Lonza

Application for the Approval of L-carnitine as a Nutritive Substance under the Australia New Zealand Food Standards Code

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ACRONYMS AND ABBREVIATIONS

ADHD	Attention-Deficit Hyperactivity Disorder
ADI	Acceptable daily intake
ARs	Analytical Reports
AUC	Area-under-the-curve
CAAet	Ethyl 4-chloroacetoacetate
CAS	Chemical Abstracts Services
CCI	Confidential Commercial Information
CDC	Centers for disease control and prevention
CFR	Code of Federal Regulations
cGMP	Current Good Manufacturing Practice
CoA	Certificate of Analysis
CRN	Council of Responisble Nutrition
CSFII	Continuing Survey of Food Intake by Individuals
CV	Coefficient of variation
CVD	Cardiovascular Disease
DSHEA	Dietary Supplement Health Education Act
EC	European Commission
EFSA	European Food Safety Authority
EWG	Europäische Wirschaftsgemeinschaft
FAO/WHO	Food and Agriculture Organization of the United Nations and the World Health Organisation
FCC	Foods Chemical Codex
FSANZ	Food Standards Australia New Zealand
FSSC	Food Safety System Certification
GBB	Gamma-Butyrobetaine
GFSI	Global Food Safety Initiative
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GNPD	Mintel´s Global New Products Database
GRAS	Generally Recognized as Safe
HACCP	Hazard Analysis Critical Control Point
HCl	Hydrochloride
HPLC	High-performance liquid chromatography
JECFA	Joint FAO/WHO Expert Committee on Food Additives
KFDA	Korean Food and Drug Administration
LC	L-carnitine

LCFA	Long-chain fatty acids
LCLT	L-carnitine L-tartrate
LSRO	Life Science Research Office
NHANES	National Health and Nutrition Examination Surveys
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
OECD	Organization for Economic Co-operation and Development
OSL	Observed safe level
PARNUTs	Food for Particular Nutritional Uses
QCRs	Reports of Lonza´s Quality Control
RACC	Reference amount customarily consumed per eating occasion
SCF	Scientific Committee on Food
TAMC	Total Aerobic Microbial Count
TGA	Therapeutic Goods Administration
TMA	Trimethylamine
TMAO	Trimethylamine-N-oxide
TML	N-trimethyl-L-lysine
U.S.	United States
USDA	U.S. Department of Agriculture
U.S. FDA	U.S. Food and Drug Administration
USP/NF	United States Pharmacopoeia/National Formulary

ADMINISTRATIVE DATA

Applicant Details

(As per section 3.1.2 (a-e) of the Application Handbook 1 September 2013)

Applicant:

Organisation Name: Lonza Ltd

Street Address: Muenchensteinerstasse 38
CH-4052 Basel,
Switzerland

Postal Address: Postfach, CH-4002 Basel, Switzerland

Direct Telephone:

Local Telephone:

Email:

Nature of Business

(As per section 3.1.2(f) of the Application Handbook 1 September 2013)

Lonza Ltd (Lonza) is a Swiss-based company that serves the pharmaceutical, healthcare and nutrition industries. Its products and services span its customers' needs from research to final product manufacture. In the pharmaceutical field, Lonza is the leading company in the production of active pharmaceutical ingredients, produced both chemically as well as biotechnologically. Lonza also has a leading position in cell-based research, endotoxin detection and cell therapy manufacturing. In the area of nutrition, Lonza also is one of the world's largest manufacturers/suppliers of vitamin B_3 (niacin and niacinamide), docosahexaenoic acid (from algae oil), arabinogalactan (from larch tree), phosphatidylserine (from soy) and L-carnitine. All of Lonza's ingredients are sold globally to the feed, food, cosmetic and pharmaceutical industry. Lonza is headquartered in Basel, Switzerland and is listed on the SWX Swiss Exchange. Additional information is available at: www.lonza.com.

Details of Other Parties Associated with the Application

(As per section 3.1.2(g) of the Application Handbook 1 September 2013

Assessment Procedure

(As per section 3.1.6 of the Application Handbook 1 September 2013

Lonza seeks to proceed with an unpaid application that adopts a general assessment procedure up to a maximum of 650 hours.

Confidential Commercial Information

(As per section 3.1.7 of the Application Handbook 1 September 2013

Lonza requests the information contained within Appendix B, C, D, H, I, J and M be considered confidential commercial information (CCI). The Appendices B, C and D provide detailed information on analyses performed for L-carnitine crystalline and L-carnitine L-tartrate including an impurity profile for both materials. The specifications for both products are shown in Section B. 5 (pp.28). Appendix H provides Lonza´s proprietary analytical method for the determination of L-carnitine in L-carnitine and Lcarnitine L-tartrate. Public methods for the determination of L-carnitine are specified in Section B.6 (pp.30). Appendix I includes a summary of the US Expert Panel Consensus Report on GRAS status of L-carnitine and L-carnitine L-tartrate for use in food. In general such reports are not open to the public. Appendix J provides a report on the so called Koeth study originally prepared for Health Canada to support the Novel food application of L-carnitine and L-carnitine L-tartrate in Canada. The report was compiled in response to the aforementioned study (published in 2013) to substantiate L-carnitine´s safety. A summary of the study is given in Section C.2 (p.38). Appendix M includes the supporting application letters received from companies.

FORMAT OF THE APPLICATION

This application is prepared pursuant to Sections 3.1 (General Requirements) and 3.3.3 (Nutritive Substances) of the Food Standards Australia New Zealand *Application Handbook* (FSANZ, 2013b) which requires the following structured format to assess an application for a nutritive substance:

A. General Information on the application
B. Technical Information on the nutritive substance
C. Information on the safety of the nutritive substance
D. Information on dietary exposure to the nutritive substance
E. Information on the nutritional impact of the nutritive substance
F. Information related to potential impact on consumer understanding and behaviour

The application is presented in this format. At the start of each section the information that must be addressed therein is specified in more detail.

A. GENERAL INFORMATION ON THE APPLICATION

A.1 Purpose of the Application

(As per section 3.1.3 of the Application Handbook 1 September 2013)

Lonza Ltd (Lonza) is making this application to amend the *Australia New Zealand Food Standards Code* to permit the sale of L-carnitine as L-carnitine (TGA: Levocarnitine) and L-carnitine L-tartrate (TGA: Levocarnitine tartrate) within Australia and New Zealand as a nutritive substance.

Lonza wishes to market refined L-carnitine and L-carnitine L-tartrate under the trade names Carnipure™ crystalline and Carnipure™ tartrate, respectively, as ingredients.

Lonza's L-carnitine (trade name Carnipure™ crystalline) is a purified, crystallized powder produced through a 2-step chemical process using ethyl 4-chloroacetoacetate as the starting material. Lonza's L-carnitine L-tartrate (trade name Carnipure™ tartrate) is the crystallized stable salt of Carnipure™ crystalline free base and food-grade L-tartaric acid, in a molar ratio of 2:1 (*i.e.,* approximately 68% L-carnitine and 32% L-tartaric acid).

A.2 Proposed Amendments

L-carnitine is proposed for use as a dietary source of L-carnitine in a variety of food categories including dairy products (excluding butter and butter fat), confectionary, cereal and cereal products, foods intended for particular nutritional uses, non alcoholic beverages and gels as set out in Table D.2-1 (Appendix L).

The following standards will require amendment to permit the use of the nutritive substance or to increase the maximum permitted amount:

i. ***Standard 1.3.4 – Identity and Purity*** (The information on the specifications for L-carnitine and L-carnitine L-tartrate is provided in Section B.5 (pp.28).

ii. ***Standard 2.1.1 – Cereal & Cereal products***

iii. ***Standard 2.5.1 – Milk***

iv. ***Standard 2.5.3 – Fermented Milk Products***

v. ***Standard 2.6.1 – Fruit Juice and Vegetable Juice***

vi. ***Standard 2.6.2 – Non alcoholic beverages and brewed soft drinks***

vii. ***Standard 2.6.4 – Formulated caffeinated drinks***

viii. ***Standard 2.9.3 – Formulated meal replacements and formulated supplementary food***

ix. ***Standard 2.9.4 – Formulated Supplementary Sports Foods***

The information requirements for changes to the commodity standards to allow the addition of L-carnitine and L-carnitine L-tartrate to these foods are provided in all Sections of this application.

A.3 Justification for the Application

(As per section 3.1.4 of the Application Handbook 1 September 2013)

A.3.1 Purpose of Using the Nutritive Substance

The purpose of using L-carnitine as an ingredient in foods is to maintain the normal carnitine status of the body, particularly in those individuals consuming foods with minimal L-carnitine content and/or inadequate supply of micronutrients caused by certain forms of nutrition or changed eating habits. Carnipure™ crystalline and Carnipure™ tartrate are proposed for use in Australia and New Zealand in a wide range of foods as detailed in Table D.2-1 (Appendix L). Further details of the anticipated intakes and nutritional information are provided in Section D (pp.86).

L-carnitine was first isolated from meat extracts in 1905, and subsequently identified as the naturally-occurring and biologically active form of carnitine in 1927 (Tanphaichitr and Leelahagul, 1993). L-carnitine is an essential co-factor for fatty acid metabolism and other metabolic pathways, with body stores maintained primarily in skeletal muscle. The majority of the body's L-carnitine is supplied in the diet from meats and meat-based foods; however, L-carnitine is also synthesized endogenously from lysine and methionine supported by certain vitamins and minerals. Thus, L-carnitine cannot be considered an essential nutrient, *per se*, although the term "conditionally essential nutrient" is often encountered in the scientific literature.

L-carnitine is present in the human diet in a variety of food sources, with the concentration dependent on the type of food source (Tanphaichitr and Leelahagul, 1993; Demarquoy *et al.,* 2003). Animal products, such as lamb, beef and pork, are the richest sources of dietary L-carnitine (Tanphaichitr and Leelahagul, 1993; Demarquoy *et al.,* 2003). In comparison, lower levels of L-carnitine are found in dairy products and most fruits and vegetables contain minimal amounts of L-carnitine (Rebouche and Engel, 1984; Demarquoy *et al.,* 2003). The consumption of L-carnitine from the diet has been estimated to range from 100 to 300 mg/day (Broquist, 1994); however, the consumption of L-carnitine may be considerably higher in heavy consumers of meats and meat products vice versa lower in people avoiding meat like vegetarians/ vegans or people lacking appetite for it like elderly people.

A.3.2 Need for the Proposed Change

L-carnitine is currently permitted to be added to a limited range of food products in Australia and New Zealand as follows:

1. Standard 2.9.1 Infant Formula Products - Table to clause 7:
    - Minimum amount for claim per 100 kJ – 0.21 mg
    - Maximum amount per 100 kJ – 0.8 mg
2. Standard 2.9.4 Formulated Supplementary Sports Foods – Table to Paragraph 2(c):
    - Maximum amount added per one-day quantity – 100 mg
3. Standard 2.9.5 Food for Special Medical purposes

Lonza is seeking approval to extend the use of L-carnitine to a range of other food products to widen the possibilities for innovation by national manufacturers and allow them to benefit from increased market development both domestically and overseas.

A.3.3 Advantages and Disadvantages of the Proposed Change

It is anticipated that the introduction of a range of food products containing Carnipure™ crystalline or Carnipure™ tartrate would provide greater opportunities for innovation by manufacturers and allow them to benefit from increased market development both domestically and overseas. Considering the range of products that Carnipure™ crystalline and Carnipure™ tartrate are added to, consumers would be provided with an increased choice of products.

L-carnitine is not currently produced within Australia or New Zealand and therefore approval will not disadvantage local L- carnitine manufactures.

Imported L-carnitine containing final products will be required to be labelled with country of origin so consumers will be able to make a choice as to whether they purchase or not.

A.3.4 Status of Similar Applications

In India a similar application is in progress. In Canada Lonza currently has an application for the approval of Carnipure™ crystalline and Carnipure™ tartarate in food.

A.3.5 Regulatory Impact Information

A.3.5.1 Cost and Benefits

Consumers

The proposed amendments place no additional economic cost on consumers – L-carnitine will be labelled and consumers can chose if they wish to purchase a product containing this material.

The benefit to consumers is additional choice of an alternative ingredient in a product or additional products which become available due to the larger number of food categories permitted to contain L-carnitine.

Consumers will also have access to food products containing L-carnitine that are currently manufactured overseas – these products will contribute to consumer choice.

Industry and Business

The addition of L-carnitine will cause a food to be different from other commodity foods of its type, and will provide the industry with greater opportunities for innovation and expanding their markets. It is not anticipated that there will be any added cost to the industry that won't be passed through the distribution channel to the final retail price, which the consumer wanting L-carnitine (from Carnipure™ crystalline or Carnipure™ tartrate) will be willing to bear.

In terms of cost, consumer products containing Carnipure™ crystalline or Carnipure™ tartrate will be sold with a slightly higher retail price than products without the aforementioned ingredients, as these products are marketed as value-added products. It is expected that the consumer interested in L-carnitine-containing foods will generally accept the added cost to be a good value proposition. If not, the consumer will simply choose a product that does not contain L-carnitine within the given category.

Government

There is no apparent impact on government agencies by the controlled addition of L-carnitine as an ingredient to foods beyond the initial regulatory cost of approving further uses as a nutritive substance.

A.3.5.2 Impact on International Trade

L-carnitine is intended for use as a food ingredient in a specific range of currently available food products to impart a specific functional property. The impact on international trade is anticipated to be the following:

- Approval of L-carnitine as a nutritive substance may, in the future, promote international trade and reduction of technical barriers to trade, while continuing to protect public health and safety.
- Manufacturers and importers wishing to sell L-carnitine containing foods would benefit.
- The global marketing opportunities for the development and sale of L-carnitine containing foods would be expanded.
- Approval of L-carnitine as a nutritive substance gives the domestic industry the chance to serve consumers with products containing L-carnitine made in Australia and New Zealand. Currently, there are some examples of individual consumers buying L-carnitine-containing products online for personal use. The approval of L-carnitine will make it possible for Australian and New Zealand brands to participate in this demand.

A.4 Information to Support the Application

(As per section 3.1.5 of the Application Handbook 1 September 2013)

Information is provided in this application to enable the objectives specified in Section 18 of the FSANZ Act to be addressed as follows:

(a) **The protection of public health and safety**: Information to support objective (a) is provided in Section C (pp.32) of the application, in which the safety of Carnipure™ crystalline and Carnipure™ tartrate, based on the available pre-clinical and human safety data, is discussed in detail.

(b) **The provision of adequate information relating to food to enable consumers to make informed choices**: Data to support objective (b) are provided in Section E (pp.103), in which the full compositional information and nutrient profiles of foods containing Carnipure™ crystalline and Carnipure™ tartrate are described in detail.

(c) **The prevention of misleading or deceptive conduct**: Information to support objective (c) is provided in Section F (pp.110), in which the consumer awareness and potential behaviour in response to Carnipure™ crystalline and Carnipure™ tartrate-containing products are described in detail. This objective can also be further supported by human safety data contained in Section C (pp.32).

A.4.1 Public Health and Safety Issues

The safety of L-carnitine is based on knowledge regarding the historical consumption of L-carnitine as a common component of the typical human diet, the endogenous biosynthesis of L-carnitine as a normal body metabolite, the absorption, distribution, metabolism and excretion of L-carnitine, published toxicological safety data in rats, rabbits and dogs, as well as human studies without indication of adverse effects on human health. The safety of L-carnitine, Carnipure™ crystalline and Carnipure™ tartrate is discussed in detail in Section C (pp.32).

A.4.2 Consumer Choice

L-carnitine is required to be labelled enabling consumers to choose if they wish to purchase a product containing this ingredient.

A.4.3 Support for the Proposed Change

The company letters of the companies who have an interest in marketing products which would contain L-carnitine are provided in Appendix M and considered as confidential.

A.4.4 Policy Guidelines

Information is provided in this section to address the high order and specific principles in the Ministerial Council Policy Guideline - Addition to Food of Substances other than Vitamins and Minerals[1].

The addition of substances other than vitamins and minerals to food where the purpose of the addition is for other than to achieve a solely technological function should be permitted where:

Specific Order Policy Principles – Any Other Purpose	Section of Application
a) the purpose for adding the substance can be articulated clearly by the manufacturer (i.e. the 'stated purpose'); and	A.3.1 / E.4
b) the addition of the substance to food is safe for human consumption; and	A.4.1 / C.1
c) the substance is added in a quantity and a form which is consistent with delivering the stated purpose; and.	D.4 / E.4
d) the addition of the substance is not likely to create a significant negative public health impact to the general population or sub population; and	C.1/ F.3
e) the presence of the substance does not mislead the consumer as to the nutritional quality of the food.	A.3.5.1/ E.1

A.5 International and Other National Standards

(As per section 3.1.9 of the Application Handbook 1 September 2013)

This application meets the FSANZ objective of promoting consistency between domestic and international standards.

Please see also chapter D.5 "Information Relating to the Nutritive Substance in Other Countries" (pp.100).

A.5.1 International Standards

Global: Codex Alimentarius

- L-carnitine is regarded as an essential nutrient in infant formula and formulas for special medical purposes intended for infants (Codex, 2011)
- L-carnitine and L-carnitine L-tartrate are listed on the Advisory list of nutrient compounds for use in foods for special dietary uses intended for infants and young children (Codex, 2009)

1 http://www.foodstandards.gov.au/code/fofr/fofrpolicy/documents/Addition%20to%20Food%20of%20Substances%20other%20than%20Vitamins%20and%20Minerals%20May%202008.pdf, accessed 05.10.13

A.5.2 Other National Standards

USA

- L-carnitine and L-carnitine L-tartrate are self-affirmed as Generally Recognized as Safe (GRAS) for use in a number of foods, including beverages and beverage bases, coffee and tea, dairy product analogs, grain products and pastas, hard candies, milk products, processed fruits and fruit juices and soft candies (Lonza, 2011)
- L-carnitine and L-carnitine L-tartrate are regulated according to the "Dietary Supplement Health and Education Act" of 1994 (DSHEA) and allowed to be used as a food supplement – status "grandfathered" (CRN, 1998)

Canada

- L-carnitine and L-carnitine L-tartrate have been approved by Health Canada for use in dietary supplements.
- Prior to 2011, Levocarnitine (L-carnitine) and its salts and derivatives were only allowed to be used in pharma products. Since 2011, L-carnitine has been revised to retain prescription status for levocarnitine and its salts and derivatives when sold for the treatment of primary or secondary levocarnitine deficiency. Levocarnitine and its salts and derivatives for any other uses at any strength, dosage form or route of administration are exempt from prescription status (Canada Gazette, 2011).

European Union (EU)

- L-carnitine crystalline and L-carnitine L-tartrate have been marketed in the EU before May 15, 1997. They are grandfathered and thus, not regarded as Novel Food according to Regulation (EC) No 258/97 of 27 January 1997 concerning novel foods and novel food ingredients. (EC, 2006a)
- L-carnitine and L-carnitine L-tartrate are listed in Commission Directive 2006/141/EC on infant formulae and follow-on formulae. (EC, 2006b)
- L-carnitine is approved as a nutrient for use in processed cereal-based foods and baby foods for infants and young children under Commission Directive 2006/125/EC. (EC, 2006c)
- L-carnitine and L-carnitine L-tartrate are listed in Commission Regulation (EC) No 953/2009 on substances that may be added for specific nutritional purposes in foods for particular nutritional uses (PARNUTS) (EC, 2009) and subsequent regulation "Regulation (EU) No 609/2013 on food intended for infants and young children, food for special medical purposes, and total diet replactement for weight control" (EU, 2013).
- The EFSA Scientific Committee on Food (SCF) evaluated the use of L-carnitine L-tartrate as a source of L-carnitine in PARNUTS. The Panel concluded that consumption of up to 3 g/day of L-carnitine L-tartrate (equivalent to 2 g/day of L-carnitine) presents no safety concerns when used as a source of L-carnitine for use in PARNUTS, provided

that the acceptable daily intake (ADI) for L-tartaric acid from all sources in the diet is not regularly exceeded. (EFSA, 2003)

China

- Carnipure™ crystalline and Carnipure™ tartrate are regulated according to
  - Standard for the use of nutritional fortification substances in foods (GB14880-2012)
  - National food safety standard Infant formula (GB 10765-2010)
  - National food safety standard follow-up infants and young children (GB 10767- 2010).

  Both substances are allowed to be used in milk powder, beverages on the basis of fruit/vegetable juice and milk, flavored beverages, solid beverages (instant powder), sports beverages, athletic nutrition food and Health Food (covering food supplements).
- L-carnitine and L-carnitine L-tartrate are approved for use in Infant formulae, follow-on formulae and young children: 0.3 mg/100 kJ or 1.3 mg/100 kcal.

Japan: L-carnitine and L-carnitine L-tartrate are allowed to be used in foods and dietary supplements. Maximum daily intake was suggested by Ministry of Health and Welfare to be up to 1000 mg/day or 20 mg/kg body weight/day. (Japan, 2002)

Korea: L-carnitine crystalline is listed in the Korean Food Additive List.
In 2011, the Korean Food and Drug Administration (KFDA) approved Carnipure™ tartate as a health food functional ingredient for weight management products. The approved daily dosage is about 2 g as L-carnitine, the allowed health claim is read “Can reduce body fat”. (KFDA, 2012)

Malaysia: L-carnitine crystalline and L-carnitine L-tartrate have been officially classified under category of amino acids in the Food Regulation Amendment September 2009 (effective from 1/1/2010). They can be freely added to products without any classification by Ministry of Health.

Singapore: L-carnitine crystalline and L-carnitine L-tartrate are allowed to be added to foods formulated for specific nutritional needs/target groups like infant formulas, follow-on formulas and energy drinks. In energy drinks, the addition of L-carnitine should not exceed 100 mg/day. For dietary supplements L-carnitine crystalline and L-carnitine L-tartrate can be added as long as level is safe for the target consumers.

Brazil: Carnipure™ crystalline and Carnipure™ tartate are approved as Novel Food.

Australia and New Zealand

- L-carnitine is approved in Australia and New Zealand as a permitted nutritive substance for use in infant formula products at levels up to 0.8 mg per 100 kJ in Standard 2.9.1 – Infant Formula Products (FSANZ, 2013c).
- L-carnitine is approved as a permitted nutritive substance for use in supplementary sports food at levels up to 100 mg/day in Standard 2.9.4 - Formulated Supplementary Sports Foods (FSANZ, 2013d).
- L-carnitine crystalline and L-carnitine L-tartrate are allowed to be used in products regulated by Therapeutic Goods Administration (TGA) (Australia only) (see following "Published Specifications").

Published Specifications:

- The specification for Lonza's L-carnitine ingredient conforms to those listed in the United States Pharmacopoeia/National Formulary (USP36/NF31) monograph for Levocarnitine (USP, 2013).
- The specification for Lonza´s L-carnitine ingredient conforms to the Foods Chemical Codex (FCC) 8th version published in March 2012. (FCC 2012)
- Lonza's L-carnitine and L-carnitine L-tartrate meet the draft compositional guideline published by the TGA for L-carnitine (Levocarnitine) and L-carnitine L-tartrate (Levocarnitine tartrate) (TGA, Levocarnitine).*

*Note: The draft compositional guidelines, for which the comment periods have closed, are being reviewed in light of submissions made to the TGA and are being reformatted for consistency with the TGA website redevelopment. Once this review has been completed, finalized documents will be included with the 'Current compositional guidelines' or revised drafts will be published and opened for further comment.

Until the draft guidelines are progressed in either of these two ways, information on the following documents can be obtained from the Office of Complementary Medicines.[2]

[2] TGA website, http://www.tga.gov.au/newsroom/consult-cm-cg-draft.htm, accessed 29.05.13.

B. TECHNICAL INFORMATION ON THE NUTRITIVE SUBSTANCE

In accordance with Section 3.3.3 – Nutritive Substances of the Food Standards Australia New Zealand *Application Handbook* (FSANZ, 2013b) the following technical information is provided:

1. Information to enable identification of the nutritive substance
2. Information on the physical and chemical properties of the nutritive substance
3. Information on the impurity profile
4. Manufacturing process
5. Specification for identity and purity
6. Analytical method for detection
7. Information on the proposed food label

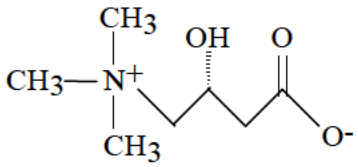
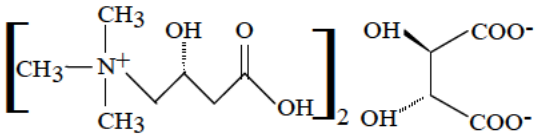
Each point is addressed in turn in the Section that follows.

B.1 Information on the Type of Nutritive Substance

Carnipure™ crystalline and Carnipure™ tartrate are single chemical compounds produced synthetically.

B.2 Information on the Physical and Chemical Properties of the Nutritive Substance

Carnipure™ crystalline is a purified, crystallized powder produced through a 2-step chemical process using ethyl 4-chloroacetoacetate as the starting material, whereas Carnipure™ tartrate is the crystallized stable salt produced from the combination of Carnipure™ crystalline free base and food-grade equivalent L-tartaric acid, in a molar ratio of 2:1 (*i.e.,* approximately 68% L-carnitine and 32% L-tartaric acid). The common names, trade names, chemical names, Chemical Abstracts Services (CAS) numbers, structural formulae, solubility in water, and melting points for Carnipure™ crystalline and Carnipure™ tartrate are presented in Table B.2-1 (p.25).

Table B.2- 1 Descriptions of Carnipure™ crystalline and Carnipure™ tartrate						
Common Name	**Trade Name**	**Chemical Name**	**CAS NO.**	**Structural Formula**	**Solubility in Water**	**Melting Point**
L-carnitine	Carnipure™ crystalline	4-Amino-3-hydroxybutyric acid trimethylbetain; 1-Propanaminium, 3-carboxy-2-hydroxy-N,N,N-trimethyl-, hydroxide, inner salt, (R)-; (R)-(3-carboxy-2-hydroxypropyl) trimethylammonium hydroxide, inner salt; γ-amino-β-hydroxybutyric acid trimethylbetaine; γ-trimethyl-β-hyroxybutyrobetaine; 3-hydroxy-4-(trimethyl-ammonio)butanoate	541-15-1		Entirely miscible 250g/100ml (20℃)	185 to 195℃, decomposition without melting
L-carnitine L-tartrate	Carnipure™ tartrate	1-Propanaminium, 3-carboxy-2-hydroxy-N,N,N-trimethyl-, (R)-, salt with [R-(R*,R*)]-2,3-dihydroxybutanedioic acid (2:1)	36687-82-8		>1000 g/L (20℃)	171.1 to 173.7℃

B.3 Information on the Impurity Profile for a Typical Preparation

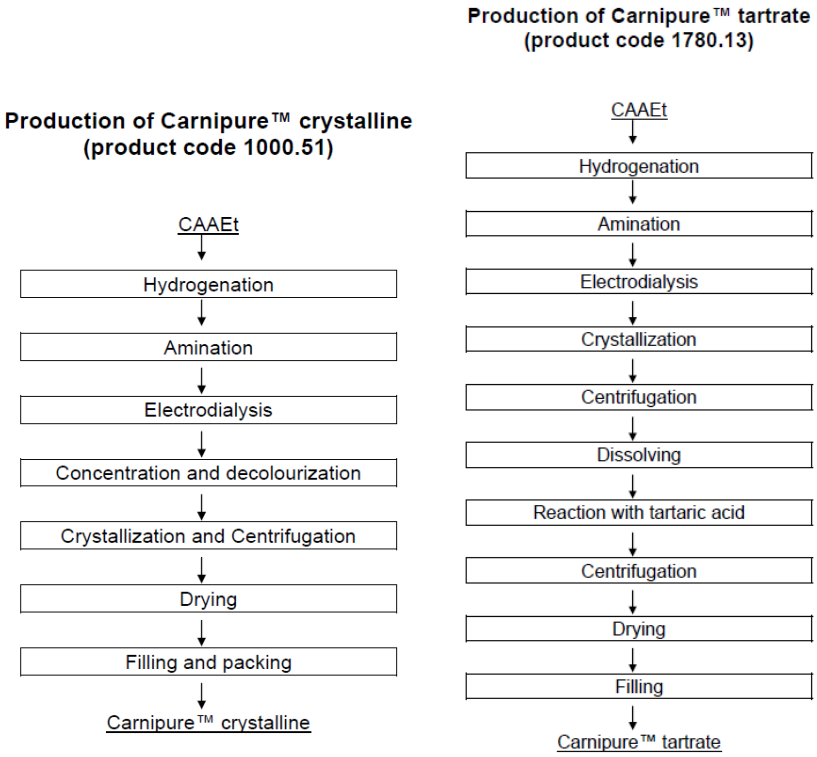
The manufacturing process of Carnipure™ crystalline and Carnipure™ tartrate involves a combination of food-grade/food grade equivalent materials and highly pure chemicals that are not typical food ingredients. However, the starting materials and processing aids used in the manufacturing of Carnipure™ crystalline and Carnipure™ tartrate are removed during the purification of the L-carnitine and L-carnitine L-tartrate products and are demonstrated through analysis to be well below the specification limits defined for the final Carnipure™ crystalline and Carnipure™ tartrate products. In addition, all the potential impurities or by-products accounted for by the production process are shown through analytical results to be below the limit values for pertinent specifications. Lot samples are routinely assayed to verify compliance with specifications. Detailed information is provided in the Appendices B, C and D.

B.4 Manufacturing Process for Carnipure™ crystalline and Carnipure™ tartrate

B.4.1 Overview of Production Process

An overview of the production process is provided in Figure B.4.1-1(p.26).

Figure B.4.1- 1 Schematic Overview of the Manufacturing Process for Carnipure™ crystalline and Carnipure™



B.4.2 Control of the Production Process and Product Quality

The production of Carnipure™ crystalline and Carnipure™ tartrate is conducted in accordance with the principles of Good Manufacturing Practice (GMP) for dietary supplements and Food Safety System Certification (FSSC) 22000:2011. Lonza's L-carnitine manufacturing site is FSSC 22000:2011 certified to assure the safety and quality of Carnipure™ crystalline and Carnipure™ tartrate. FSSC 22000 was developed by the Foundation for Food Safety Certification. This development is supported by the Confederation of the FoodDrinkEurope. The scheme is recognised by the Global Food Safety Initiative (GFSI). The GFSI is a business-driven initiative for the continuous improvement of food safety management systems to ensure confidence in the delivery of safe food to consumers worldwide (www.mygfsi.com).
FSSC 22000 has as mission to be the globally leading, independent, non-profit, ISO-based and GFSI-accepted food safety certification scheme for the whole supply chain. The appropriate certification is provided in Appendix E

B.4.3 History of the Production Process

In 1984, Lonza sold the first commercial quantities of L-carnitine to infant nutrition companies, who began to include L-carnitine in their soy-based infant nutrition products. In 1986, Lonza developed a fermentation process for the manufacture of L-carnitine, resulting in a product with excellent purity and safety profile, which was marketed under the Carnipure™ brand name. After 2005, Lonza developed a new chemical process for the synthesis of L-carnitine involving a 2-step enantioselective process.

B.4.5 Potential for Substances Hazardous to Health

It is not anticipated that the production process results in the formation of any substances that might present a safety concern. This is supported by detailed analytical information on the potential impurities, by-products and environmental and microbiological contaminants (see Appendix D), and by a comprehensive product specification (see Section B.5 (p.28)). The final Carnipure™ crystalline and Carnipure™ tartrate products undergo several purification steps including distillation, crystallization, and washing in order to remove any potential impurities, excess reagents or by-products that may pose a health hazard.

B.5 Specification for Identity and Purity for the Nutritive Substance

The specifications for Carnipure™ crystalline conform to the relevant published specifications listed in the USP/NF monograph for L-carnitine (USP, 2013) and in the FCC (FCC, 2012), and as a result, comply with Clause 2 of Standard 1.3.4 – Identity and Purity. These specifications are presented in Table B.5-1 (p. 28).

Table B.5- 1 Product Specifications for L-carnitine crystalline (Levocarnitine)

Specification Parameter	USP Specifications	FCC Specifications	TGA Draft Comp.	Lonza's Specifications	Method (Lonza)
Appearance	n/a	Crystalline powder	Crystalline powder	Crystalline powder	Visual
Colour	n/a	White	White	White	Visual
Identity	L-carnitine	L-carnitine	L-carnitine	L-carnitine	IR spectroscopy
Water (% w/v)	≤4.0	≤4.0	≤1.0	≤4.0 (CoA ≤1.0)	Titration (Karl Fischer)
Purity (% w/v)	97.0 – 103.0	97.0 – 103.0	98 – 102	99.0 – 101.0	Titration (acidimetric)
pH	5.5 – 9.5	5.5 – 9.5	6.5 – 8.5	6.5 - 8.5	2.5 g/50 mL water
Specific rotation (^{o})	-29.0 to -32.0	-29.0 to -32.0	29.0 to -32.0	-29.0 to -32.0	Polarometric determination
Residue on ignition [% (w/v)]	≤0.5	≤0.5	≤0.5	≤0.1	USP 281 (residue on ignition)
Microbiological Count					
Total aerobic microbial count (CFU/g)	n/a	n/a	n/a	≤ 50	USP 61 (plate count/ membrane filtration)
Heavy Metals					
Heavy metals (as Pb) (mg/kg)	≤20	Lead: ≤ 1 mg/kg	≤10	≤10	USP 231 (limit test)

CFU = colony-forming units; CoA = Certificate of Analysis, FCC = Food Chemicals Codex; IR = infrared; Pb =lead; n/a = not applicable; USP = United States Pharmacopeia

The TGA draft compositional guideline for L-carnitine L-tartrate (TGA 2010) and Lonza's specifications for Carnipure™ tartrate are provided in Table B.5-2 (p.29).

Table B.5- 2 Product Specifications for L-carnitine L-tartrate (Levocarnitine tartrate)

Specification Parameter	**TGA Draft Comp.**	**Lonza´s Specifications**	**Method (Lonza)**
Appearance	White crystalline powder	White crystalline powder	Visual
L-carnitine [% (w/w)]	68.7 ± 2%	67.2 - 69.2	Titration (acidimetric)
L-Tartaric acid [% (w/w)]	31.1± 1%	30.8 - 32.8	Titration (acidimetric)
Water [% (w/w)]	≤1.0%	≤0.50	Titration (Karl Fischer)
pH	3 - 4	3.0 - 4.5	2.5 g/50 mL water
Specific rotation (^{o})	-9.5 to -11.0	-9.5 to -11.0	Polarometric determination
Residue on ignition [% (w/w)]	≤0.5%	≤0.1	USP 281 (residue on ignition)
Microbiological Count			
Total aerobic microbial count (CFU/g)	n/a	≤ 50	USP 61 (plate count/ membrane filtration)
Heavy Metals			
Heavy metals (as Pb) (mg/kg)	≤10	≤10	USP 231 (limit test)

CFU = colony-forming units; Pb =lead; USP = United States Pharmacopeia

B.6 Analytical Method for Detection

For L-carnitine crystalline (Levocarnitine) two official test methods are available to determine L-carnitine in the raw material itself

- Food Chemical Codex (FCC, 2012)
- United States Pharmacopeia (USP, 2013).

For L-carnitine L-tartrate no official test method is published. Lonza´s test method related to the determination of L-carnitine in L-carnitine and L-carnitine L-tartrate is proprietary and provided in Appendix H.

There is no official test method available to determine L-carnitine in food/food matrices.

The only document describing a method for the determination of L-carnitine in different food is the study run by Demarquoy in 2003 “Radioisotopic determination of L-carnitine content in foods commonly eaten” (Demarquoy et al. 2003).

B.6.1 Stability Data for Carnipure™ crystalline

The stability of one lot of Carnipure™ crystalline (Lot No.03803) under ambient conditions (*i.e.,* 25±2℃, 60±5% relative humidity) was evaluated for a period of 48 months. Carnipure™ crystalline was shown to be stable for at least 48 months, as L-carnitine levels were within the specification limits of 99.0 to 101.0%. In addition, one lot of Carnipure™ crystalline (Lot No. 04704) was evaluated under accelerated conditions (*i.e.,* 40±2℃, 75±5% relative humidity) and was determined to be stable for a period of 12 months. Analyses for appearance, transparency, turbidity, pH, water content, impurities (*i.e.,* γ-butyrobetaine, crotonobetaine, betaine, norcarnitine, TMA), and microbiological contaminants also indicated that both lots met the specification limits for these parameters. The results of the stability tests are presented in Table B.6.1-1 (p.30), and in Appendix F.

Table B.6.1- 1 L-carnitine Content (%) of Carnipure™ crystalline

Batch	Specification (w/w %)	Months								
		0	3	6	9	12	18	24	36	48
25°C, 60±5% RH										
03803[a]	99.0-101.0	100.0	100.7	100.2	100.0	99.9	99.5	101.0	99.8	99.6
40℃, 75±5% RH										
04704[b]	99.0-101.0	99.6	n/a	100.1	100.4	99.8	n/a	n/a	n/a	n/a

n/a = not applicable; RH = relative humidity

[a] Test initiated on 06/08/2003; [b] Test initiated on 03/05/2004

B.6.2 Stability Data for Carnipure™ tartrate

The stability of 3 non-consecutive lots of Carnipure™ tartrate (Lot No. 08003, 09002, and 09202), stored at temperatures ranging from 25 to 40℃, was evaluated for periods of 24 to 60 months. As shown in Table B.6.2-1 (p.31), Carnipure™ tartrate was stable for at least 24 months under all conditions. Stored under ambient conditions (*i.e.,* 25±2℃, 60±5% relative humidity) L-carnitine levels remained within the specification limits of 67.2 to 69.2% even for 60 month. Other parameters assessed included appearance, transparency, turbidity, pH, water content, impurities (*i.e.,* γ-butyrobetaine, crotonobetaine, betaine, norcarnitine, TMA), and microbiological contaminants. Carnipure™ tartrate was shown to meet the specification limits for these parameters over a period of 60 months, regardless of temperature or humidity. Results of these analyses are presented in Appendix F.

Table B.6.1- 2 L-carnitine Content (%) of Carnipure™ tartrate

Batch	Specification (w/w %)	Months									
		0	3	6	9	12	18	24	36	48	60
25°C, 60±5% RH											
08003[a]	67.2 – 69.2%	68.1	67.8	67.8	68.3	68.2	68.5	68.3	67.6	68.0	67.8
30℃, 60±5% RH											
09002[b]		68.0	68.4	668.0	68.1	68.2	n/a	68.2	n/a	n/a	n/a
40℃, 75±5% RH											
09202[c]		68.1	68.4	67.9	68.2	68.1	n/a	68.0	n/a	n/a	n/a

n/a = not applicable; RH = relative humidity

[a] Test initiated on 31/10/2003; [b] Test initiated on 13/11/2002; [c] Test initiated on 13/11/2002

B.7 Information on the Proposed Food Label

L-carnitine / L-carnitine L-tartrate needs to be listed in the ingredients list when used.

C. INFORMATION RELATED TO THE SAFETY OF THE NUTRITIVE SUBSTANCE

In accordance with Section 3.3.3 – Nutritive Substances of the Food Standards Australia New Zealand *Application Handbook* (FSANZ, 2013b) the following safety information is provided:

1. Information on the toxicokinetics and metabolism of the nutritive substance, and if necessary, its degradation products and major metabolites.
2. Information from studies in animals or humans that is relevant to the toxicity of the nutritive substance, and if necessary, its degradation products and major metabolites.
3. Safety assessment reports prepared by international agencies or other national government agencies.

These points are addressed in the Section that follows.

C.1 Information on the Toxicokinetics and Metabolism of the Nutritive Substance and, if necessary, its Degradation Products and Other Metabolites

C.1.1 Endogenous Production of L-carnitine

L-carnitine is present in the human body as a result of exposure from dietary sources or is synthesized endogenously. Dietary sources contribute to approximately 75% of the body's store of L-carnitine, while the remaining 25% is formed endogenously (Arenas *et al.*, 1998). Endogenous L-carnitine is biosynthesized from L-lysine and L-methionine, in which the carbon backbone of L-carnitine is derived from L-lysine, while the 4-N-methyl groups are provided by L-methionine (Vaz and Wanders, 2002). The first product of L-carnitine biosynthesis, N-trimethyl-L-lysine (TML), is produced *via* initial series of methylation reactions involving S-adenosylmethionine. The production of TML has been shown to be the major rate-determining step in carnitine biosynthesis (Stanley, 1998). TML is enzymatically transformed into 3-hydroxy-TML in a reaction requiring α-ketoglutarate, oxygen, ascorbic acid, and iron. Glycine is cleaved from 3-hydroxy-TML to form γ-trimethylaminobutyraldehyde (γ-butyrobetaine; deoxycarnitine) using pyridoxal 5'-phosphate (vitamin B6) as a co-factor. The final step in the synthesis of L-carnitine involves the hydroxylation of γ-butyrobetaine in a reaction that requires α-ketoglutarate, oxygen, ascorbic acid, and iron. This final step is catalysed by γ-butyrobetaine hydroxylase, an enzyme present in the liver, kidney, and brain (Haeckel *et al*., 1990).

The majority of endogenous L-carnitine is synthesized in the liver and, to a lesser extent, in the kidney and brain (Siliprandi *et al*., 1989; Ramsay, 1994; Vaz and Wanders, 2002). The kidney also has a high level of uptake of TML from the blood for conversion to γ-butyrobetaine through β-hydroxylase activity (Siliprandi *et al*., 1989). Some of the γ-butyrobetaine released from the kidney may subsequently be hydroxylated to L-carnitine in the liver. Tissues, such as heart and muscle, derive a large amount of their energy from the oxidation of long-chain fatty acids, but are lacking γ-butyrobetaine hydroxylase. These tissues receive L-carnitine transported either systemically from sites of biosynthesis or following absorption from the gastrointestinal tract (Borum, 1991).

L-carnitine exists in both free and esterified forms in all tissues, including plasma. Approximately 80 to 90% of the intracellular and extracellular L-carnitine exists in free, unesterified form (Stanley, 1998). The esterified fraction increases when fatty acid oxidation is activated (*e.g*., during fasting) and the ratio of intracellular acyl coenzyme A to free coenzyme A is elevated (Stanley, 1998). L-carnitine is stored predominantly in skeletal muscle, the heart, sperm, and the brain. The total L-carnitine pool of a typical 70 kg adult is approximately 15 to 21 g, with more than 98% present in muscle tissues, less than 1.5% in the liver and kidneys, and smaller amounts (~0.4%) in plasma and extracellular fluids (Siliprandi *et al*., 1989; Evans and Fornasini, 2003). Plasma concentrations of total L-carnitine (including L-carnitine and acetyl-L-carnitine) in healthy adults have been reported to range from 40 to 50 µmol/L (Evans and Fornasini, 2003). L-carnitine is more concentrated in tissues than in blood, and L-carnitine concentrations in skeletal and cardiac muscle have been reported to be up to 100 times higher than that in plasma

(Evans and Fornasini, 2003). In addition, the concentration of L-carnitine is higher in the heart (4 mM) compared to liver (2 mM) (Ramsay, 1994).

L-carnitine is transported into tissues *via* an active saturable transport system OCTN2 (Tanphaichitr and Leelahagul, 1993; Rebouche and Seim, 1998). The active transport systems are tissue-specific and sodium-dependent mechanisms, located in cell membranes function to maintain a higher intracellular concentration of L-carnitine (Ramsay, 1994; Rebouche and Seim, 1998).

L-carnitine homeostasis in humans is maintained by dietary L-carnitine intake, a modest rate of endogenous L-carnitine synthesis, and efficient conservation of L-carnitine by the kidney (Rebouche and Seim, 1998). The rate and quantity of L-carnitine synthesis may be affected by the amount of L-carnitine absorbed from the diet, with endogenous synthesis reduced with higher dietary intakes (Rebouche and Chenard, 1991). Strict vegetarians have been reported to obtain more than 90% of L-carnitine through biosynthesis, while omnivorous individuals derive approximately 25% of L-carnitine from biosynthesis (Vaz and Wanders, 2002). In male rats, Rebouche (1983) reported that L-carnitine biosynthesis from [methyl-^{3}H]-TML was reduced following 32 days of dietary supplementation with 1.0% of L-carnitine, D-carnitine, or γ-butyrobetaine. Rebouche (1983) attributed the lower amount of radiolabelled L-carnitine in tissues and urine to inhibition of γ-butyrobetaine transport into the liver for subsequent hydroxylation.

C.1.2 Absorption and Distribution

Dietary L-carnitine is absorbed through active transport and passive diffusion mechanisms, while the unabsorbed L-carnitine may be degraded in the gastrointestinal tract (Rebouche and Chenard, 1991; Tanphaichitr and Leelahagul, 1993; Rebouche, 2004). A number of different values for the bioavailability of L-carnitine have been reported. The oral bioavailability of L-carnitine from normal Western diets has been reported to range from 54 to 87%, and is suggested to be dependent on the quantity of L-carnitine ingested (Rebouche and Chenard, 1991; Rebouche, 2004). Specifically, the bioavailability of L-carnitine was reported to range from 66 to 86% in individuals consuming a low-carnitine diet (*i.e.,* 1.53 to 2.12 µmol/kg body weight/day or 247 to 342 µg/kg body weight), and from 54 to 72% in those consuming a high-carnitine diet (8.4 to 11.8 µmol/kg body weight/day or 1,354 to 1,902 µg/kg body weight/day). In contrast, Sahajwalla *et al*. (1995) reported an absolute oral bioavailability of approximately 18% in 15 healthy adult male volunteers for 3 dosage forms of L-carnitine, a 330 mg L-carnitine tablet, a 1,000 mg L-carnitine chewable tablet, and an oral solution of 1,000 mg L-carnitine/ 10 mL, each provided as a total dose of 2,000 mg L-carnitine every 12 hours for 4 days. This was compared with the bioavailability of a single intravenous dose of 20 mg L-carnitine/kg body weight. After correction for baseline (endogenous) plasma concentrations, the absolute bioavailability ranged between 14 to 16% based on free L-carnitine concentrations in plasma. Similarly, a low oral bioavailability of 16% (ranged from 9 to 25%) and 5% (ranged from 4 to 10%) following administration of 2,000 and 6,000 mg of L-carnitine, respectively, were reported in 6 healthy subjects (Harper *et al*., 1988). No significant difference was observed between the

areas under the plasma L-carnitine concentration-time curves for both dose levels, and it was concluded that L-carnitine absorption was saturated at the 2,000 mg dose (Harper *et al*., 1988). The mean plasma carnitine concentrations after a single oral administration of 2 and 6 g L-carnitine to 6 healthy human subjects were 46 µmol/L and 51 µmol/L, respectively, 24 h post-dose. While there was no significant difference between the areas under the plasma carnitine concentration-time curves of the 2 g and 6 g doses, the bioavailability of the 2 g oral dose was 16% (mean) and that of the 6 g dose was about 5% (mean). Of the oral doses of 2 and 6 g, 1,021 and 1,580 µmol/L was excreted over 24-hour post-dose (basal values subtracted), *i.e.*, 8 and 4% of ingested dose, respectively (Harper *et al.,* 1988). Interestingly the bioavailability of a 2 g oral dose, which is in the region of the maximum estimated daily intake from the use of L-carnitine in the specified food and beverage categories, equates to approximately 15% of the administered concentration. The low bioavailability of large doses of L-carnitine supplements (*i.e.,* 1 g or more), in comparison to the higher bioavailability of dietary L-carnitine, has been attributed to the high polarity of L-carnitine, which limits its passive diffusion across gastrointestinal membranes, as well as the saturation of active intestinal transporters (Evans and Fornasini, 2003).

The uptake of ingested L-carnitine by intestinal cells is not only saturable but also a slow process as maximum plasma levels are reported after some delay (Harper *et al.,* 1988). In summarizing pharmacokinetic studies with L-carnitine, Goa and Brogden (1987) reported normal plasma concentrations ranging from 39 to 46 and 50 to 57 µmol/L in healthy non-obese and obese females and males, respectively. In comparison, peak plasma concentrations of 48.5 and 69 µmol L-carnitine/L have been reported at 5 and 3.5 hours following single oral doses of 500 and 2,000 mg L-carnitine, respectively (Goa and Brogden, 1987). Harper *et al.* (1988) reported peak plasma concentrations at 3 to 9 and 2.5 to 7 hours following oral doses of 2,000 and 6,000 mg of L-carnitine, respectively. In addition, peak plasma concentrations of L-carnitine were reported to be reached at approximately 3.4 hours in healthy volunteers ingesting a single oral dose of 2.0 g L-carnitine in solution (Cao *et al.,* 2009).

The turnover time of L-carnitine in the liver and kidneys is short (11.6 hours) compared with skeletal muscle and heart (8 days), where carnitine is stored but not synthesized (Tanphaichitr and Leelahagul, 1993; Ramsay, 1994). In comparison, the turnover time of L-carnitine is approximately 11.3 hours in extracellular fluid and 66 days in the whole body (Tanphaichitr and Leelahagul, 1993). In review of studies conducted with healthy volunteers and in patients with coronary heart disease, the estimated excretion half-life of L-carnitine ranges from 2 to 15 hours following single oral or intravenous doses of 500 to 2,000 mg L-carnitine (Goa and Brogden, 1987). In a more recent study, however, the biological half-life of L-carnitine was reported to be 60.3±14.9 hours following a single oral dose of 2.0 g L-carnitine in solution (Cao *et al.*, 2009).

In a study to evaluate changes in free- and acylcarnitine concentrations in plasma, whole blood, red blood cells, and urine, subjects were given a single oral dose of 500 or 2,500 mg of L-carnitine, or 10 daily doses of 2,500 mg/day (Baker *et al*., 1993). An increased concentration of free L-carnitine was observed in the urine following all dosage regimens. Single or consecutive daily doses of 2,500 mg of L-carnitine significantly increased free- and acylcarnitine in plasma, whole blood, and urine, compared to the 500 mg dose (Baker *et al*., 1993). However, L-carnitine

levels in red blood cells did not change significantly at either dosage level (Baker *et al.*, 1993). Kelly (1998) suggested this was indicative of slow repletion of tissue stores of L-carnitine following an oral dose, or a low capability to transport L-carnitine into tissues under normal conditions.

The available data on metabolic fate of L-carnitine L-tartrate are limited; however, it is expected that the pattern of absorption of L-carnitine L-tartrate is similar to that of L-carnitine base. This is further corroborated by the results of a study undertaken to investigate the bioavailability of various L-carnitine esters (acetyl-L-carnitine and lauroyl-L-carnitine) and salts (L-carnitine L-tartrate, L-carnitine fumarate, L-carnitine magnesium citrate) relative to base of free L-carnitine (Eder *et al.,* 2005). Six groups of 5 or 6 piglets each were administered orally a single dose of 40 mg L-carnitine equivalents/kg body weight of each of those L-carnitine compounds, while a 7^{th} group served as a control. Free and total plasma carnitine concentrations were measured 1, 2, 3.5, 7, 24, and 32 hours post administration. The bioavailability of the L-carnitine compounds was assessed based on the calculations of the area-under-the-curve (AUC) values. The AUC values, calculated for the time interval between 0 and 32 hours, for both free and total carnitine were similar for base of free L-carnitine and the 3 L-carnitine salts (L-carnitine L-tartrate, L-carnitine fumarate, and L-carnitine magnesium citrate), while those of the 2 esters (acetyl-L-carnitine, lauroyl-L-carnitine) were lower. Overall, the results suggested that L-carnitine salts have a similar bioavailability to that of free L-carnitine, while L-carnitine esters have been shown to have a lower bioavailability. The fact that the differing salts of L-carnitine are seen to have similar AUC values following oral administration suggests that they all have similar pharmacokinetic properties. Considering that the various salts of L-carnitine have similar pharmacokinetic properties permits the use of studies conducted on differing salts to support or bridge to the safety of L-carnitine in general.

C.1.3 Metabolism and Excretion

L-carnitine is eliminated *via* the kidney mostly as unchanged carnitine and acylcarnitine (Tanphaichitr and Leelahagul, 1993; Evans and Fornasini, 2003). The kidney is crucial in regulating the plasma and tissue levels of L-carnitine, adapting to higher L-carnitine intakes by reducing the efficiency of L-carnitine reabsorption (Rebouche and Seim, 1998). L-carnitine is readily filtered through the glomeruli; however, active transport of L-carnitine across the proximal renal tubule minimizes the loss of L-carnitine in the urine and greater than 90% of filtered L-carnitine is reabsorbed (Harper *et al.,* 1988; Rebouche and Seim, 1998). Stanley (1998) reported that 95 to 98% of the filtered load of free carnitine is reabsorbed under conditions of normal plasma carnitine concentration. The renal clearance of acylcarnitine is higher than that of free carnitine, and excess acylcarnitine is rapidly cleared from the serum (Coulter, 1991). Unabsorbed oral L-carnitine undergoes bacterial degradation in the gastrointestinal tract to form Trimethyl amine (TMA) and γ-butyrobetaine (GBB) (Evans and Fornasini, 2003). TMA is subsequently absorbed and metabolized to form trimethylamine-N-oxide (TMAO), which is primarily excreted in the urine, whereas GBB is mainly excreted in the faeces.

Following oral administration of 1 g L-carnitine, 3 times per day to athletes before exercise, Nüesch *et al*. (1999) reported the excretion of free and esterified carnitine to increase

approximately 15fold and fivefold, respectively. Stanley (1998) reported a renal threshold for free carnitine excretion in adult humans to be approximately 35 µmol/L (8 mg/kg); a value close to the normal plasma concentration, above which the rate of carnitine excretion soon parallels the increase of filtered load. This suggests that under normal conditions, the plasma carnitine concentration is regulated by the renal threshold, and as the filtered load of carnitine increases above normal, the efficiency of carnitine reabsorption decreases, and the rate of carnitine excretion increases rapidly (Broquist, 1994; Rebouche, 2004).

Parrák and Hromadová (1993) reported reference values for daily urinary excretion of total carnitine of 270 to 498 (43 to 80 mg) and 195 to 323 (31 to 52 mg) µmol/day, in adult males and females, respectively. The reported lower levels of free urinary carnitine in women compared to men are likely associated with the lower muscle mass in women (Mitchell, 1978). The portion of a dose of carnitine excreted in the urine within 24 hours varies depending on the route of administration; 80% of an intravenous dose of 40 or 60 mg/kg of DL-carnitine was recovered in urine within 24 hours of dosing in comparison to 7% of an oral dose of 2,000 mg L-carnitine (Goa and Brogden, 1987). Faecal elimination accounts for less than 2% of the elimination of L-carnitine (Goa and Brogden, 1987).

Rebouche and Chenard (1991) investigated the excretion of L-carnitine using a tracer dose of [methyl-^{3}H] L-carnitine taken with a meal in subjects who had been adapted to a low-carnitine- or a high-carnitine-based diet for 15 days. The low-carnitine diet provided a daily intake of 1.5 to 2.2 µmol/kg body weight/day (0.24 to 0.35 mg/kg body weight/day), while the high-carnitine diet provided 8.4 to 11.9 µmol/kg body weight/day (1.4 to 1.9 mg/kg body weight/day). Total L-carnitine intake and excretion for the 2 groups were reported to be significantly different. For example, individuals in the low-carnitine diet excreted more carnitine (mean 217%) than they ingested, and those on the high-carnitine diet excreted less carnitine (mean 69%) than they consumed. However, in both groups >99% of total carnitine excretion was measured in the urine. Rebouche and Chenard (1991) suggested the proportional difference in intake and excretion between the 2 groups was reflective of the rate of endogenous carnitine synthesis. The cumulative excretion of radiolabelled urinary and faecal carnitine metabolites were followed for 5 to 11 days following administration of the radiolabelled dose. Rebouche and Chenard (1991) reported that the single dose of [methyl-^{3}H] L-carnitine was partly degraded and excreted predominantly as [^{3}H] TMAO in the urine, and as [^{3}H] GBB in the faeces, in both groups. Other unidentified minor metabolites of lesser occurrence were also reported. The mean cumulative excretion of [^{3}H] TMAO in the urine ranged from 17 to 28% of the administered dose in both groups, and the mean cumulative excretion of [^{3}H] GBB in the faeces was approximately 2% of the administered dose in both groups. Rebouche and Chenard (1991) concluded that although dietary carnitine is highly absorbed, metabolic degradation by gut microflora may account for a measurable portion of excretion, dependent upon the level of dietary L-carnitine intake.

C.1.4 Summary

Humans can synthesize L-carnitine from the amino acids L-lysine and L-methionine in a multi-step process. While the bioavailability of L-carnitine from the diet is quite high, absorption from oral L-carnitine supplements is considerably lower. This is attributed to relatively high polarity of this compound, which impedes its free diffusion across lipid membranes, together with the limited capacity of intestinal transporters. Renal reabsorption of L-carnitine is normally very efficient; in fact, an estimated 95% is thought to be reabsorbed by the kidneys. However, when circulating L-carnitine levels increase, as in the case of oral L-carnitine supplementation, renal reabsorption of L-carnitine becomes saturated, resulting in increased urinary excretion of L-carnitine. Dietary or supplemental L-carnitine that is not absorbed by enterocytes is degraded by colonic bacteria to form 2 principal products, TMA and GBB. GBB is eliminated in the faeces, while TMA is readily absorbed and metabolized to TMAO, which is excreted in the urine. L-carnitine L-tartrate is expected to readily dissociate into L-carnitine and L-tartaric acid in the gastrointestinal tract, and the bioavailability of L-carnitine from L-carnitine-L-tartrate has been shown to be similar to L-carnitine given as the free base.

C.2 Association between Dietary L-carnitine and Intestinal Microbiota in Promotion of Cardiovascular Disease

A recent publication in Nature Medicine by Koeth et al. (2013) suggests that trimethylamine N oxide (TMAO) may play a role in the development of atherosclerosis. The authors identified that metabolism of dietary L-carnitine by gut microbes is a potential source of TMAO. The study further states that intestinal microbiota are the essential factor in TMAO production from carnitine.

A comprehensive report investigating this thesis is provided in Appendix J.
In summary, the weight of scientific evidence indicates that an association between L-carnitine consumption and cardiovascular disease (CVD) cannot be established. L-carnitine is considered to be neither the driver nor the initiator of arteriosclerosis for the following reasons:

- Several limitations are present in the mice study model including genetic variability and species differences that make this rodent model unsuitable for extrapolation to human health. Furthermore and most importantly, the doses of L-carnitine used in the animal model were about 470 times greater than heavy consumer (90^{h} percentile) all-user intake of L-carnitine by the total Canadian population from all proposed food-uses of Carnipure™ crystalline or Carnipure™ tartrate, which was estimated to be 484 mg/day. Therefore, since this dose levels bear no similarity to human exposure levels, it can be viewed as having little if any human relevance.
- Koeth *et al*. did not take into account the impact of other TMAO-generating substances such as choline and betaine that are readily present in both the omnivores and vegans/vegetarians diets.

- Seafood contains high concentrations of TMAO and consumption of seafood increases plasma levels of TMAO; however, regular consumption of seafood especially fish has been associated with a decrease rather than an increase in the risk of CVD.
- Based on the small sample size used in some of the experiments such as comparison of TMAO levels in vegans/vegetarians *versus* omnivores, no valid conclusions can be drawn, and after adjustment for some of the confounding factors the association between L-carnitine and major adverse cardiac event risk was completely abolished. This suggests factors other than meat consumption can be involved including genetic variations.
- A review of the histopathological data from several oral and dietary subchronic and chronic studies in rats failed to reveal any treatment-related inflammatory or degenerative changes of the heart, aorta, or arteries at doses up to 3,356 mg L-carnitine/kg body weight/day.
- A large number of published literature on L-carnitine suggests that the beneficial effects of carnitine on heart function outweigh any potential deleterious effects of its breakdown product TMAO.

C.3 Preclinical Toxicological Studies

In vitro assays conducted in bacterial and mammalian cells indicated no mutagenic or genotoxic activity. The animal toxicity studies conducted with L-carnitine have generally used L-carnitine chloride as the test substance. Bioequivalence of L-carnitine and L-carnitine L-tartrate to L-carnitine chloride can be supported by the complete dissociation of L-carnitine salts upon absorption, based on the results of a study which demonstrated that L-carnitine salts have a similar bioavailability to that of free L-carnitine (Eder *et al.,* 2005). Therefore, toxicity studies using L-carnitine chloride are considered appropriate to support or bridge to the safety of L-carnitine.

Overall, the results from animal studies provide evidence that L-carnitine has a low order of toxicity. The administration of L-carnitine L-tartrate or L-carnitine chloride *via* the diet or by gavage was not associated with toxicologically-significant adverse effects in rats, rabbits or dogs. Gastrointestinal effects observed at high doses were self-limiting, physiological responses, typically associated with large bolus doses, rather than evidence of systemic toxicity. The no-observed-adverse-effect levels (NOAELs) determined from the repeated-dose toxicity studies ranged from 601 to 3,162 mg L-carnitine/kg body weight/day, for a 1-year chronic oral toxicity study in rats to a 90-day subchronic dietary study in rats, respectively. The effects noted were generally functional and morphological manifestations of a physiological response to high doses, rather than evidence of systemic toxicity. Although a formal 2-year carcinogenicity study of L-carnitine in animals has not been conducted, there was no evidence of carcinogenicity from 1-year studies in rats and dogs in which L-carnitine chloride was administered at doses up to 2,000 and 1,600 mg/kg body weight/day, respectively (Kikumori *et al.,* 1988c; Kudow *et al.*, 1988b). L-carnitine also was not associated with any reproductive toxicity or teratogenic effects in studies conducted with rats or rabbits (Itabashi *et al.*, 1988a,b; Nakamura *et al.*, 1988; Toteno *et al.*, 1988; Brandsch and Eder, 2003; Ramanau *et al.*, 2005).

C.3.1 Acute Toxicity Studies

An acute oral toxicity study using Carnipure™ crystalline was conducted in rats (Harlan Laboratories, 2008a). This unpublished study was conducted in accordance with the Organization for Economic Co-operation and Development (OECD) Guidelines for the Testing of Chemicals Test No. 423 (OECD, 2001) and OECD Principles of Good Laboratory Practice (GLP) (OECD, 1998a). Six (6) female HanRcc:Wist (SPF) rats were orally administered a single dose of 2,000 mg/kg body weight of L-carnitine *via* gavage and were observed for a period of 14 days thereafter. Mortality, viability, and clinical signs were recorded following administration, and during the observation period. Body weights were measured prior to administration, and on Days 8 and 15 of the observation period. All animals were killed and examined for macroscopic and microscopic abnormalities. No deaths, clinical signs, changes in body weight, or macroscopic findings were reported during the study. Thus, the oral LD_{50} of Lonza's L-carnitine ingredient was determined to be >2,000 mg/kg body weight in female rats. The results of this unpublished study further corroborate the safety of L-carnitine following oral administration of L-carnitine at a single dose of up to 2,000 mg/kg body weight.

In addition, several studies investigating the acute oral toxicity of L-carnitine chloride were identified in the literature.

The reported oral LD_{50} values for Carnipure™ crystalline and L-carnitine chloride in mice, rats, rabbits and dogs are presented in Table C.2.1-1 (p.40).

Table C.2.1- 1 Reported Oral LD_{50} Values for Carnipure™ crystalline and L-carnitine chloride

Species (Strain, Sex)	LD_{50} (mg/kg body weight)	Reference
L-carnitine (Carnipure™ crystalline)		
Rat (HanRcc:Wist, F)	>2,000	Harlan Laboratories, 2008a
L-carnitine chloride		
Mouse (ddy, M)	8,200	Toshida and Wada, 1988
Mouse (ddy, F)	8,000	
Rat (Crj:CD, M)	6,900	Narita *et al.*, 1988
Rat (Crj:CD, M)	6,127	Kudow *et al.*, 1988a
Rat (Crj:CD, M)	4,374	
Rat (Crj:CD, F)	6,890	Narita *et al.*, 1988
Rat (Crj:CD, F)	6,299	Kudow *et al.*, 1988a
Rat (Crj:CD, F)	4,578	
Rabbit (Japanese white, M)	5,400	Toshida and Wada, 1988
Rabbit (Japanese white, F)	6,000	
Dog (beagle, M)	>1,600	Kikumori *et al.*, 1988a
Dog (beagle, F)	>1,600	

F = female; M = male

C.3.2 Short-Term Toxicity Studies

Several short-term toxicity studies on L-carnitine, L-carnitine L-tartrate, and L-carnitine chloride have been conducted in rats and dogs. The results of these studies are summarized below and in Table C.2.2-1 (p.45).

In 2 metabolic feeding studies, male Wistar rats were fed diets containing L-carnitine at levels providing 0 (control) or 14 mg L-carnitine/kg body weight/day for 10 and 30 days (Clouet *et al*., 1996). No mortality was reported in either study, and oral administration of L-carnitine was reported to have no significant effects on total body weights, or absolute and relative liver and skeletal muscle weights. A significant decrease in absolute and relative weights of periepididymal fat tissue without histological findings was reported in L-carnitine groups compared to the control. In addition, no adverse effects on lipid metabolism, including no evidence of increased ketone body formation (ketosis) in rats fed L-carnitine for up to 30 days were noted.

A subchronic (13-week) toxicity study of L-carnitine L-tartrate (manufactured by Lonza) was undertaken in male and female Crl:CD® rats (LPT Laboratory of Pharmacology and Toxicology, 2003). This study was conducted in accordance with the OECD Guidelines for the Testing of Chemicals No. 408 (OECD, 1998b) and in compliance with the U.S. Food and Drug Administration (U.S. FDA)'s GLP regulations (21 §58 – U.S. FDA, 2011a). Groups of 10 male and female Crl:CD® rats were fed diets containing 0 (control), 2,500 (low-dose), 12,500 (mid-dose), or 50,000 (high-dose) ppm of L-carnitine L-tartrate for a period of 90 days. A 4-week recovery period was included wherein 5 rats/sex selected from the control and high-dose groups were provided the control diet. The mean intakes of L-carnitine L-tartrate for the low-, mid-, and high-dose groups during the study were 196, 1,018, and 4,365 mg/kg body weight/day for male rats (equivalent to 133, 692, and 2,968 mg/kg body weight/day of L-carnitine), and 218, 1,118, and 4,935 mg/kg body weight/day for female rats, respectively (equivalent to 148, 760, and 3,356 mg/kg body weight/day of L-carnitine). Parameters evaluated included mortality, clinical signs, body weight, food and water intake, haematology, clinical biochemistry, urinalysis, ophthalmology, gross pathology, organ weights, and histopathology.

No compound-related deaths or ophthalmological abnormalities were reported among groups. In addition, there were no significant differences in body weight, and haematology and clinical biochemistry parameters among groups. Soft faeces were observed in all high-dose animals from Day 6 onwards, but were not observed at the end of the recovery period. Food consumption and water intake were reported to be significantly increased in high-dose males and females compared to their respective controls. Although the increased water consumption disappeared at the end of the recovery period, food consumption in high-dose males was reported to be significantly increased during the recovery period compared to controls. Compared to the control group, high-dose males and females were reported to exhibit significantly decreased urine pH values, and significantly increased urine specific gravity; however, these effects were not observed at the end of the recovery period. Absolute and relative seminal vesicle weights were reported to be significantly decreased in high-dose males compared to their respective controls; however, this effect was transient, and disappeared at the end of the recovery period. In addition, there were no macroscopic findings observed in any of

the test groups, and no compound-related morphological lesions were observed in the high-dose group upon histopathological examination. Based on these findings, and in contrast to the NOAELs reported in the study report, the NOAEL for L-carnitine L-tartrate was determined to be 50,000 ppm in the diet, equivalent to 4,365 and 4,935 mg/kg body weight/day for males and females, respectively (approximately 2,968 and 3,356 mg L-carnitine/kg body weight/day for males and females, respectively), the highest dose tested.

In a 91-day subchronic toxicity study, Crj:CD rats (15 to 25 rats/sex/group) were administered doses of 0 (control), 100, 450, 1,500, and 5,000 mg/kg body weight of L-carnitine chloride per day (equivalent to 0, 82, 367, 1,223, or 4,077 mg L-carnitine/kg body weight/day) *via* gastric intubation (Yamate *et al*., 1988). All females and 17 out of 18 males in the highest dose group (*i.e.,* 5,000 mg/kg body weight/day) died. Congestion of the liver, kidneys, and lungs was reported in almost all male and female rats that died and the authors noted that these deaths was due to administration errors. Several histopathological changes in the dead animals were reported, including marked dilatation of the cecum with fluid-like contents, dilatations of splenic sinuses and medullary sinuses of the mesenteric lymph node, epithelial cell degeneration in the choroid plexus, cornea, prostate gland, and epididymis, degeneration of pancreatic acinar cells, hyperplasia of transitional epithelial cells in the urinary bladder, and haemorrhage in the adrenal cortex. The authors suggested that the dilatations of splenic sinuses and medullary sinuses of the mesenteric lymph node, and the haemorrhage in the adrenal cortex may be associated with the hemodynamic actions of L-carnitine. Adverse effects, including diarrhoea, increased water intake, aciduria, distension of the cecum, and increased absolute and relative liver weights were reported in rats administered doses of 1,500 mg/kg body weight/day and greater; however, no compound-related histopathological changes were reported in this dose group. Significant increases in absolute and relative caecal weights were reported in male rats in the 450 mg/kg body weight/day dose group, as well as both sexes in the 1,500 mg/kg body weight/day dose group compared to the control group; however, the authors noted that the increases in caecal weight were small in magnitude and thus, did not consider this effect to be of toxicological significance. Based on the results of this study, a NOAEL of 450 mg/kg body weight/day for L-carnitine chloride (equivalent to 367 mg L-carnitine/kg body weight/day) in rats was established.

The toxicity of L-carnitine chloride capsules was further evaluated in male and female beagle dogs orally administered capsules providing doses of 0 (control), 50 (low-dose), 200 (mid-dose), and 800 (high-dose) mg L-carnitine chloride/kg body weight/day (equivalent to 0, 41, 163, or 652 mg L-carnitine/kg body weight/day) for a period of 13 weeks (Kikumori *et al.*, 1988b). Two animals in the mid- and high-dose groups were used in a 5-week recovery portion of the study. Vomiting and diarrhoea were reported in the high-dose group; however, these symptoms were not observed during the recovery period. No other clinical signs were seen in the experimental groups or the control groups. No compound-related effects on organ weights, clinical chemistry, and histopathology were reported, and no mortalities were reported in any test group. The study authors set the NOAEL upon the vomiting and diarrhoea effects. However, there were no systemic effects noted, and the vomiting and diarrhoea are considered physiological effects rather than toxicological responses. As such, the true NOAEL was revised to be the highest dose administered, and the systemic NOAEL for L-carnitine chloride in dogs was determined to

be 800 mg/kg body weight (equivalent to 652 mg L-carnitine/kg body weight/day) (Kikumori *et al.,* 1988b).

The effect of chronic oral administration of L-carnitine chloride capsules was studied in beagle dogs (20/sex/group) at doses of 0 (control), 50, 200, 800, or 1,600 mg/kg body weight L-carnitine chloride (equivalent to 0, 41, 163, 652, or 1,305 mg L-carnitine/kg body weight) daily for 53 weeks (Kikumori *et al*., 1988c). No mortality occurred in any dose group. Diarrhoea was reported in 4 males and 4 females of the 1,600 mg/kg body weight group, and in 3 males and 4 females, of the 800 mg/kg body weight group. The occurrence of vomiting and diarrhoea noted in animals of the 200 mg/kg body weight and 50 mg/kg body weight groups was not significantly different compared to the control group. No other signs of toxicity were noted in the experimental and control groups. Electrocardiography, ophthalmology, otology, urinalysis, haematology, biochemistry, and organ weight analyses did not reveal any dose-dependent changes. Necropsy and histological results revealed slight oedema, local mucosal necrosis, and congestion in the cardiac region and fundus ventriculi in the 800 and 1,600 mg/kg body weight dose groups; however, these results were considered to be physiologic and species-related, and as a result are not toxicologically significant. Once more, the authors based the NOAEL upon effects which were considered physiological in nature and not related to systemic toxicity. As a result, the NOAEL was re-adjusted to the top dose tested. The NOAEL for L-carnitine chloride in this study was therefore, determined to be 1,600 mg/kg body weight, equivalent to 1,305 mg L-carnitine/kg body weight/day, the highest dose tested.

In a chronic study, the toxicity of L-carnitine chloride was investigated in Crj:CD rats (30/sex/group) administered daily doses of 0 (control), 100, 272, 737, or 2,000 mg L-carnitine chloride/kg body weight (equivalent to 0, 82, 222, 601, or 1,631 mg L-carnitine/kg body weight/ day) by gastric intubation for 12 months (Kudow *et al*., 1988b). Rats in the 100 and 272 mg/kg body weight/day groups did not demonstrate any compound-related abnormalities. Mortalities among males in the control group and the 100, 272, 737, and 2,000 mg/kg body weight/day groups were respectively, 3.6, 4.2, 0, 5.3, and 35.5%; mortalities among females were respectively 3.3, 0, 0, 0, and 28.6%. A significant increase in absolute and standardized cecum weight was noted in males in the 737 mg/kg body weight/day group; however, these effects are considered to be physiological rather than toxicological response.

Rats of both sexes in the 2,000 mg/kg body weight/day group showed abnormal breathing sounds, loose bowels, an increase in mortality and water intake, and a decrease in food consumption resulting in significant suppression of body-weight gain. A significant increase in urine volume and chloride, and a significant decrease in sodium in rats of both sexes were also reported in the 2,000 mg/kg body weight/day group. Significant decreases in urinary sodium and potassium were observed in females administered 2,000 mg/kg body weight/day. Diminished vigour was reported among male rats of the 2,000 mg/kg body weight/day group. Female rats in the 2,000 mg/kg body weight/day group demonstrated an increase in segmented neutrophils and a decrease in lymphocytes, and both sexes showed a decrease in triglyceride level. At necropsy, rats given 2,000 mg L-carnitine chloride/kg body weight/day demonstrated significant decreases in weight of the whole body, heart, and thymus, and an increase in weight of the cecum of both sexes. The liver, prostate gland, and epididymis of males significantly decreased

in weight. Histopathological examination was conducted on rats of all dose groups. There was no observed trend in the induction of neoplasms in L-carnitine-treated animals. The NOAEL for this study was determined to be 737 mg/kg L-carnitine chloride body weight/day, corresponding to 601 mg L-carnitine/kg body weight/day.

Based upon a close examination of the data from repeated-dose studies on L-carnitine, a number of effects which were deemed adverse within the published manuscripts are considered physiological in nature and of no toxicological significance. The resulting change in the interpretation of the data led to a recalculation of the NOAEL for L-carnitine to be 601 mg/kg body weight/day, based upon the chronic study conducted in Crj:CD rats fed L-carnitine chloride. While the NOAEL (*i.e.,* 450 mg L-carnitine chloride/kg body weight/day, equivalent to 367 mg L-carnitine/kg body weight/day) from the 90-day subchronic dietary study in Crj:CD rats was lower than that from the chronic study, it was felt that this was a less accurate indication of the true NOAEL based upon the stepping dose range within this study (*i.e.*, 100, 450, 1,500, and 5,000 mg/kg/day in comparison to 100, 272, 737 and 2,000 mg/kg/day). In reality, the true NOAEL should fall somewhere between 737 and 1,500 mg/kg body weight/day for L-carnitine chloride.

Table C.3.2- 1 Summary of Short-Term Toxicity Studies on L-carnitine and Related Compounds						
Species (Strain), Sex, and Number of Animals	**Route of Administration and Study Duration**	**Chemical**	**Dose (mg/kg bw/d) (concentration)**	**Reported Effects** [a,b]		**Reference**
L-carnitine (n = 2)						
Rat (Wistar) 10 M/group	Oral (diet) 10 days	L-carnitine	0 or 14	General condition/ survival	• No mortality	Clouet *et al.*, 1996
				Food and water intake	• NE	
				Body weight	• NSD in total body weight	
				Organ and tissue effects	• NSD in absolute and relative liver and skeletal muscle weights • NSD in lipid contents of heart, muscle, and kidney • ↓ absolute and relative weights of periepididymal fat tissue	
				Haematology, clinical chemistry, and urinalysis	• NE	
				NOAEL	• Not identified	
Rat (Wistar) 10 M/group	Oral (diet) 30 days	L-carnitine	0 or 14	General condition/ survival	• No mortality	Clouet *et al.*, 1996
				Food and water intake	• NE	
				Body weight	• NSD in total body weight	
				Organ and tissue effects	• NSD in absolute and relative liver and skeletal muscle	

Table C.3.2- 1 Summary of Short-Term Toxicity Studies on L-carnitine and Related Compounds						
Species (Strain), Sex, and Number of Animals	**Route of Administration and Study Duration**	**Chemical**	**Dose (mg/kg bw/d) (concentration)**	**Reported Effects [a,b]**		**Reference**
					weights • NSD in lipid contents of heart, muscle, and kidney • ↓ absolute and relative weights of periepididymal fat tissue	
				Haematology, clinical chemistry, and urinalysis	• NE	
				NOAEL	• Not identified	
L-carnitine L-tartrate (n = 1)						
Rat (Crl:CD) 10/sex/group	Oral (diet) 90 days, and a 4-week recovery period	L-carnitine L-tartrate [Provided in diet at concentrations of 0 (control), 2,500, 12,500, and 50,000 ppm]	M: 0, 196, 1,018, or 4,365 F: 0, 218, 1,118, 4,935 Equivalent to 0, 133, 692, and 2,968 mg L-carnitine/kg bw/d for M, and 0, 148, 760, and 3,356 mg L-carnitine/kg bw/d for F, respectively	General condition/ survival	• No compound-related mortality • No clinical signs of systemic toxicity [M, ≤1,018; F, ≤1,118] • No compound-related ophthalmological abnormalities • Soft faeces from Day 6 onwards, but disappeared during recovery period [M, 4,368; F, 4,935]	LPT Laboratory of Pharmacology and Toxicology, 2003
				Food and water intake	• ↑ food intake on Weeks 7 and 13 [M, 4,368], and Weeks 6 and 8 [F, 4,935] • ↑ food intake during recovery period [M, 4,368] • ↑ water intake at Weeks 6 and 12, but disappeared during recovery period [M, 4,368; F, 4,935]	

Table C.3.2- 1 Summary of Short-Term Toxicity Studies on L-carnitine and Related Compounds						
Species (Strain), Sex, and Number of Animals	**Route of Administration and Study Duration**	**Chemical**	**Dose (mg/kg bw/d) (concentration)**	**Reported Effects** [a,b]		**Reference**
				Body weight	• NSD in body weight	
				Organ and tissue effects	• No compound-related macroscopic or microscopic findings at end of study period and recovery period • ↓ absolute and relative weight of seminal vesicles at end of study period, but not at end of recovery period [M, 4,368]	
				Haematology, clinical chemistry, and urinalysis	• No compound-related effects on haematology and clinical chemistry parameters • ↑ urine excretion (SS NR) [M, 4,368; F, 4,935] • ↓ urine pH at Week 13, but not at end of recovery period [M, 4,368; F, 4,935] • ↑ urine specific gravity at Week 13, but not at end of recovery period [F, 4,935]	
				NOAEL	• 50,000 ppm in the diet [4,365and 4,935 mg/kg bw/d for M and F, respectively] (equivalent to 2,968 and 3,356 mg L-carnitine/kg bw/d)	
L-carnitine Chloride (n = 4)						
Rat (Crj:CD) 15 to	Oral (gavage) 91 days	L-carnitine chloride	0, 100, 450, 1,500, and 5,000	General condition/ survival	• No compound-related mortalities [M, F, ≤1,500]	Yamate *et al.*, 1988

Table C.3.2- 1 Summary of Short-Term Toxicity Studies on L-carnitine and Related Compounds						
Species (Strain), Sex, and Number of Animals	**Route of Administration and Study Duration**	**Chemical**	**Dose (mg/kg bw/d) (concentration)**	**Reported Effects [a,b]**		**Reference**
25/sex/group			Equivalent to 0, 82, 367, 1,223, or 4,077 mg L-carnitine/kg bw/d		• 17/19 M and 18/18 F died during the study [5,000] • Loose faeces [M, F, ≥1,500]	
				Food and water intake	• ↑ water intake [M, F, 5,000]	
				Body weight	• ↓ body weight gain [M, F, 5,000]	
				Organ and tissue effects	• No compound-related histopathological abnormalities [M, F, ≤1,500] • ↑ absolute and relative liver weights [M, F, 1,500] • ↑ absolute and relative caecal weights [M, 450; M, F, 1,500] • Distension of cecum in 9/15 M and 7/8 F rats [1,500] • Dead animals exhibited: o Congestion of liver, kidneys, lungs, and adrenal glands o Distension of stomach and small intestine o Dilatation of cecum with fluid-like contents o Dilatation of splenic sinuses and medullary sinuses of mesenteric lymph nodes o Degeneration of epithelial cells of the choroid	

Table C.3.2- 1 Summary of Short-Term Toxicity Studies on L-carnitine and Related Compounds						
Species (Strain), Sex, and Number of Animals	**Route of Administration and Study Duration**	**Chemical**	**Dose (mg/kg bw/d) (concentration)**	**Reported Effects [a,b]**		**Reference**
					plexuses of the brain, corneal epithelial cells, epithelial cells of the prostate gland and epididymis, o Haemorrhaging of the adrenal cortex	
				Haematology, clinical chemistry, and urinalysis	• Aciduria [M, F, ≥1,500]	
				NOAEL	• 450 mg L-carnitine chloride/kg bw/d (equivalent to 367 mg L-carnitine/kg bw/d)	
Rat (Crj:CD) 30/sex/group	Oral (gavage) 12 months	L-carnitine chloride	0, 100, 272, 737, or 2,000 Equivalent to 82, 222, 601, or 1,631 mg L-carnitine/kg bw/d	General condition/ survival	• ↑ frequency of abnormal breathing sounds and loose bowels [M, F, 2,000] • ↑ percent mortality [M, F, 2,000]	Kudow *et al.*, 1988b
				Food and water intake	• ↑ water intake [M, F, 2,000] • ↓ food intake [M, F, 2,000]	
				Body weight	• ↓ body weight gain [M, F, 2,000]	
				Organ and tissue effects	• ↑ absolute and relative cecum weights [M, 737] • ↓ absolute heart and thymus weights [M, F, 2,000] • ↑ absolute cecum weight [M, F, 2000]	

Table C.3.2- 1 Summary of Short-Term Toxicity Studies on L-carnitine and Related Compounds						
Species (Strain), Sex, and Number of Animals	**Route of Administration and Study Duration**	**Chemical**	**Dose (mg/kg bw/d) (concentration)**	**Reported Effects [a,b]**		**Reference**
					• ↓ absolute liver, prostate gland, and epididymis weights [M, 2000] • ↑ relative kidney, heart, lungs, adrenal glands, brain, and cecum [M, F, 2000] • ↑ relative liver weight [F, 2000] • ↑ relative epididymis and testes weights [M, 2000] • No neoplastic lesions in any organs	
				Haematology, clinical chemistry, and urinalysis	• ↑ urine volume and chloride [M, F, 2,000] • ↓ urinary sodium [M, F, 2,000] • ↓ urinary sodium and potassium [F, 2,000] • ↑ segmented neutrophil count [F, 2,000] • ↓ lymphocyte count [F, 2,000] • ↓ serum triglycerides [M, F, 2,000] • ↑ BUN [M, 2,000] • ↓ serum creatinine [M, 2,000] • ↑ serum ALP [F, 2000] • ↓ blood glucose and FFA [F, 2000]	
				NOAEL	• 737 mg L-carnitine chloride/kg bw/d (equivalent to 601 mg L-carnitine/kg bw/d)	

Table C.3.2- 1 Summary of Short-Term Toxicity Studies on L-carnitine and Related Compounds						
Species (Strain), Sex, and Number of Animals	**Route of Administration and Study Duration**	**Chemical**	**Dose (mg/kg bw/d) (concentration)**	**Reported Effects [a,b]**		**Reference**
Dog (beagle) 4 to 6/sex/group	Oral (capsule) 13 weeks with 5-week recovery period	L-carnitine chloride	0, 50, 200, or 800 Equivalent to 0, 41, 163, or 652 mg L-carnitine/kg bw/day	General condition/ survival	• Vomiting and diarrhoea [800]; these symptoms were not observed during the recovery period	Kikumori *et al.*, 1988b
				Food and water intake	• No compound-related changes in food intake • Transient changes in water intake (data not shown); however, this effect was not dose-dependent	
				Body weight	• Slight ↓ in body weight gain [F, 800] (SSNR)	
				Organ and tissue effects	• No compound-related effects on organ weights and histopathology	
				Haematology, clinical chemistry, and urinalysis	• No compound-related effects on clinical chemistry	
				NOAEL	• 800 mg L-carnitine chloride/kg bw/d (equivalent to 652 mg L-carnitine/kg bw/d)	
Dog (beagle) 4/sex/group	Oral (capsule) 53 weeks	L-carnitine chloride	0, 50, 200, 800, or 1,600 Equivalent to 0, 41, 163, 652, or 1,304 mg L-carnitine/kg bw/day	General condition/ survival	• No compound-related deaths • Vomiting and diarrhoea [≥800]; however, the general condition of these animals was not compromised • No compound-related adverse effects on electrocardiography,	Kikumori *et al.,* 1988c

Table C.3.2- 1 Summary of Short-Term Toxicity Studies on L-carnitine and Related Compounds						
Species (Strain), Sex, and Number of Animals	**Route of Administration and Study Duration**	**Chemical**	**Dose (mg/kg bw/d) (concentration)**	**Reported Effects [a,b]**		**Reference**
					ophthalmology, otology	
				Food and water intake	• Slight ↓ in food intake [M, 1,600] (SSNR) • Slight ↑ in water intake [M, 1,600; F, ≥800] (SSNR)	
				Body weight	• Slightly ↓ body weight gains at day 16 [F, 200, 1,600] (SSNR)	
				Organ and tissue effects	• No compound-related adverse effects on organ weights • Slight oedema, congestion, and local mucosal necrosis with congestion in the cardiac region and fundus ventriculi [≥800]	
				Haematology, clinical chemistry, and urinalysis	• No compound-related adverse effects on haematology, clinical chemistry, and urinalysis	
				NOAEL	• 1,600 mg L-carnitine chloride/kg bw/d (equivalent to 1,304 mg L-carnitine/kg bw/d)	

F = female; M = male; NOAEL = no-observed-adverse-effect level; NE = not evaluated; NSD = no significant differences; SSNR = statistical significance not reported; n = number of studies

[a] unless stated otherwise, all reported effects are relative to control group(s)

[b] numbers in [ ] correspond to the dose(s) at which the reported effects were observed

C.3.3 Long-Term Toxicity Studies, Immunotoxicity Studies and Carcinogenicity Studies

No long-term toxicity, immunotoxicity or carcinogenicity studies on L-carnitine or L-carnitine L-tartrate were identified.

The results from animal studies provide evidence that L-carnitine is of low order of toxicity. The administration of L-carnitine and L-carnitine L-tartrate was not associated with toxicologically-significant adverse effects in rats, rabbits or dogs. Although a formal 2-year carcinogenicity study was not conducted with L-carnitine, some tumour data were available from 12-month chronic toxicity studies that indicate no carcinogenic potential. There was no evidence of carcinogenicity from 1-year studies in rats and dogs in which L-carnitine was administered at doses up to 2,000 and 1,600 mg/kg body weight/day, respectively. In addition, L-carnitine was not associated with any reproductive toxicity or teratogenic effects in studies conducted with rats or rabbits. No systemic adverse effects were observed were administered by oral routes in acute and chronic human clinical testing, which are well above the dietary intakes including all current and proposed food and beverage uses for L-carnitine and L-carnitine L-tartrate. *In vitro* assays conducted in bacterial and mammalian cells indicated no mutagenic or genotoxic activity.

The European Food Safety Authority conducted a safety evaluation (EFSA, 2003) and did not raise further requirements for any toxicity studies.

Given that L-carnitine is a body´s own substance, 100 to 300 mg are ingested by the daily diet (culture depending, countries with a high meat consumption ingest far more), L-carnitine has to be added to soy based infant formula to give a certain level of L-carnitine since 1991 (EWG, 1991), it can be anticipated that L-carnitine has neither cancerogenic nor immunotoxic potential due to its long history of safe use.

C.3.4 Reproductive and Developmental Toxicity Studies

A number of studies examining the potential reproductive and developmental toxicity of L-carnitine and L-carnitine chloride have been conducted in rats and rabbits (Itabashi *et al.*, 1988a,b; Nakamura *et al.*, 1988; Toteno *et al*., 1988; Brandsch and Eder, 2003; Ramanau *et al*., 2005). These studies are summarized below and in Table C.3.4-1 (p.57).

A reproductive study to evaluate the effects of L-carnitine on the dams, foetuses and newborns of Crj:CD rats was conducted by Itabashi *et al*. (1988a). L-carnitine chloride was administered by gastric intubation at doses of 0 (control) 100, 548, or 3,000 mg/kg body weight (equivalent to 0, 82, 447, or 2,447 mg L-carnitine/kg body weight) to 25 rats/group during the perinatal and lactating periods. Administration of L-carnitine chloride to the dams began on the 17^{h} day of gestation and continued until 21 days postpartum. Control rats received distilled water. The parent rats were necropsied 22 days after giving birth. Four days after birth, the F_1 rats were culled into litters of 8 rats/each. They were weaned at 22 days postpartum; however, they were not directly administered L-carnitine chloride. No abnormal symptoms or deaths were observed during the gestation period in either the control group or the treatment groups. L-carnitine chloride administration was well tolerated by the dams and did not result in any symptoms of

toxicity or mortality. No changes were noted in body weight and food consumption. Rats in all treatment groups showed increased water consumption after treatment; a significant increase in water consumption was noted in dams given 3,000 mg/kg body weight during the perinatal and lactating periods. Necropsy results revealed an increase in absolute and standardized liver weight in the 3,000 mg/kg body weight group. Atrophy and haemorrhaging of the thymus were reported in 3 dams in the 3,000 mg/kg body weight; however, 1 animal in the control group also exhibited haemorrhaging, as such these findings were likely not attributed to L-carnitine chloride treatment. There were no reports of L-carnitine-related changes in the number of live pups at birth, viability index at 4 days of age, lactation index at 21 days of age, and morphological and functional developments of F_1 rats in any of the treated groups. Body weight gain was significantly retarded in F_1 generation females of dams administered 548 and 3,000 mg/kg body weight L-carnitine. No adverse effects were reported in the reproductive function of the F_1 generation with respect to the mating index, fertility, ovulation, and implantation, growth and development of F_2 foetuses. The NOAEL for parental toxicity was 100 mg/kg body weight of L-carnitine chloride (equivalent to 82 mg L-carnitine/kg body weight/day), based on reduced body weight gain, and the NOAEL for the reproductive performance of parent rats was established to be 3,000 mg/kg body weight/day (equivalent to 2,447 mg L-carnitine/kg body weight/day, respectively).

In a reproductive toxicity study, the effect of oral administration of L-carnitine chloride on reproductive performance was investigated in rats prior to mating and in the early stage of gestation (Itabashi *et al*., 1988b). L-carnitine chloride was administered by gastric intubation at dosage levels of 0 (control), 100, 520, or 2,700 mg/kg body weight/day (equivalent to 0, 82, 424, 2,202 mg L-carnitine/kg body weight/day) to 25 male and 25 female Crj:CD rats/group. The male rats received L-carnitine chloride starting at 6 weeks of age, for a total of 9 weeks prior to mating. The female rats received L-carnitine chloride starting at 8 weeks of age, for a total of 2 weeks before mating. Both males and females continued to receive L-carnitine chloride during the pairing period, and after copulation was confirmed, the females continued receiving L-carnitine chloride up to the 7^{th} day of gestation. Rats of either sex in the 100 mg/kg body weight group did not demonstrate abnormal clinical signs. A transient decrease in food consumption was noted in the male and female rats in the 520 mg/kg body weight group, accompanied by a slight reduction of body-weight gain in males. Rats of both sexes in the 2,700 mg/kg body weight group exhibited diarrhoea during the administration period, a transient decrease in food consumption, and a significant increase in water intake. Body-weight gain was significantly suppressed in males of the 2,700 mg/kg body weight group; however, the body weight of female rats in the same dose group was not affected. Males in this group also exhibited a significant increase in testes:body weight ratio that was attributed to the reduction in body weight. There were no adverse effects on the oestrous cycle, mating, fertility, and foetal growth of rats at any of the doses used. Compound-related abnormalities were not reported after external, visceral, and skeletal examinations. The NOAEL for parental effects was estimated to be 520 mg/kg body weight of L-carnitine chloride and the NOAEL for the reproductive performance of parent rats and for the development of the foetuses were 2,700 mg/kg body weight/day, which are equivalent to 424 and 2,202 mg L-carnitine/kg body weight/day, respectively.

Brandsch and Eder (2003) examined the effect of L-carnitine on the reproductive performance of rats in a multigenerational study. In this study, 30 female rats of 4 weeks of age received diets containing 0 (control) or 1 g L-carnitine/kg for a period of 34 weeks. Consumption of the diets provided a daily L-carnitine dose of approximately 50 mg/kg body weight/day. After 8 weeks of feeding, the female rats were mated the first time and the pups were allowed to be delivered naturally. Two more reproductive cycles followed, with 3-week intervals in between and pups were delivered in each phase. No significant differences were observed between the body weight gain of the adult female rats in the L-carnitine and the control groups. Consumption of the diets supplemented with L-carnitine was not reported to affect the number of pregnancies, the number of total rat pups, the number of live births, or the number of stillborn pups. Dietary L-carnitine did not have any effects on the body weight of individual pups and the litter weights at birth. Weight development of litters differed between both groups on several days, but no uniform effect of L-carnitine was observed. Body weight development of weaned rats fed a commercial diet was different between both groups, but only in one reproduction cycle. Overall, the authors concluded that L-carnitine supplemented diets providing 50 mg L-carnitine/kg body weight/day did not affect the reproductive performance of rats.

A reproductive study examining the effect of L-carnitine-supplemented diets on the birth weight of piglets was identified, and although this was not a toxicity study *per se*, some endpoints related to the safety of L-carnitine were examined. In this study, sows were administered low-energy and low-protein diets providing 125 mg L-carnitine/day during pregnancy and 250 mg L-carnitine/day during lactation (Ramanau *et al.,* 2005). No adverse effects were noted to result from the consumption of the diets for either the sows or the piglets, and sows consuming the L-carnitine-supplemented diets produced more milk and their piglets displayed larger litter weight gains as compared to control animals.

The teratogenic effect of oral L-carnitine chloride was investigated in Crj:CD rats (21 to 24 dams/group) administered L-carnitine chloride at doses of 0, 100, 548, or 3,000 mg/kg body weight (equivalent to 0, 82, 447, or 2,447 mg L-carnitine/kg body weight) by gastric intubation during the foetal organ development period, from the 7^{h} day through the 17th day of gestation (Nakamura *et al*., 1988). No mortality was reported among the dams. Two cases of ptyalism (excess production of saliva), 5 cases of soft stools, and 1 case of soiling of the underbelly were observed in the 3,000 mg/kg body weight group. A significant suppression of body weight gain during the 7th through 17^{h} days of gestation, significant increase in water consumption, and a compound-related decrease in food consumption on the 8th day were noted in dams of the 3,000 mg/kg body weight group. No effect on the body weight was found in either the 548 or 100 mg/kg body weight groups. L-carnitine chloride did not cause foetal death or inhibit offspring growth and development. External and skeletal examinations revealed no significant difference in the rates of occurrence of abnormalities or variations in the offspring. No effects were noted in terms of the number of live births, birth index, external abnormality index, or body weight at birth. The offspring viability, weaning, and growth indexes were all normal. Skeletal examination at the time of weaning revealed no compound-related effects in the progress of ossification, or in the rates of occurrence of deformations or variations. The NOAELs for

maternal toxicity and teratogenicity were 548 and 3,000 mg L-carnitine chloride/kg body weight/day (corresponding to 447 and 2,447 mg L-carnitine/kg body weight/day, respectively).

The teratogenic effects of L-carnitine chloride were examined in pregnant female Japanese white rabbits orally administered L-carnitine chloride at dose levels of 0, 100, 316, or 1,000 mg/kg body weight (equivalent to 0, 82, 258, or 816 mg L-carnitine/kg body weight) from Day 6 to 18 of pregnancy (Toteno *et al*., 1988). Fourteen females were used in each dose group, except for the 100 mg L-carnitine chloride/kg body weight dose group in which 13 animals were included. Foetuses were examined on Day 29 of pregnancy for external, visceral, and skeletal abnormalities. No deaths occurred during the study. Diarrhoea occurred in 12 of 14 dams in the 1,000 mg/kg body weight dose group and decreased pinna temperature was noted in 2 of these affected dams. Dams receiving 1,000 mg/kg body weight were found to have significant decreases in food and water consumption, suppression of weight gain, and haemorrhaging and ulceration of the mucous membrane of the stomach and small intestine. The foetuses in the high-dose group did not demonstrate any compound-related external, visceral, or skeletal abnormalities. Administration of 100 or 316 mg L-carnitine chloride/kg body weight had no effect in dams, or on foetal growth and development. It was concluded by the authors that L-carnitine chloride does not cause adverse effects on foetal development at concentrations up to 1,000 mg/kg body weight, corresponding to 816 mg L-carnitine/kg body weight.

Table C.3.4- 1 Summary of Teratogenicity and Reproductive Toxicity Studies on L-carnitine and L-carnitine chloride						
Species (Strain) and Number of Animals	**Route of Administration and Exposure Period**	**Chemical**	**Dose (mg/kg bw/d) (concentration)**	**Reported Effects[a,b]**		**Reference**
L-carnitine (n = 1)						
Rat (Sprague-Dawley) 15 F/group	Oral (diet) 8 weeks prior to mating, during a 6-day mating period, and a 21-day lactation period Both groups underwent 3 reproductive cycles, each separated by a 3-week reproduction-free period	L-carnitine [Provided in the diet at concentrations of 0 or 0.1%]	0 or 257.2[c]	Maternal Effects	• No compound-related effects on number of pregnancies, maternal body weight, and total number of live pups	Brandsch and Eder, 2003
				Offspring Effects	• No compound-related effects on number of live pups, stillborn pups, pup body weight at birth, and litter weights at birth	
				NOAEL	• Not identified	
L-carnitine Chloride (n = 4)						
Rat (Crj:CD) 21 to 24/group	Oral (gavage) GD 7 to 17	L-carnitine chloride	0, 100, 548, or 3,000 Equivalent to 0, 82, 447, or 2,447 mg L-carnitine/kg body weight	Maternal Effects	• No compound-related mortality • ↓ body weight gain [3,000] • ↑ water intake on GD 8 [3,000] • ↓ food intake on GD 8 [3,000] • ↑ incidence of soft stools [3,000]	Nakamura *et al.*, 1988
				Offspring Effects	• No compound-related effects on number of live births, birth index, external abnormality index, or body weights at birth and lactation • No compound-related effects on the progress of ossification or occurrence of skeletal deformities • No compound-related effects on reproductive ability, or on the viability, development, and growth of F2 offspring	

Table C.3.4- 1 Summary of Teratogenicity and Reproductive Toxicity Studies on L-carnitine and L-carnitine chloride						
Species (Strain) and Number of Animals	**Route of Administration and Exposure Period**	**Chemical**	**Dose (mg/kg bw/d) (concentration)**	**Reported Effects[a,b]**		**Reference**
				NOAEL	• 548 mg/kg bw/d for maternal toxicity • 3,000 mg/kg bw/d for teratogenicity and reproductive toxicity	
Rat (Crj:CD) 25 F/group	Oral (gavage) GD 17 to 21 days post-partum	L-carnitine chloride	0, 100, 548, or 3,000 Equivalent to 0, 82, 447, or 2,447 mg L-carnitine/kg body weight	Maternal Effects	• No adverse clinical signs or mortality during gestation or lactation periods • NSE on body weight or food intake • ↑ water intake after administration of test article [≥100] • ↑ water intake during perinatal and lactating periods [3,000] • Atrophy or haemorrhaging of thymus in 3 dams [3,000]; however, haemorrhaging also was observed in 1 control dam • ↑ absolute and relative liver weights [3,000] • ↓ relative right ovary weight [3,000] • NSD in total relative ovary weight	Itabashi *et al.*, 1988a
				Offspring Effects	• No test article-related effects on number of live pups at birth, viability index at 4 days of age, lactation index at 21 days of age, or morphological and functional development • ↓ body weight gain in F offspring	

Table C.3.4- 1 Summary of Teratogenicity and Reproductive Toxicity Studies on L-carnitine and L-carnitine chloride						
Species (Strain) and Number of Animals	**Route of Administration and Exposure Period**	**Chemical**	**Dose (mg/kg bw/d) (concentration)**	**Reported Effects[a,b]**		**Reference**
					[≥548] • ↓ absolute and relative liver and brain weights in 10-week-old F offspring [548] • NSD in body weight of M offspring • ↓ absolute and relative testes weights in 10-week-old M offspring [3,000] • NSE on physical or behavioural development, reproductive function (mating index, fertility, ovulation, implantation, and growth and development of F_2 foetuses)	
				NOAEL	• 548 mg L-carnitine chloride/kg bw/d for maternal toxicity (equivalent to 447 mg L-carnitine/kg bw/d) • 3,000 mg L-carnitine chloride/kg bw/d for teratogenicity (equivalent to 2,447 mg L-carnitine/kg bw/d)	
Rat (Crj:CD) 25/sex/group	Oral (gavage) M: 9 weeks prior to mating and during mating F: 2 weeks prior to mating, during gestation, and up to GD 7	L-carnitine chloride	0, 100, 520, or 2,700 Equivalent to 0, 82, 424, 2,202 mg L-carnitine/kg body weight/day	Parental Effects	• Transient ↓ in food intake [M, F, 520; M, 2,700] • ↓ body weight gain [M, 2,700] • Diarrhoea [M, 2,700] • ↑ relative testes weight [M, 2700]; however, this was attributed to the reduction in body weight • NSE on reproductive ability,	Itabashi *et al.*, 1988b

Table C.3.4- 1 Summary of Teratogenicity and Reproductive Toxicity Studies on L-carnitine and L-carnitine chloride						
Species (Strain) and Number of Animals	**Route of Administration and Exposure Period**	**Chemical**	**Dose (mg/kg bw/d) (concentration)**	**Reported Effects[a,b]**		**Reference**
					oestrous cycle, mating performance, or fertility	
				Foetal Effects	• No compound-related effects on growth or external, visceral, and skeletal abnormalities	
				NOAEL	• 520 mg L-carnitine chloride/kg bw/d for parental toxicity (equivalent to 424 mg L-carnitine/kg bw/d) • 2,700 mg L-carnitine chloride/kg bw/d for reproductive and developmental effects (equivalent to 2,202 mg L-carnitine/kg bw/d)	
Rabbit (Japanese white) 13 to 14 F/group	Oral (gavage) GD 6 to 18 Foetuses examined on GD 29	L-carnitine chloride	0, 100, 316, or 1,000 Equivalent to 0, 82, 258, or 816 mg L-carnitine/kg body weight	Parental Effects	• No mortality • Diarrhoea in 12/14 dams [1,000] • ↓ food and water intake [1,000] • Slight suppression in body weight gain [1,000] • Haemorrhaging and ulceration of mucous membranes of stomach and small intestine [1,000]	Toteno *et al.*, 1988
				Foetal Effects	• No compound-related external, visceral, and skeletal abnormalities	

Table C.3.4- 1 Summary of Teratogenicity and Reproductive Toxicity Studies on L-carnitine and L-carnitine chloride						
Species (Strain) and Number of Animals	**Route of Administration and Exposure Period**	**Chemical**	**Dose (mg/kg bw/d) (concentration)**	**Reported Effects[a,b]**		**Reference**
				NOAEL	• 316 mg L-carnitine chloride/kg bw/d for maternal toxicity (equivalent to 258 mg L-carnitine/kg bw/d) • 1,000 mg L-carnitine chloride/kg bw/d for foetal development (equivalent to 816 mg L-carnitine/kg bw/d)	

bw = body weight; F =female; GD = gestation day; M = male; NOAEL = no-observed-adverse-effect level; NSE = no significant effects ; n = number of studies

[a] unless stated otherwise, all reported effects are relative to control group(s)

[b] numbers in [ ] correspond to the dose(s) at which the reported effects were observed

[c] Mean L-carnitine intake prior to mating, and during the pregnancy and lactation periods. The average L-carnitine intake was reported to be 138, 180, and 453.5 mg/kg bw/d prior to mating, and during pregnancy and lactation periods.

C.3.5 Genotoxicity Studies

Several *in vitro* mutagenicity and genotoxicity studies on L-carnitine chloride have been conducted in prokaryotic and eukaryotic test systems. In addition, the potential mutagenicity and genotoxicity of Carnipure™ crystalline has been evaluated in *in vitro* bacterial and mammalian assays. The results of these studies are summarized below and in Table C.3.5-1 (p.63).

The potential mutagenicity of Carnipure™ crystalline was evaluated in an *in vitro* bacterial reverse mutation assay (Ames test) using *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, and *Escherichia coli* strain WP2uvrA-pKM101 (SafePharm Laboratories, 2008). This unpublished study was conducted in compliance with GLP and the OECD Guidelines for the Testing of Chemicals No. 471 (OECD, 1997a). The bacterial strains were tested with Carnipure™ crystalline at concentrations of 50, 150, 500, 1,500, and 5,000 µg/plate, in the presence and absence of a rat liver homogenate metabolizing system (S9). Sterile distilled water was used as the negative control, while *N*-ethyl-*N*'-nitro-*N*-nitrosoguanidine, 9-aminoacridine, and 4-nitroquinoline-1-oxide were used as positive controls in the absence of S9, and 2-aminoanthracene, and benzo(a)pyrene were used as positive controls in the presence of S9. No significant increases in the frequency of revertant colonies were reported in any of the bacterial strains tested with up to 5,000 µg/plate of Carnipure™ crystalline, with and without S9. As expected, treatment with the negative control did not increase the number of revertant colonies, while all of the positive controls induced significant increases in the frequency of revertant colonies in the presence or absence of metabolic activation. Carnipure™ crystalline was therefore considered to be non-mutagenic under the conditions of the Ames test.

The potential genotoxicity of Carnipure™ crystalline was further investigated in a chromosomal aberration assay (consisting of 2 independent experiments) performed in human lymphocytes (Harlan Laboratories, 2008b). This unpublished study was conducted in accordance with the OECD Guidelines for Testing of Chemicals No. 473 (OECD, 1997b), as well as the OECD Principles of GLP (OECD, 1998a). The positive controls used were ethylmethane sulfonate and cyclophosphamide in the absence and presence of S9, respectively, and a vehicle control (deionised water) also was included in both experiments as a negative control. The cells were incubated with Carnipure™ crystalline at concentrations of 529, 925.7, and 1,620 µg/mL, in both experiments. The exposure period was 4 hours with and without S9 in the first experiment, while the exposure period was 4 hours with S9 and 22 hours without S9 in the second experiment. Treatment with Carnipure™ crystalline did not significantly increase the number of cells with structural chromosomal aberrations in either experiment, with or without metabolic activation. A dose-dependent increase in chromosomal aberrations was observed in the absence of metabolic activation in both experiments; however, these values were within historical control data and were considered by the investigators to be biologically irrelevant. As expected, the positive controls resulted in significant increase in cells with structural chromosomal aberrations, while no increase in chromosomal aberrations was observed with the vehicle control. Based on the results of the experiment, Carnipure™ crystalline was considered

to have no clastogenic activity in human lymphocytes when tested at concentrations up to 1,620 µg/mL.

Negative results were reported for L-carnitine chloride in the Ames test using various *Salmonella typhimurium* strains, including TA98, TA100, TA1535, TA1537, and TA1538, and *Escherichia coli* strain *WP2 uvrA*, with and without metabolic activation when tested at concentrations up to 10,000 µg/plate (Hamai *et al.*, 1988). L-carnitine chloride (at concentrations of up to 5,000 µg/disk) was reported to test negative in the Rec assay using *Bacillus subtilis* in the presence and absence of metabolic activation (Hamai *et al.*, 1988). In addition, L-carnitine chloride was reported to be non-genotoxic in the chromosomal aberration assay conducted in Chinese hamster V79 cells, in the presence and absence of metabolic activation, at concentrations up to 10.0 mg/mL.

Table C.3.5- 1 Summary of *In Vitro* Mutagenicity Studies on L-carnitine and L-carnitine chloride in Prokaryotic and Eukaryotic Test Systems

Strain	Test	Concentration	Results	Reference
Prokaryotic Test Systems				
L-carnitine (Carnipure™ crystalline)				
Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537	Mut (+/-S9)	Up to 5,000 µg/plate	Negative	SafePharm Laboratories, 2008
Escherichia coli strain *WP2 uvrA*	Mut (+/-S9)	Up to 5,000 µg/plate	Negative	
L-carnitine chloride				
S. typhimurium strains T98, TA100, TA1535, TA1537, and TA1538	Mut (+/-S9)	Up to 10,000 µg/plate	Negative	Hamai *et al.*, 1988
E. coli strain *WP2 uvrA*	Mut (+/-S9)	Up to 10,000 µg/plate	Negative	
Bacillus subtilis H17 and M45	Mut (+/-S9)	Up to 5,000 µg/disk	Negative	
Eukaryotic Test Systems				
L-carnitine (Carnipure™ crystalline)				
Human lymphocytes	CA (+/-S9)	Up to 1,620 µg/mL	Negative	Harlan Laboratories, 2008b
L-carnitine chloride				
Chinese hamster V79 cells	CA (+/-S9)	Up to 33.3 mg/mL (+S9) Up to 10.0 mg/mL (-S9)	Negative	Hamai *et al.*, 1988

CA = chromosomal aberration; Mut = mutation; S9 = metabolic activation

C.4 Clinical Studies

Numerous human studies have been conducted to examine the effects of L-carnitine in HEALTHY INDIVIDUALS (Uematsu *et al*., 1988; Drăgan *et al*., 1989; Rebouche *et al.*, 1993; Vukovich *et al*., 1994; Colombani *et al*., 1996; Nüesch *et al*., 1999; Maggini *et al.*, 2000; Villani *et al*., 2000; Rubin *et al.,* 2001; Müller *et al.,* 2002; Volek *et al.*, 2002, 2008; Wächter *et al.*, 2002; Pistone *et al.*, 2003; Wutzke and Lorenz, 2004; Abramowicz and Galloway, 2005; Broad *et al.*, 2005, 2008; Sugino *et al.,* 2007; Spiering *et al.,* 2007, 2008; de Faria Coelho *et al.,* 2010; Ho *et al.*, 2010), INDIVIDUALS WITH ATTENTION-DEFICIT HYPERACTIVITY DISORDER (ADHD) (Van Oudheusden and Scholte, 2002), HEART CONDITIONS (Cacciatore *et al.,* 1991; Davini *et al.*, 1992; Iyer *et al.,* 1999; Singh *et al.*, 1996), HYPERLIPOPROTEINAEMIA (Stefanutti *et al.*, 1998), ESSENTIAL HYPERTENSION (Digiesi *et al.*, 1994), OLIGOASTHENOTERATOZOOSPERMIA OR ASTHENOZOOPSERMIA (Vitali *et al*., 1995; Lenzi *et al.*, 2004; Balercia *et al.*, 2005; Sigman *et al.*, 2006; Moradi *et al.*, 2010; Wang *et al.*, 2010a,b), HEPATIC ENCEPHALOPATHY (Malaguarnera *et al.*, 2003, 2006), NON-ALCOHOLIC STEATOHEPATITIS (Malaguarnera *et al.*, 2010), DIABETES (Derosa *et al.*, 2003, 2010; Molfino *et al.*, 2010), HEPATITIS (Neri *et al.*, 2003), ADVANCED CANCER AND/OR CANCER-RELATED ANOREXIA/CACHEXIA SYNDROME (Cruciani *et al.*, 2006; Mantovani *et al.*, 2008), PREGNANT WOMEN (Lohninger *et al.*, 2005; Keller *et al.*, 2009), AND HAEMODIALYSIS PATIENTS (Hakeshzadeh *et al.*, 2010; Sabry *et al.*, 2010; Shakeri *et al.*, 2010). Although the majority of studies were not conducted to specifically examine safety-related endpoints, the absence of reported adverse effects in these studies support the safety of the intended use of L-carnitine and L-carnitine L-tartrate in foods. Specifically, supplementation with up to 6 g/day of L-carnitine (as L-carnitine or L-carnitine L-tartrate) was reported to be well-tolerated in all identified studies and any reported adverse effects were either mild or were not related to L-carnitine consumption. The results of these studies are summarized in Table C.4-1 (p.66).

In addition, Hathcock and Shao (2006) conducted a review of numerous clinical trials to evaluate the safety of L-carnitine supplementation. No adverse effects attributed to L-carnitine consumption at doses up to 6,000 mg/day were identified in their review, with the exception of unpleasant body or urine odour reported by some subjects and thus, the authors were unable to establish a NOAEL for L-carnitine. Based on the absence of a NOAEL, the authors used the observed safe level (OSL) approach established by the Food and Agriculture Organization of the United Nations and the World Health Organisation (FAO/WHO). The authors concluded that the evidence from well-designed randomized clinical trials demonstrates that the upper safe level for L-carnitine supplements is 2,000 mg/day of L-carnitine equivalents. Hathcock and Shao (2006) also identified several clinical trials that used higher doses of L-carnitine (*i.e*., 4,000 to 6,000 mg/day) with no adverse effects reported; however, due to the limitations of these studies (*i.e.,* extreme diseased subject population, small sample size, modest duration, lack of sufficient detail on adverse effects), the authors stated that “the data for intakes above 2,000 mg/day are not sufficient for a confident conclusion of long-term safety”.

The safety of L-carnitine and L-carnitine L-tartrate is further corroborated by the current use of formulations of L-carnitine (Levocarnitine; Carnitor®) in the medical treatment of primary and secondary carnitine deficiencies. L-carnitine deficiency is defined as “a state of carnitine

concentration in the plasma or tissues that is below the requirement for the normal function of the organism" (Famularo *et al.,* 1997). Clinically, carnitine deficiency is characterized by low plasma concentrations of free L-carnitine, and low red blood cell and/or tissue levels. Primary carnitine deficiency results from a genetic deficiency of plasma membrane carnitine transporter activity (Flanagan *et al.*, 2010). Secondary carnitine deficiency is caused by the accumulation of organic acids, which leads to increased carnitine excretion in the urine; this condition is associated with a number of factors, including liver or kidney disorders, use of certain pharmacological agents, defects in fatty acid metabolism, and malabsorption of carnitine (Flanagan *et al.*, 2010). The recommended oral dose of L-carnitine for the treatment of L-carnitine deficiency is 1,980 to 2,970 mg/day in adults, provided in repeated doses of 990 mg, and 50 to 100 mg/kg body weight in infants and children, up to a total of 3,000 mg/day.

Moreover, in a comprehensive review of the pharmacological uses of L-carnitine, L-carnitine was reported to be well-tolerated at daily doses of up to 15 g/day, with no notable side effects other than infrequent diarrhoea, stomach aches, and nausea (Goa and Brogden, 1987). The occurrence of gastrointestinal effects at high doses is consistent with the observations at high doses in animal studies, and would be considered "self-limiting" for the consumption of higher amounts of L-carnitine. The estimated intakes of L-carnitine, for all consumer categories through the proposed uses of L-carnitine and L-carnitine L-tartrate in food, do not approach a level suggestive of a physiological self-limiting effect. Furthermore, the estimated intake of L-carnitine through all proposed food uses are lower than prescribed doses of L-carnitine under medical conditions of carnitine deficiency, and would not be consumed in a bolus amount.

Table C.4- 1 Summary of Human Studies on L-carnitine and L-carnitine L-tartrate							
Study Design	**Population (age, sex, health status)**	**Duration of Treatment**	**Test item**	**Daily Dose**	**Parameters Measured**	**Significant Results Related to Safety**	**Reference**
L-carnitine (n = 30)							
Randomized, double-blind, placebo-controlled, parallel-arm	18 subjects (age and sex NR; healthy athletes)	Single-dose	Vials	4 g of LC	Serum free fatty acids, triglycerides, cholesterol, creatinine Blood lactate	No adverse events attributed to LC consumption	Drăgan *et al.*, 1989, study 3
Randomized, double-blind, placebo-controlled, crossover	17 subjects (aged 16 to 24 years; F; healthy athletes)	Single-dose	Tablets	2 g of LC	Serum free fatty acids, triglycerides, cholesterol, creatinine Blood lactate	No adverse events attributed to LC consumption	Drăgan *et al.*, 1989, study 4
Double-blind, placebo-controlled, crossover	7 subjects (age NR; M; endurance athletes)	Single-dose	Tablets	2 g of LC	Carbohydrate and lipid metabolism Hormone levels Enzyme activity	No adverse events reported by authors	Colombani *et al.*, 1996
Randomized, double-blind, placebo-controlled, crossover	12 subjects (age range NR; mean age of 25.7±4 years; 2 F, 10 M; healthy)	5 days	NR	2 g/d of LC (as LCLT)	Mean power output during a period of 25 min. following strenuous exercise	No adverse events reported by authors	Maggini *et al.*, 2000
Uncontrolled, non-blinded	9 subjects (aged 21 to 23 years; sex NR; healthy professional athletes)	7 days	NR	3 g/d of LC	Plasma and urine concentrations of total, free and esterified carnitine before and after maximal exercise	No adverse events reported by authors	Nüesch *et al.*, 1999

Table C.4- 1 Summary of Human Studies on L-carnitine and L-carnitine L-tartrate							
Study Design	**Population (age, sex, health status)**	**Duration of Treatment**	**Test item**	**Daily Dose**	**Parameters Measured**	**Significant Results Related to Safety**	**Reference**
Uncontrolled, non-blinded	27 subjects (mean age of 59.7±14 years; 10 F, 17 M; advanced cancer, fatigue, carnitine deficiency)	7 days	NR	0.25, 0.75, 1.25, 1.75, 2.25, 2.75, or 3 g/d of LC	Side effects Plasma carnitine levels, complete blood count, electrolytes Severity of fatigue Depressed mood Quality of sleep	No patient reported significant side effects No signs of toxicity observed	Cruciani *et al.*, 2006
Randomized, controlled, parallel-arm Blinding NR	16 subjects (mean age of 66.7 years; 12 M, 4 F; impaired fasting glucose or T2DM)	10 days	Vials	2 g/d of LC, in combination with a hypocaloric diet	Body weight Adverse events OGTT HOMA-IR Plasma insulin Serum glucose	No adverse reactions reported in patients given LC.	Molfino *et al.,* 2010
Randomized, double-blind, placebo-controlled, parallel-arm	30 subjects (aged 17 to 26 years; F; healthy athletes)	20 days	Vials	3 g/d of LC	Serum free fatty acids, triglycerides, cholesterol, creatinine Blood lactate Urine protein	No adverse events attributed to LC consumption	Drăgan *et al.*, 1989, study 1
Randomized, double-blind, placebo-controlled, parallel-arm	20 subjects (aged 21 to 33 years; M; healthy athletes)	3 weeks	Vials	3 g/d of LC	Serum free fatty acids, triglycerides, cholesterol, creatinine Blood lactate Urine protein	No adverse events attributed to LC consumption	Drăgan *et al.*, 1989, study 2

Table C.4- 1 Summary of Human Studies on L-carnitine and L-carnitine L-tartrate							
Study Design	**Population (age, sex, health status)**	**Duration of Treatment**	**Test item**	**Daily Dose**	**Parameters Measured**	**Significant Results Related to Safety**	**Reference**
Randomized, double-blind, placebo-controlled, crossover	8 M subjects (age range NR; mean age of 22±3 years; healthy, weight-trained)	3 weeks with a 3-week washout period	Capsules	1 or 2 g/d of LC	Squat exercise test Perceived muscle soreness Post-exercise serum concentrations of hypoxanthine, xanthine oxidase, and myoglobin Handgrip test Serum carnitine concentration	No adverse events reported by authors.	Spiering *et al.*, 2007
Non-randomized, uncontrolled, open study	10 subjects (mean age of 36.4±12.8 years; aged 22 to 56 years; 5 M, 5 F; healthy)	10 days	NR	3 g/d of LC	Fatty acid metabolism	No adverse events reported by authors. NSD in serum glucose, HbA_{1c}, TG, TC, HDL-C, and LDL-C compared to baseline.	Müller *et al.*, 2002
Randomized, double-blind, placebo-controlled, parallel-arm	101 subjects (age range NR; mean age of 50 years; 91 M, 10 F; suspected acute myocardial infarction)	28 days	Capsules	1.98 g/d of LC	Myocardial infarct size Cardiac CK and CK-MB activities Cardiac enzymes and lipid peroxides Cardiac events and complications Adverse events	No subject withdrew from the study. There were no treatment-related adverse events, with the exception of loose stools in 3 subjects.	Singh *et al.*, 1996

Table C.4- 1 Summary of Human Studies on L-carnitine and L-carnitine L-tartrate							
Study Design	**Population (age, sex, health status)**	**Duration of Treatment**	**Test item**	**Daily Dose**	**Parameters Measured**	**Significant Results Related to Safety**	**Reference**
Randomized, double-blind, placebo-controlled, parallel-arm	84 subjects (aged 70 to 92 years; mean age of 81.5±6.7 years; 46 M, 38 F; with onset of fatigue followings light physical activity)	30 days	Vials	4 g/d of LC	Total fat and muscle mass BMI Serum lipids (TC, TG, HDL-C, LDL-C, apo A1, apo B) Physical and mental fatigue Hepatic and renal function (parameters NR)	No subject dropped out of the study. No adverse events of laboratory abnormalities were reported.	Pistone *et al.*, 2003
Randomized	21 subjects (aged 40 to 58 years; mean age of 46±5 years; 9 M, 12 F; overweight)	30 days	Capsules	1.8 g/d of LC	Caloric intake Anthropometry Resting metabolic rate Respiratory exchange ratio Plasma FFA VO_{2max}	No adverse effects reported by the authors.	de Faria Coelho *et al.*, 2010 [English abstract, article in Portuguese]
Randomized, double-blind, placebo-controlled, parallel-arm	36 subjects (mean age of 27.2±9.6 years; F; healthy)	8 weeks	Powder (mixed with a drink)	4 g/d of LC, with daily exercise	Anthropometric measures Adverse events	Half of the subjects in the LC group had self-reported symptoms of nausea and diarrhoea 4 subjects in LC group withdrew due to chronic diarrhoea 5 additional subjects receiving LC reported diarrhoea, which subsided during the study	Villani *et al*., 2000

Table C.4- 1 Summary of Human Studies on L-carnitine and L-carnitine L-tartrate							
Study Design	**Population (age, sex, health status)**	**Duration of Treatment**	**Test item**	**Daily Dose**	**Parameters Measured**	**Significant Results Related to Safety**	**Reference**
Uncontrolled	12 subjects (mean age of 27.5±6.4 years; 8 M, 4 F; healthy strict vegetarians)	60 days	Capsules	120 mg/d of LC	Plasma free and total carnitine concentrations	No adverse effects reported by the authors.	Rebouche *et al.*, 1993
Randomized, double-blind, placebo-controlled, parallel-arm	120 subjects (mean age of 52.1 years; 78 M, 42 F; hepatic encephalopathy)	60 days	NR	4 g/d of LC	NCT-A (measures cognitive and motor ability) Serum ammonia concentrations Haematology (Hb, Hct, WBC, thrombocyte count) Liver function tests (ALT, AST, GGT, serum bilirubin, PT, PTT)	Treatments reported to be well-tolerated by authors. 5 subjects in LC group complained of nausea (n=1), slight headache (n=2), and abdominal pain (n=2). 3 subjects in placebo group complained of diarrhoea (n=2) and moderate headache (n=1). No subjects withdrew from the study.	Malaguarnera *et al.*, 2003
Uncontrolled	24 subjects (14 M; 10 F; ages 39 to 64 years; mean age of 51.3±7.8 years; primary hyperlipoproteinaemia)	90 days	NR	1 g/d of LC	Plasma TG, TC, LDL-C, HDL-C, apo AI, and apo B Plasma free fatty acids P/S ratio	2 subjects dropped out of the study – one due to non-compliance, and one died due to reasons unrelated to study treatment. No adverse events were reported by the authors	Stefanutti *et al.*, 1998
Randomized, double-blind, placebo-controlled, parallel-arm	60 subjects (mean age 56±11 years; 34 M, 26 F; patients with acute anterior wall myocardial infarction)	3 months	Capsules	3 g/d of LC (following i.v. administration of 6 g/d of LC for 7 days)	Cardiac function Clinical condition	No adverse reactions to LC treatment in any subject	Iyer *et al.,* 1999

Table C.4- 1 Summary of Human Studies on L-carnitine and L-carnitine L-tartrate							
Study Design	**Population (age, sex, health status)**	**Duration of Treatment**	**Test item**	**Daily Dose**	**Parameters Measured**	**Significant Results Related to Safety**	**Reference**
Uncontrolled, non-blinded	47 subjects (age NR: M; idiopathic asthenozoospermia)	3 months	Oral solution	3 g/d of LC	Sperm motility Blood chemistry	Treatment was well-tolerated with no adverse effects reported NSE on blood chemistry and "other blood tests" (parameters NR)	Vitali *et al.*, 1995
Non-randomized, uncontrolled, open study	8 subjects (aged 23 to 25 years; M; healthy)	3 months	Tablets	4 g/d of LC	Muscle biopsy Exercise test Mitochondrial activity Skeletal muscle fibre composition	No adverse events reported by authors.	Wächter *et al.*, 2002
Randomized, controlled, parallel-arm Blinding NR	135 subjects (age NR; M; asthenozoospermia)	3 months	NR	2 g/d of LC plus 200 mg/d of vitamin E	Semen analyses Adverse effects Pregnancy rates	No adverse events observed during the study period.	Wang *et al.*, 2010a [English abstract, article in Chinese]
Randomized, controlled, parallel-arm Blinding NR	103 subjects (age NR; M; oligoasthenozoospermia)	3 months	NR	2 g/d of LC plus 20 mg/d of tamoxifen	Semen analyses Adverse effects Pregnancy rates	No side effects reported in any subject.	Wang *et al.*, 2010b [English abstract, article in Chinese]
Randomized, double-blind, active control, parallel-arm	52 M subjects (age range of 22 to 35 years; mean age of 28.46±2.67 years; idiopathic infertility)	3 months	Tablets	2 g/d of LC	Sperm count Semen volume Sperm motility and morphology	No adverse events reported by authors.	Moradi *et al.*, 2010

Table C.4- 1 Summary of Human Studies on L-carnitine and L-carnitine L-tartrate							
Study Design	**Population (age, sex, health status)**	**Duration of Treatment**	**Test item**	**Daily Dose**	**Parameters Measured**	**Significant Results Related to Safety**	**Reference**
Uncontrolled study	9 subjects (ages 49 to 68 years; mean age of 63 years; 7 F, 2 M; essential hypertension)	10 weeks	NR	2 g/d of LC	Blood cell count Serum TC, HDL-C, TG, Na, K, glucose, creatinine, apoA1, apoB Urinalysis Resting and dynamic ECG Blood pressure Asthenia Radionuclide angiocardiographic value	NSD in blood pressure. No adverse events reported by authors.	Digiesi *et al.*, 1994
Randomized, double-blind, placebo-controlled, parallel-arm	36 subjects (aged 20 to 74 years; mean age of 50 years; 15 M, 21 F; undergoing haemodialysis)	12 weeks	Vials	1 g/d of LC	Body weight Serum free carnitine, CRP, and PAI-1 to tpA ratio Plasma coagulation and anti-coagulation factors Dietary intakes	No adverse events reported by authors. NSD in plasma coagulation and anti-coagulation factors.	Hakeshzadeh *et al.*, 2010
Uncontrolled, non-blinded, randomized, parallel-arm	36 subjects (23 M, 13 F; aged 24 to 80 years; mean age of 54±13 years; undergoing haemodialysis)	12 weeks	NR	1 g/d of LC	Serum free carnitine, CRP, IL-1β, IL-6, TNF-α, Lp(a), and ox-LDL Body weight Dietary intakes	All subjects completed the study. No adverse events reported by authors.	Shakeri *et al.*, 2010

Table C.4- 1 Summary of Human Studies on L-carnitine and L-carnitine L-tartrate							
Study Design	**Population (age, sex, health status)**	**Duration of Treatment**	**Test item**	**Daily Dose**	**Parameters Measured**	**Significant Results Related to Safety**	**Reference**
Randomized, double-blind, placebo-controlled, parallel-arm	150 subjects (95 M, 55 F; mean age of 52.5 years; hepatic encephalopathy)	90 days	Vials	4 g/d of LC	Neurological function Liver function assessment (Child-Pugh score) Serum ammonia concentrations Haematology (Hb, Hct, WBC, thrombocyte count) Liver function tests (ALT, AST, GGT, serum bilirubin, PT, PTT)	No adverse events reported by authors.	Malaguarnera *et al.*, 2006
Two-centre, randomized, comparator trial	24 subjects (aged 18 to 80 years; sex NR; advanced cancer and cancer-related anorexia/cachexia syndrome)	4 months	Vials	4 g/d of LC	Lean body mass, resting energy expenditure, physical activity Serum IL-6 and TNF-α Clinical response Progression-free survival Appetite Grip strength Blood levels of ROS and GPx QOL Adverse events	No serious side effects attributed to treatment. No patients withdrawn from the study. No toxicity of any grade according to NCI common terminology criteria reported.	Mantovani *et al.*, 2008

Table C.4- 1 Summary of Human Studies on L-carnitine and L-carnitine L-tartrate							
Study Design	**Population (age, sex, health status)**	**Duration of Treatment**	**Test item**	**Daily Dose**	**Parameters Measured**	**Significant Results Related to Safety**	**Reference**
Controlled study Randomization and blinding NR	83 F subjects (age NR; healthy, pregnant)	~20 weeks (from 20th week of gestation until term)	NR	500 mg or 1 g/d of LC, or 1.45 g/d of LC (as LCLT)	Free fatty acids	No adverse events reported by authors.	Lohninger *et al.*, 2005
Randomized, controlled, non-blinded, parallel-arm	28 subjects (ages 31 to 68 years; mean age of 57 years; 21 F, 7 M; essential hypertension)	22 weeks	NR	2 g/d LC	Blood cell count Serum TC, HDL-C, TG, Na, K, glucose, and creatinine Urinalysis Resting and dynamic ECG Blood pressure Asthenia	No adverse events reported by authors.	Digiesi *et al.*, 1994
Randomized, non-blinded	200 subjects (aged 40 to 65 years; 125 M, 75 F; patients with exercise-induced stable angina)	6 months	Capsules	2 g LC/d, in addition to pre-existing pharmacological therapy	Cardiac function, blood glucose, triglyceride, total cholesterol, HDL	NSD in blood glucose or HDL concentrations between groups ↓ triglyceride and cholesterol compared to baseline in LC group. No adverse events reported by authors.	Cacciatore *et al.*, 1991
Randomized, double-blind, placebo-controlled, double-crossover	22 subjects (aged 6 to 13 years; M; ADHD)	6 months	Vials	100 mg/kg bw/d of LC (maximum of 4 g/d)	Child Behaviour Checklist score Conners teacher-rating score Haematology (Hb, Hct, RBC, WBC, platelet count) Clinical chemistry (urea, creatinine,	1 subject dropped out during first placebo period due to familial circumstances 1 subject dropped out during first carnitine period due to unpleasant body odour, a well-known side effect due to formation	Van Oudheusden and Scholte, 2002

Table C.4- 1 Summary of Human Studies on L-carnitine and L-carnitine L-tartrate							
Study Design	**Population (age, sex, health status)**	**Duration of Treatment**	**Test item**	**Daily Dose**	**Parameters Measured**	**Significant Results Related to Safety**	**Reference**
					sodium potassium, AST, ALT, GGT, ALP, free carnitine, acetyL-carnitine) Physical examination	of TMA 2 subjects broke their arm No abnormalities observed during physical examination ↑ plasma creatinine levels in one subject during last placebo period, but levels dropped to normal range 2 weeks after the trial 1 subject had unexplained period of fainting during last placebo period; no explanation	
Randomized, double-blind, placebo-controlled, parallel-arm	94 subjects (mean age of 51 years; 47 M, 47 F; recently diagnosed with type 2 diabetes mellitus)	6 months	Tablets	2 g/d of LC	Body weight Clinical chemistry [FPG, PPG, HbA_{1c}, FPI, TC, LDL-C, HDL-C, TG, apoA1, apoB, and Lp(a)]	NSD in body weight and clinical chemistry parameters. ↓ plasma Lp(a) at 6 months No clinically relevant adverse events – only 1 patient (2.2%) was reported to experience slight nausea, which resolved after 3 days of treatment	Derosa *et al.*, 2003
Randomized, uncontrolled, parallel-arm	50 subjects (mean age of 48.7 years; 30 M, 20 F; chronic hepatitis C)	6 months	NR	2 g/d of LC	Physical fatigue Mental fatigue	No adverse events reported by authors.	Neri *et al.*, 2003

Table C.4- 1 Summary of Human Studies on L-carnitine and L-carnitine L-tartrate							
Study Design	**Population (age, sex, health status)**	**Duration of Treatment**	**Test item**	**Daily Dose**	**Parameters Measured**	**Significant Results Related to Safety**	**Reference**
Randomized, double-blind, placebo-controlled, parallel-arm	60 M subjects (aged 20 to 40 years; infertile with oligoasthenotera-tozoospermia)	6 months	Vials and tablets	2 g/d of LC and 1 g/d of LAC	Microscopic semen analysis Seminal carnitine concentration Sperm lipid peroxidation potential Side effects Haematology (parameters NR)	4 subjects in placebo group dropped out of study (reasons NR). No adverse events reported by authors.	Lenzi *et al.*, 2004
Randomized, double-blind, placebo-controlled, parallel-arm	60 M subjects (ages 24 to 38 years; mean age of 30 years; idiopathic asthenozoospermia)	6 months with 3-month follow-up	Vials and/or tablets	3 g/d of LC (vials), 3 g/d of LAC (tablets), or 2 g/d of LC (vials) in combination with 1 g/d of LAC (tablet)	Sperm total motility Sperm forward motility Sperm concentration Atypical sperm cells Semen volume Curvilinear velocity Straight progressive velocity Total oxyradical scavenging capacity	One subject dropped out of the study (reason NR). No adverse events reported by authors.	Balercia *et al.*, 2005
Randomized, double-blind, placebo-controlled, parallel-arm	21 M subjects (ages 18 to 65 years; mean age of 36.2±1.7 years; idiopathic asthenospermia)	24 weeks	Capsules	2 g/d of LC and 1 g/d of LAC	Sperm motility Total motile sperm count Seminal plasma and sperm free, acetyl, and total LC concentrations Adverse events Liver function tests	5 subjects dropped out of the study; none dropped out because of adverse reactions. NSD in liver function tests, TC, serum creatinine, or BUN values compared to baseline values. There were no adverse events reported	Sigman *et al.*, 2006

Table C.4- 1 Summary of Human Studies on L-carnitine and L-carnitine L-tartrate							
Study Design	**Population (age, sex, health status)**	**Duration of Treatment**	**Test item**	**Daily Dose**	**Parameters Measured**	**Significant Results Related to Safety**	**Reference**
Randomized, controlled, parallel-arm Blinding NR	55 subjects (mean age of 42.8 years; 36 M, 19 F; chronic haemodialysis patients)	6 months	NR	1.5 g/d of LC	Adverse events Serum Hb and ferritin Transthoracic echocardiographs Erythropoietin dose and duration	No adverse events reported by authors.	Sabry *et al.*, 2010
Randomized, double-blind, placebo-controlled, parallel-arm	74 subjects (aged 28 to 60 years; mean age of 47.9 years; 40 M, 34 F; non-alcoholic steatohepatitis)	24 weeks	Vials	2 g/d of LC	Serum AST, ALT, GGT, albumin, TC, LDL-C, HDl-C, TG, insulin, C-peptide, CRP, TNF-α, ALP, and PT Liver biopsy HOMA-IR Hepatitis B serology Antibody to hepatitis C Hepatitis C RNA polymerase chain reaction Autoantibodies Serum iron, transferring saturation, and ferritin	LC reported to be well-tolerated in all subjects. Side effects reported in LC group included nausea (n=1), moderate headache (n=2), and abdominal pain (n=2) Side effects reported in placebo group included diarrhoea (n=2), moderate headache (n=1), and fatigue (n=2). ↓ serum AST, ALT, and GGT activities. ↓ serum TC and LDL-c levels. ↓ plasma glucose and HOMA-IR. ↓ serum CRP and TNF-α levels.	Malaguarnera *et al.*, 2010

Table C.4- 1 Summary of Human Studies on L-carnitine and L-carnitine L-tartrate							
Study Design	**Population (age, sex, health status)**	**Duration of Treatment**	**Test item**	**Daily Dose**	**Parameters Measured**	**Significant Results Related to Safety**	**Reference**
Randomized, single-blind, placebo-controlled, parallel-arm	26 F subjects (ages 22 to 40 years; mean age of 30.5 years; healthy and pregnant)	~27 weeks (from 13th week of gestation until term)	Tablets	500 mg/d of LC (as LCLT)	Plasma free, esterified, and total carnitine concentrations, plasma γ-butyobetaine and 6-*N*-TMA concentrations, and blood count at delivery	No treatment-related adverse effects were reported by the authors	Keller *et al.*, 2009
Randomized, controlled, non-blinded, parallel-arm	160 subjects (ages 39 to 86 years; mean age of 66 years; 124 M, 36 F; recent acute myocardial infarction)	12 months	NR	4 g/d of LC	Heart rate Systolic arterial pressure Diastolic arterial pressure Anginal attacks Rhythm disorders Clinical signs of impaired myocardial contractility Lipid Pattern Mortality	10 deaths in control group (2 due to extracardiac causes, and 8 due to cardiovascular causes). 1 death in treatment group due to thromboembolism. No other adverse events reported by authors.	Davini *et al.*, 1992

Table C.4- 1 Summary of Human Studies on L-carnitine and L-carnitine L-tartrate							
Study Design	**Population (age, sex, health status)**	**Duration of Treatment**	**Test item**	**Daily Dose**	**Parameters Measured**	**Significant Results Related to Safety**	**Reference**
Multi-centre, randomized, double-blind, placebo-controlled, parallel-arm	258 subjects (127 M, 131 F; aged ≥18 years; mean age of 52 years; obese with uncontrolled T2DM)	12 months	Capsules	2 g/d of LC plus 120 mg of orlistat	Body weight BMI HbA1c FPG and FPI Post-prandial plasma glucose HOMA-IR Serum TC, LDL-C, HDL-C, TG, RBP-4, Resistin, visfatin, Hs-CRP Adverse events	31 patients dropped out of the study due to side effects (n=29), loss to follow-up (n=1) , and withdrawal of informed consent (n=1) Side effects included flatulence, constipation, abdominal pain, fatty/oil evacuation, increased defecation, faecal urgency, and malaise NSD in frequency of adverse events between groups. LC plus orlistat reported to be generally well-tolerated and was not associated with any cardiovascular effects.	Derosa *et al.*, 2010
L-carnitine L-tartrate (n=11)							
Uncontrolled, non-blinded	8 subjects (26.8±2.3 years old; M; healthy)	7 to 14 days	Orange juice	9 g/d of LCLT (equivalent to 6 g/d of LC)	Muscle and serum carnitine Serum triglycerides, free fatty acids, glycerol, glucose Muscle glycogen content	No adverse events reported by authors	Vukovich *et al.*, 1994

Table C.4- 1 Summary of Human Studies on L-carnitine and L-carnitine L-tartrate							
Study Design	**Population (age, sex, health status)**	**Duration of Treatment**	**Test item**	**Daily Dose**	**Parameters Measured**	**Significant Results Related to Safety**	**Reference**
Randomized, double-blind, placebo-controlled, crossover	18 subjects (mean age of 36.1±9.9 years; aged 21 to 57 years; 9 M, 9 F; healthy)	8 days with 4-week washout	Capsules	1.462 g/d of LCLT (equivalent to 1 g/d of LC)	Body weight Blood pressure Heart rate Clinical chemistry (serum TP, BUN, CPK, AST, LDH) Metabolic and hormone parameters (plasma glucose, FFA, ketone bodies, cortisol, plasma ACTH, citric acid, blood lactate) Serum L-carnitine and acetyL-carnitine Physical performance test	NSD in mean body weight, systolic blood pressure, diastolic blood pressure, and clinical chemistry, metabolic, and hormone parameters. ↑ serum LC and acetyL-carnitine levels before physical load, at the end of the physical load, and after the recovery period.	Sugino *et al.,* 2007
Non-randomized, uncontrolled, open study	12 subjects (aged 18 to 30 years; 5 M, 7 F; healthy, slightly overweight)	10 days	NR	4.5 g/d of LCLT (equivalent to 3 g/d of LC)	Protein turnover Fat oxidation Body fat mass Total body water Lean body mass	Subjects in good health throughout the study. No gastrointestinal symptoms reported by any subject.	Wutzke and Lorenz, 2004
Randomized, double-blind, placebo-controlled, crossover	12 subjects (mean age of 27.5 years; 6 M, 6 F; healthy and active)	2 weeks	Capsules	3 g/d of LCLT (equivalent to 2 g/d of LC)	Respiratory exchange ratio and oxygen uptake during exercise Carbohydrate and fat oxidation Exercise heart rate and rating of perceived exertion	No adverse events reported by the authors.	Abramowicz and Galloway, 2005

Table C.4- 1 Summary of Human Studies on L-carnitine and L-carnitine L-tartrate							
Study Design	**Population (age, sex, health status)**	**Duration of Treatment**	**Test item**	**Daily Dose**	**Parameters Measured**	**Significant Results Related to Safety**	**Reference**
Randomized, double-blind, placebo-controlled, parallel-arm	20 M subjects (mean age of 33 years; healthy, endurance-trained)	2 weeks	Capsules	2.984 g/d of LCLT (L-Carnipure®) (equivalent to 2 g/d of LC)	Exercise heart rate, cadence, and rating of perceived exertion Haematology prior to and after exercise (pH, CO_2, bicarbonate, glucose, FFA, BUN, total BCAA, lactate, glycerol) Plasma ammonia concentrations Carbohydrate oxidation Urinary nitrogen excretion	No adverse events reported by the authors. NSD in haematology parameters.	Broad *et al.*, 2008
Randomized, balanced, placebo-controlled, double-blinded crossover	10 subjects (mean 23.7±0.7 years old ; M; healthy)	21 days	Capsules	3 g/d of LCLT (equivalent to 2 g/d of LC)	Haematology Biochemical markers of renal and liver function	NSD in haematology, blood chemistry, or kidney and liver function between groups. No gastrointestinal side effects in any subject.	Rubin *et al.,* 2001
Randomized, double-blind, placebo-controlled, crossover	10 subjects (mean age of 23.7±2.3 years; M; healthy, recreationally weight-trained)	3 weeks with 1-week washout	Capsules	2.944 g/d of LCLT (equivalent to 2 g/d of LC)	Serum total carnitine, free carnitine, and acetyL-carnitine Plasma lactate Purine catabolism Perceived muscle soreness Muscle tissue disruption and repair	No adverse events reported by authors.	Volek *et al.*, 2002

Table C.4- 1 Summary of Human Studies on L-carnitine and L-carnitine L-tartrate							
Study Design	**Population (age, sex, health status)**	**Duration of Treatment**	**Test item**	**Daily Dose**	**Parameters Measured**	**Significant Results Related to Safety**	**Reference**
Randomized, double-blind, placebo-controlled, crossover	30 subjects (mean age of 30±8 years; aged 20 to 50 years; 16 M, 14 F; healthy)	3 weeks with 3- and ~5-week washout periods for M and F, respectively	Capsules	2.944 g/d of LCLT (equivalent to 2 g/d of LC)	Body weight Flow-mediated dilation Post-prandial plasma TG levels Fasting and post-prandial serum IL-6, TNF-α, MDA, and insulin levels	NSD in body weight. No adverse effects reported by subjects during either phase.	Volek *et al.*, 2008
Randomized, double-blind, placebo-controlled, parallel-arm	9 subjects (mean age of 25.2±6 years; healthy, previously resistance-trained)	23 days	Capsules	2.944 g/d of LCLT (equivalent to 2 g/d of LC)	Tissue oxygenation responses Prostacyclin and MDA responses to resistance exercise	No adverse events reported by authors.	Spiering *et al.,* 2008
Randomized, double-blind, placebo-controlled, parallel-arm	18 subjects (mean age of 48.7 years; 8M, 8F; healthy, physically active, not resistance-trained)	24 days	Capsules	2.944 g/d of LCLT (Carnipure tartrate) (equivalent to 2 g/d of LC)	Muscle soreness Functional tests (strength/power, motility) Acute exercise resistance challenge Serum xanthine oxidase, MDA, myoglobin, CK, and lactate	No adverse events reported by authors.	Ho *et al.*, 2010

Table C.4- 1 Summary of Human Studies on L-carnitine and L-carnitine L-tartrate							
Study Design	**Population (age, sex, health status)**	**Duration of Treatment**	**Test item**	**Daily Dose**	**Parameters Measured**	**Significant Results Related to Safety**	**Reference**
Randomized, double-blind, placebo-controlled, crossover	15 M subjects (aged 20 to 46 years; mean age of 32 years; healthy, endurance-trained)	28 days	Capsules	2.984 g/d of LCLT (L-Carnipure®) (equivalent to 2 g/d of LC)	Haematology prior to and after exercise (pH, CO_2, bicarbonate, glucose, FFA, BUN, total BCAA, lactate, glycerol) Plasma noradrenaline and adrenaline Total fat and carbohydrate oxidation Time trial performance	No adverse events reported by authors.	Broad *et al.*, 2005

↑ = decrease(d); ↓ = increase(d) = ACTH = adrenocorticotropic hormone; ADHD = attention-deficit hyperactivity disorder; apo = apolipoprotein; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BCAA = branched chain amino acids; BUN = blood urea nitrogen; CK = creatine kinase; CPK = creatine phosphokinase; CRP = C-reactive protein; F = female; FFA = free fatty acids; FPG = fasting plasma glucose; FPI = fasting plasma insulin; GGT = *gamma*-glutamyltranspeptidase; GPx = glutathione peroxidase; Hb = haemoglobin; HbA_{1c} = glycosylated haemoglobin; Hct = haematocrit; HDL-C = high-density lipoprotein cholesterol; HOMA-IR = homeostasis model assessment insulin resistance index; hs-CRP = high sensitivity C-reactive protein; i.v. = intravenous; IL-1β = interleukin 1β; IL-6 = interleukin-6; LAC= L-acetyL-carnitine; LC = L-carnitine; LCLT = L-carnitine L-tartrate; LDH = lactate dehydrogenase; LDL-C = low-density lipoprotein cholesterol; Lp(a) = lipoprotein a; M = male; MDA = malondialdehyde; n = number of studies; NCI = National Cancer Institute; NCT-A = number connection test A; NR = not reported; NSD = no significant differences; OGTT = oral glucose tolerance test; ox-LDL = oxidized low-density lipoprotein; PAI-1 = plasminogen activator inhibitor type-1; PPG = post-prandial plasma glucose; PT = prothrombin time; PTT = partial thromboplastin time; QOL = quality of life; RBC = red blood cell; RBP-4 = retinol binding protein 4; ROS = reactive oxygen species; T2DM = type 2 diabetes mellitus; TC = total cholesterol; TG = triglycerides; TMA = trimethylamine; TNF-α = tumour necrosis factor-α; TP = total protein; tPa = tissue plasminogen activator; WBC = white blood cell

C.5 Safety of L-tartaric acid in L-carnitine L-tartrate

L-tartaric acid exists as colourless or translucent crystals, or as a white, crystalline powder. It is odourless with an acid taste and is stable in air. In the U.S., L-tartaric acid is affirmed as GRAS for direct use in foods as a firming agent, a flavour enhancer, a flavouring agent, a humectant, and a pH control agent in foods in general, with no limitation of use other than cGMP (21 CFR §184.1099, Volume 3, U.S. FDA, 2011b).

The safety of L-tartaric acid and its potassium, potassium-sodium and sodium salts have been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1977, 1978). On the basis of available toxicity studies, JECFA established an ADI (acceptable daily intake) of 0 to 30 mg/kg body weight/day for L-tartaric acid (and monosodium L-tartrate), exclusive of tartaric acid occurring naturally in the diet. This ADI was determined based on the results of 2 metabolism studies and a long-term feeding study. As summarized by JECFA (1977, 1978), studies investigating the metabolism of monosodium L-tartrate and monosodium DL-tartrate in rats demonstrated that both these substances were rapidly cleared from most tissues at the end of dosing at high levels for 7 days; however, some accumulation in the kidneys and increased kidney weights were noted for monosodium DL-tartrate. Similar tissue accumulation and effect on kidney weight were not observed with the L-tartrate salt. In a long-term feeding study conducted in rats, dietary administration of monosodium L-tartrate did not produce any evidence of carcinogenicity (HRC, 1976). The highest level fed (L-tartrate 7.68% of diet) did not result in adverse effects with respect to renal function and pathology. Growth depression was noted at all dose levels, but this effect was transient at the lowest dose level tested (2.56% of diet). JECFA (1978) reported that the study authors partially attributed the growth reduction to the unpalatability of the diet and to the dilution of caloric intake with L-tartrate, a largely non-metabolized compound. There was no evidence of organ toxicity at any dose level of L-tartaric acid and the no-observed-effect level (NOEL) was established at the highest dose tested in the study, (*i.e.,* 7.68% of the diet) (JECFA, 1978). This dose of L-tartaric acid is equivalent to 3,000 mg/kg body weight/day. The application of a 100-fold safety factor reflects the established ADI of 30 mg/kg body weight/day (JECFA, 1978).

As detailed in Section B (p.23), L-tartaric acid comprises approximately 32% of L-carnitine L-tartrate. The estimated intake of L-tartaric acid, through the intended uses of L-carnitine L-tartrate in food, is well-below JECFA's established ADI of 30 mg/kg body weight/day in 90th percentile consumers of all age categories (see Section D.4.3 (p.99)). These estimates remain below the established ADI considering the current consumption of L-tartaric acid as a food ingredient. These intakes are based on dietary survey data that includes a number of conservative assumptions that likely overestimate actual intake (see Appendix G). Furthermore, L-tartaric acid intakes generated for the proposed uses assume exclusive use of L-carnitine L-tartrate in foods. This overestimates the actual intended use of L-carnitine L-tartrate and provides a worst-case scenario of exposure, since L-carnitine alone may be used, dependent upon the food application.

C.6 Safety Assessment Reports Prepared by Other International Agencies and/or Other National Government Agencies

In 2003, the SCF evaluated the use of L-carnitine L-tartrate as a source of L-carnitine in foods for particular nutritional uses (EFSA, 2003). The Panel concluded that consumption of up to 3 g/day of L-carnitine L-tartrate (equivalent to 2 g/day of L-carnitine) presents no safety concerns when used as a source of L-carnitine for use in PARNUTS, provided that the ADI for L-tartaric acid from all sources in the diet is not regularly exceeded (EFSA, 2003).

C.7 Summary

L-carnitine tested negative in *in vitro* mutagenicity and genotoxicity studies including rec-assay, Ames test and chromosomal aberration assay, suggesting L-carnitine does not possess mutagenic potential. The animal toxicity studies conducted with L-carnitine have generally used L-carnitine chloride as the test substance. Bioequivalence of L-carnitine and L-carnitine L-tartrate to L-carnitine chloride can be supported by the complete dissociation of L-carnitine salts upon absorption, based on the results of a study which demonstrated that L-carnitine salts have a similar bioavailability to that of free L-carnitine (Eder *et al.,* 2005). Therefore, toxicity studies with L-carnitine chloride are considered appropriate for evaluating the safety of L-carnitine and L-carnitine L-tartrate. Results from studies of L-carnitine in experimental animals indicate that this compound is of a low order of toxicity. The administration of L-carnitine L-tartrate or L-carnitine chloride *via* the diet or by gavage was not associated with toxicologically significant adverse effects in rats, rabbits or dogs. Gastrointestinal effects observed at high doses were self-limiting, physiological responses, and typically associated with large bolus doses of an osmotic substance, rather than evidence of systemic toxicity. The results of the reproductive and developmental toxicity studies revealed that L-carnitine is not a teratogen or a reproductive toxin. Although a formal 2-year carcinogenicity study was not conducted with L-carnitine, some tumour data were available from 12-month chronic toxicity studies that indicate no carcinogenic potential. In addition, no systemic adverse effects were observed when L-carnitine doses of up to 6,000 mg/day or 100 mg/kg body weight/day were administered by oral or intravenous routes in acute and chronic human clinical testing. The overall evidence available from the toxicological data indicate that use of L-carnitine or L-carnitine L-tartrate under the brand names of Carnipure™ crystalline and Carnipure™ tartrate, respectively, is not associated with adverse effects at doses well above the dietary intakes including all current food and beverage uses.

D. INFORMATION ON DIETARY INTAKE OF THE NUTRITIVE SUBSTANCE

In accordance with Section 3.3.3 – Nutritive Substances of the Food Standards Australia New Zealand *Application Handbook* (FSANZ, 2013b) the following dietary intake information is provided:

1. A detailed list of the foods or food groups proposed to contain the nutritive substance, or changes to currently permitted foods.
2. The maximum proposed level of the nutritive substance for each food group or food, or the proposed changes to the currently permitted levels.
3. For foods or food groups not currently listed in the most recent Australian or New Zealand National Nutrition Surveys (NNSs), information on the likely level of consumption.
4. The percentage of the food group in which the nutritive substance is proposed to be used or the percentage of the market likely to use the nutritive substance.
5. Information relating to the use of the nutritive substance in other countries.
6. For foods where consumption has changed in recent years, information on likely current food consumption

Each point is addressed in turn in the Section that follows.

D.1/D.2 A Detailed List of the Foods or Food Groups Proposed to Contain the Nutritive Substance, or Changes to Currently Permitted Foods and Proposed Food Categories and Maximum Use Levels for Australia and New Zealand

Lonza intends to include Carnipure™ crystalline and Carnipure™ tartrate as food ingredients in various foods catories, namely dairy products (excluding butter and butter fat), confectionary, cereal and cereal products, foods intended for particular nutritional uses, non alcoholic beverages and gels.

The individual proposed food-uses and use-levels are summarized in Table D.2-1 (Appendix L), in the context of the categories set out in Schedule 1 to Standard 1.3.1 (Food Additives).

There is only very little market data available regarding the consumption of L-carnitine fortified products. The following research by Mintel´s Global New Products Database (GNPD) gives an overview at least about launched products containing L-carnitine in product categories corresponding to those applied for in Tab D.2-1 (Appendix L). Even if the data refer only to product launches and not to food consumed, they still reflect indirectly the consumption of products respectively consumer behavior.

The following six figures consider the global launches of L-carnitine containing products over a period of almost six years (2008 to November 2013). This time frame was chosen to demonstrate a constant trend in the selected categories, while the descriptor “global” considers the five regions of Asia Pacific, North America, Latin America, Europe and Middle East & Africa.

The Figures D.2-1 (p.88) and D.2-2 (p.88) include a all categories corresponding to those applied for while Figures D.2-3 to D.2-6 (pp.89) focusing on the two categories where L-carnitine is essentially used.

Asia Pacific is the global leader in launching new products containing L-carnitine followed by Europe and North America. In regard to products launched in 2012 and in 2013 it can be presumed that the number of L-carnitine fortified products will increase slow but constant in Middle East & Africa and Latin America over the next years (Figure D.2-1 p.88).

Figure D.2- 1 L-carnitine containing products (launches per region)

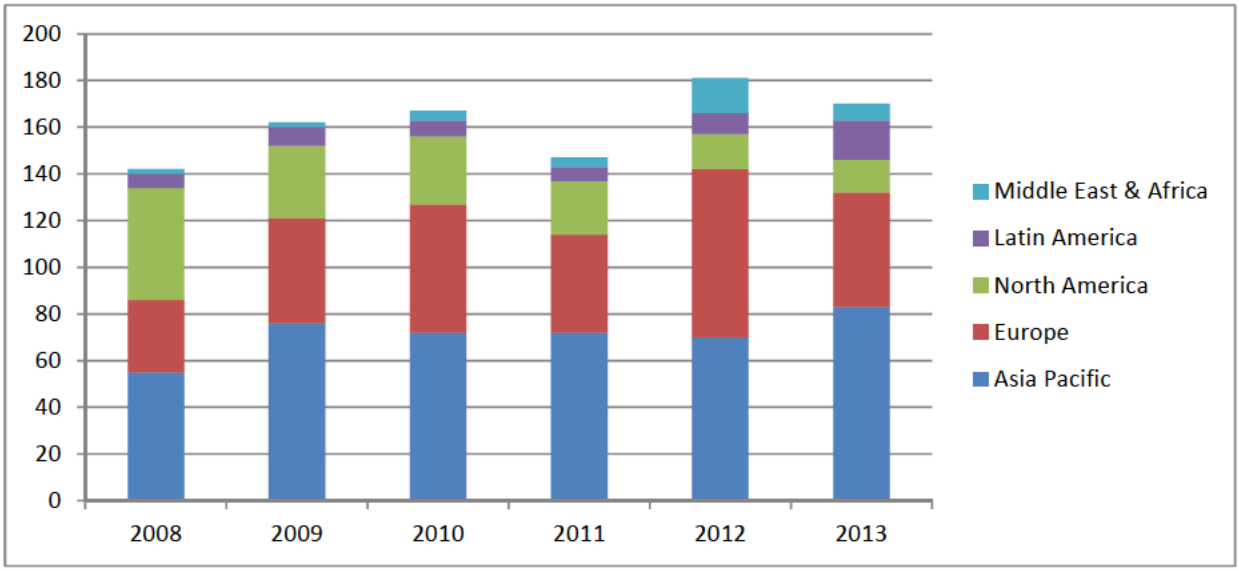
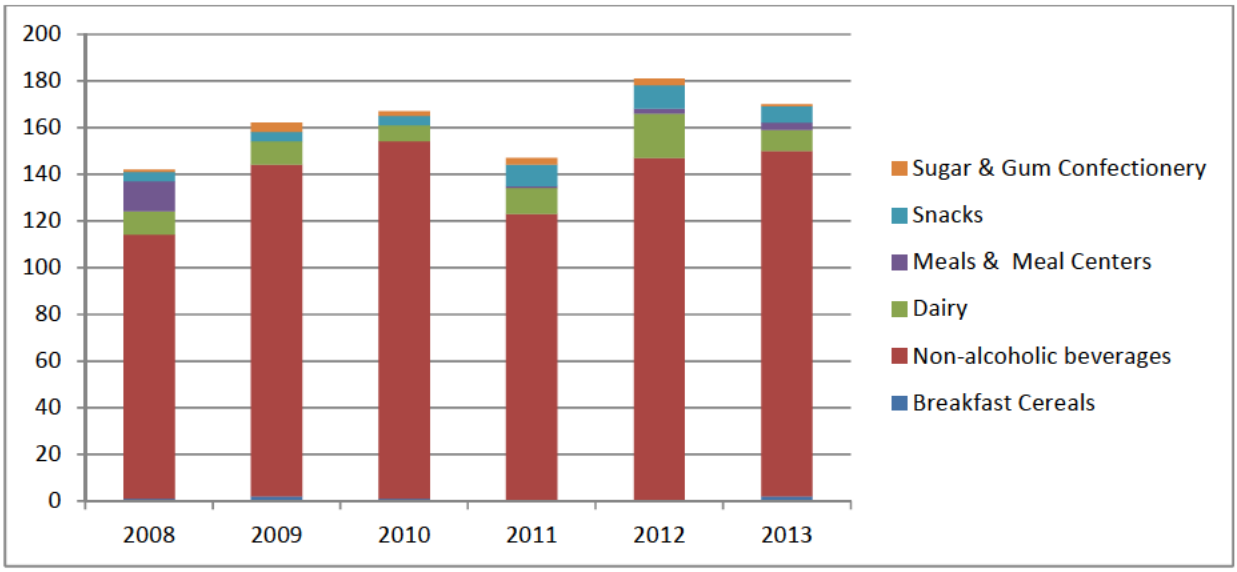


Figure D.2-2 (p.88) shows quite clearly that most of the L-carnitine containing products are launched in the category "non-alcoholic beverages", while the dairy category is a distant second.

Figure D.2- 2 L-carnitine containing products (launches per category)



The category “non-alcoholic beverages” covers the following sub-categories:

- Beverage Concentrates
- Carbonated Soft Drinks
- Energy Drinks
- Fruit/Flavoured Still Drinks
- Malt & Other Hot Beverages
- Nectars
- RTD (Iced) Tea
- Tea
- Beverage Mixes
- Coffee
- Flavoured Water
- Juice
- Meal Replacements &OtherDrinks
- RTD (Iced) Coffee
- Sports Drinks
- Water

Among the 16 sub-catogories, energy drinks, meal replacements & other drinks, fruit/flavored still drinks, coffee and sports drinks show the most L-carnitine product launches (Figure D.2-3 (p.89)).

Figure D.2- 3 Launches of L-carnitine containing “Non-alcoholic beverages” per year

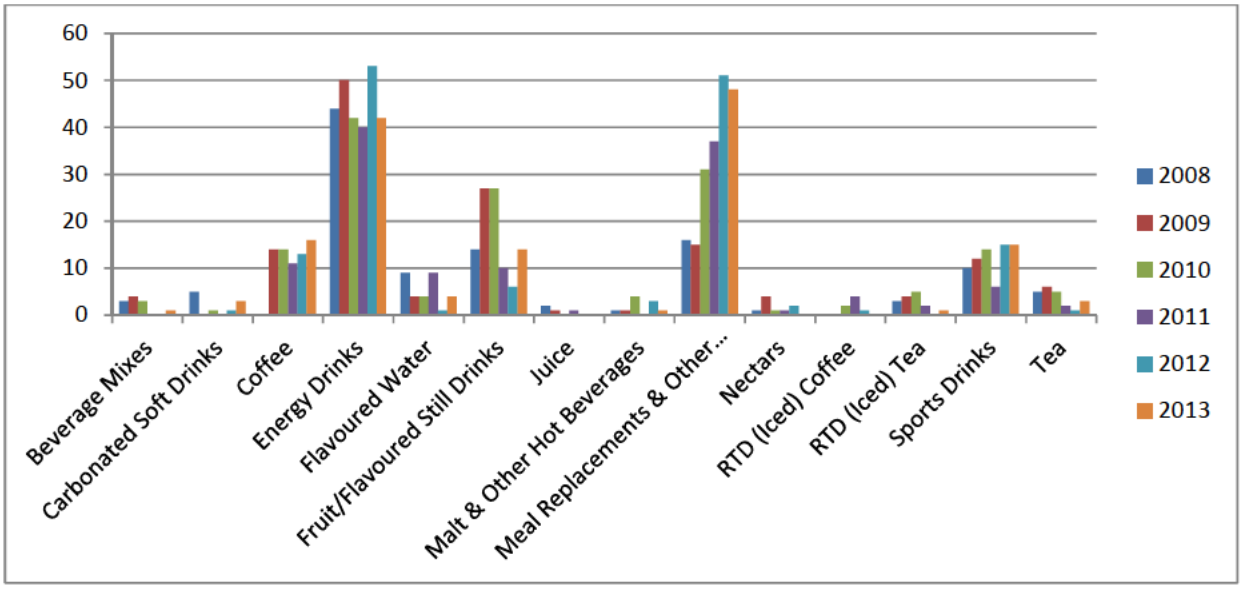
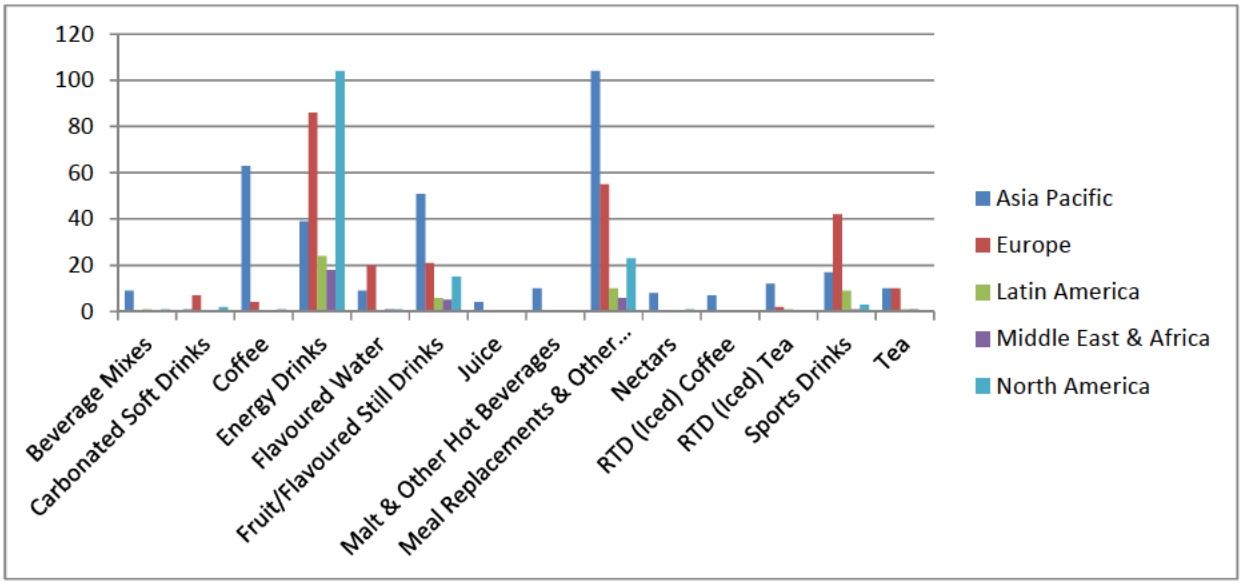


Figure D.2-4 (p.90) distinguishes L-carnitine products per region. L-carnitine containing energy drinks and meal replacements are mainly launched in North America, Europe and Asia Pacific, while coffee containing L-carnitine is more popular in Asia Pacific than other regions.

Figure D.2- 4 Launches of L-carnitine containing "Non-alcoholic beverages" per region



On a global basis and over a period of almost six years GNPD lists 119´190 product launches in the category "non-alcoholic beverages" of which only 969 include L-carnitine. To conclude, the market share of L-carnitine containing non-alcoholic beverages is about 0.8%.

Far behind non-alcoholic beverages, dairy is the second most favored product category where L-carnitine is used. The GNPD search included the following sub-categories:

- Flavoured milk
- Drinking yoghurt & liquid culturated milk
- Soy yoghurt
- Soy based drinks
- Spoonable yoghurt
- Rice, Nut, Grain & Seed based drinks

Over a period of almost six years, only 37 products containing L-carnitine were launched from a total of 33,011 products globally in the sub-categories mentioned above. The market size of dairy products launched with L-carnitine is only 0.1%.

Figure D.2-5 (p.91) shows the number of products launched per sub-category, and Figure D.2-6 (p.91) defines the region where the launches took place. Again, most of the L-carnitine dairy products are found in Asia.

Figure D.2- 5 Launches of L-carnitine containing dairy products per year

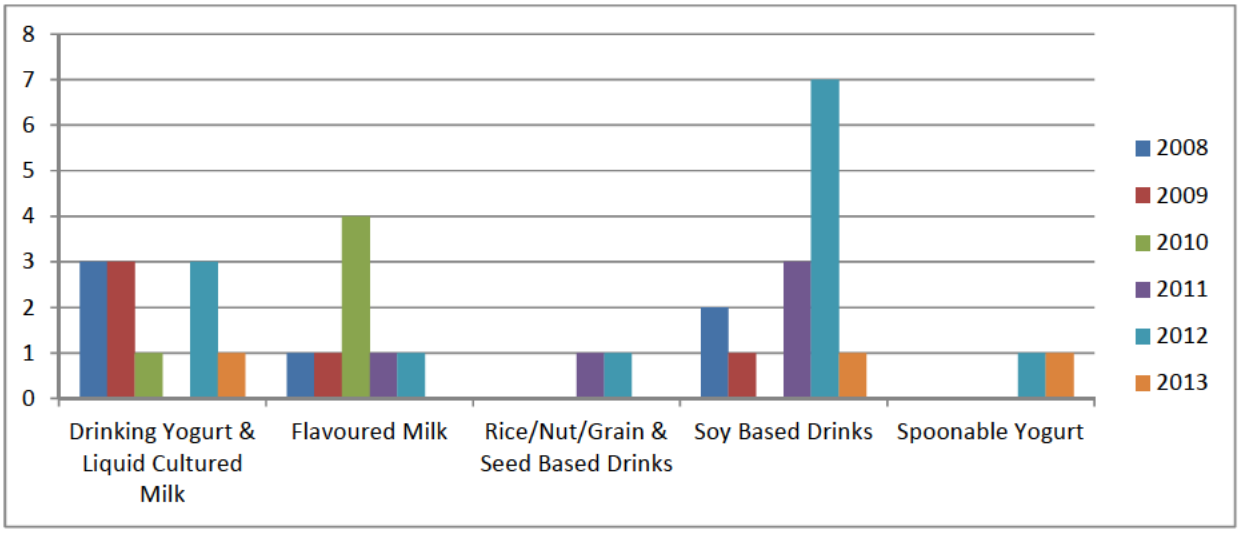
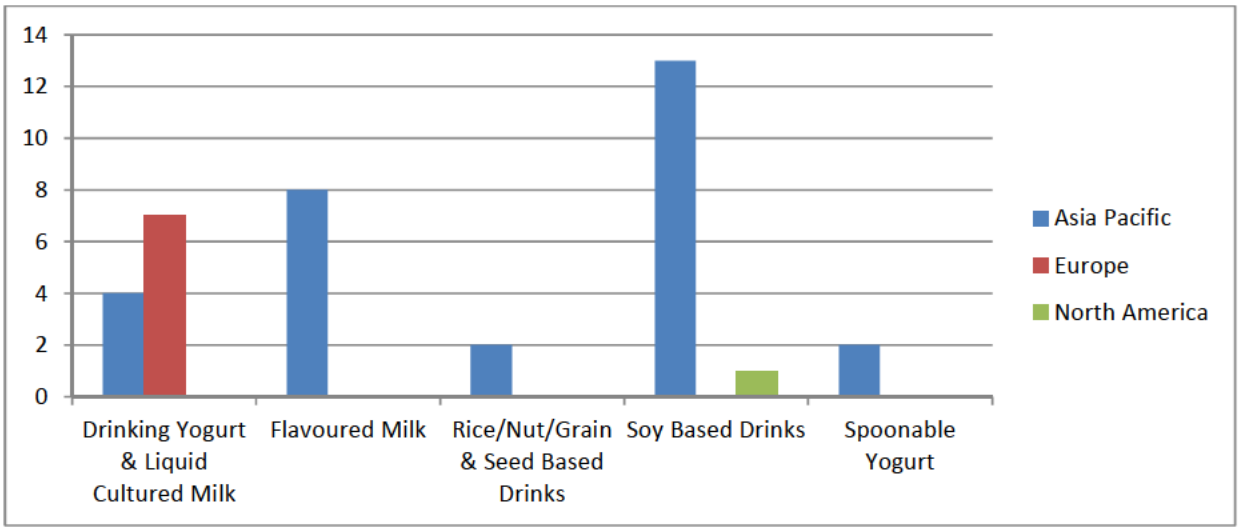


Figure D.2- 6 Launches of L-carnitine containing dairy products per region



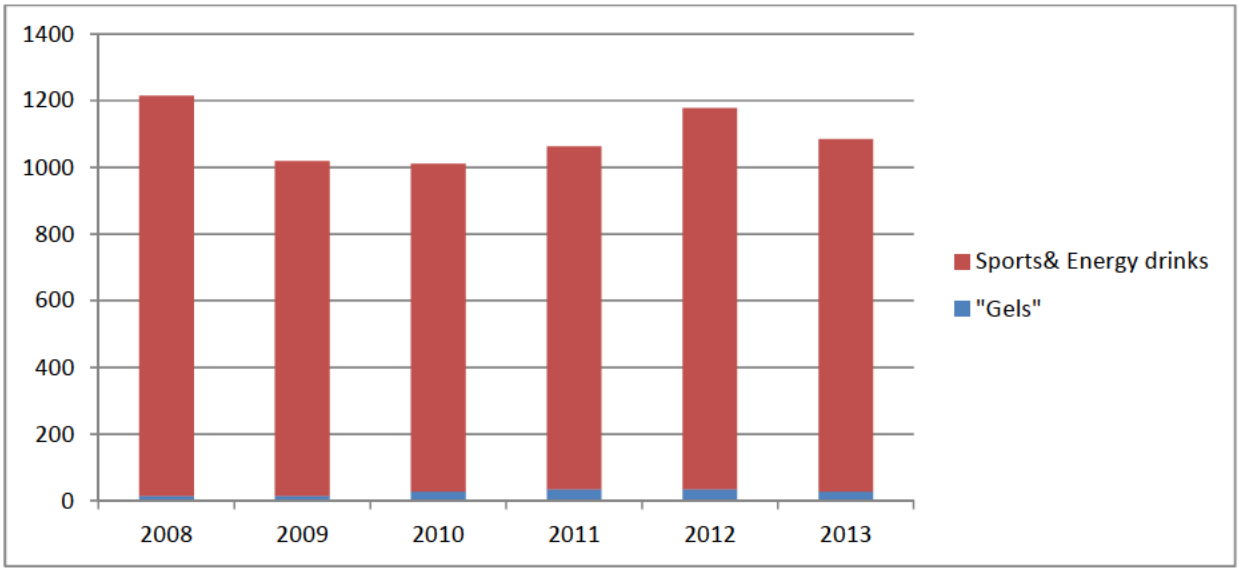
All in all it can be anticipated that even if the use of L-carnitine will be approved for certain categories in Australia New Zealand, only a few products on the market will contain L-carnitine. In regard to the global trend, the preferred product categories for the use of L-carnitine are non-alcoholic beverages and dairy. Undoubtedly, the majority of L-carnitine products are still being launched, sold and consumed in the category "food supplements".

D.3 For Foods or Food Groups not Currently Listed in the Most Recent Australian or New Zealand National Nutrition Surveys, Information on the Likely Level of Consumption.

Except its use in "gels", L-carnitine is most likely to be used in foods listed in the most recent Australian or New Zealand National Nutrition Surveys (NNSs).

There are no market data about the consumption of "gels" available. However gels are primarily consumed in the sports nutrition market as they provide a number of nutrients and functional ingredients in a concentrated form without the feeling of fullness after consumption.
Between January 2008 and November 2013, GNPD records 6413 product launches in the category "sports & energy drinks" of which 154 products can be found by searching for "gels" (Figure D.3-1 (p.92)). This results in a market share of 2.4%.

Figure D.3- 1 Product launches of "energy & sport drinks" compared to launched "gels"



D.4 The Percentage of the Food Group in which the Nutritive Substance is Proposed to be Used or the Percentage of the Market Likely to Use the Nutritive Substance

The purpose of using L-carnitine as an ingredient in food is to maintain the normal carnitine status of the body, particularly among individuals avoiding meat like vegetarians, vegans or people with reduced apetite for meat or inadequate supply of nutrients, such as elderly populations or people who might have an increased requirement of L-carnitine like athletes.

The ingredient will only be added to the foods shown in Table D.2-1 (Appendix L) and the functional purpose will be the primary reason for consumers to purchase and consume the foods. Information about the market likely to use the nutritive substance is provided in section D.1/D.2 (p.87).

D.4.1 Background Consumption in the Diet

L-carnitine was first isolated from meat extracts in 1905, and subsequently identified as the naturally occurring and biologically active form of carnitine in 1927 (Tanphaichitr and Leelahagul, 1993). L-carnitine is an essential co-factor of fatty acid metabolism and other metabolic pathways, with body stores maintained primarily in skeletal muscle. The majority of the body's L-carnitine is supplied in the diet from meat and meat-based foods; however, L-carnitine is also synthesized endogenously from L-lysine and L-methionine. Thus, L-carnitine cannot be considered an essential nutrient, *per se*, although the term "conditionally essential nutrient" is often encountered in the scientific literature.

L-carnitine is present in the human diet in a variety of food sources, with the concentration dependent on the type of food source (Tanphaichitr and Leelahagul, 1993; Demarquoy *et al.,* 2003). Animal products, such as lamb, beef and pork, are the richest sources of dietary L-carnitine (Tanphaichitr and Leelahagul, 1993; Demarquoy *et al.,* 2003). In comparison, lower levels of L-carnitine are found in dairy products, fish, and seafood, and most fruits and vegetables contain minimal amounts of L-carnitine (Rebouche and Engel, 1984; Demarquoy *et al.,* 2003). Table D.4.1-1 (pp.94) summarizes the L-carnitine content of some selected foods.

Table D.4.1- 1 L-carnitine Content of Selected Foods		
Food Source	**Type of Product**	**L-carnitine Level[1] (mg/100 g)**
Meat	Beef (steak)	65.0
	Ground beef	87.5
	Lamb chop	40.5
	Kangaroo steak	63.7
	Pork leg	17.7
	Pork shoulder	21.1
	Veal shoulder	78.2
	Veal sirloin	132.8
	Pork sausage	7.1
	Beef sausage	66.3
	Turkey meat	21.2
	Chicken meat (without skin)	10.4
Seafood Products	Shrimp	0.7
	Cod (Atlantic)	1.8
	Mussels (cooked)	2.6
	Salmon (cooked)	5.8
	Tuna	1.5
Fruits and Vegetables	Apple	0.2
	Avocado	8.1
	Banana	0.04
	Potato	2.4
Dairy Products	Goat cheese	15.3
	Feta cheese	1.8
	Parmesan cheese	0.7
	Yogurt (regular fat)	12.2
	Milk (2% fat)	2.9
	Sour cream	1.97
Other Products	Peanuts	0.2
	Egg yolk	0.8
	Egg white	0.3
	Rice	0

[1] L-carnitine values determined by Demarquoy *et al.* (2003) or Seline and Johein (2007)

The consumption of L-carnitine from the diet has been estimated to range from 100 to 300 mg/day (Broquist, 1994); however, the consumption of L-carnitine may be considerably higher in heavy consumers of meat and meat products or lesser depending on the diet. A summary of the estimated daily *Per Capita* intakes of L-carnitine from selected dietary sources is presented in Table D.4.1-2 (p. 95). Any estimation of the total daily intake of L-carnitine from the diet is dependent upon the type and quantity of food consumed.

Table D.4.1- 2 *Per Capita* L-carnitine Estimates

Dietary Source	L-carnitine Level[1] (mg/g)	Estimated Daily Mean *Per Capita* Intake of Dietary Sources[2] (g)	Estimated Daily Mean *Per Capita* Intake of L-carnitine from Dietary Sources (mg)
Meat Products			
Beef	1.43	79.5	113.7
Pork	0.27	58.4	15.8
Chicken breast	0.08	53.4	4.2
Lamb	1.90	1.4	2.6
Veal	1.05	1.1	1.2
Seafood Products			
Oysters	0.24	18.6	4.5
Dairy Products			
Processed Cheese	0.04	14	0.6
Ice Cream	0.04	19.5	0.8
Whole Milk	0.04	112	4.5
Evaporated Milk	0.10	2.6	0.3
Cottage Cheese	0.05	4.2	0.2
Yogurt	0.04	5.1	0.2
Fruits and Vegetables			
Potato	0.002	54	0.1
Avocado	0.004	1.2	0.005

[1] Representative L-carnitine values as indicated in Table 4.1-1 (p.94)

[2] Estimated Mean *Per Capita* Food Consumption data in the United States were collected in 1990/1991and reported by Jones Putnam and Allshouse, 1999.

D.4.2 Theoretical Calculation of the Consumption of L-carnitine from Carnipure™ crystalline and Carnipure™ tartrate

Intakes have been estimated for the U.S. to provide information on the projected use of foods that contain Carnipure™ crystalline and Carnipure™ tartrate and are summarised below.

Food codes representative of each intended food-use were chosen from National Health and Nutrition Examination Surveys (NHANES) 2003-2004, 2005-2006 (CDC, 2006, 2009; USDA, 2009), while food codes were grouped in food-use categories according to Title 21, Section §170.3 of the Code of Federal Regulations (CFR) (U.S. FDA, 2011c), and product-specific adjustment factors were developed based on data provided in the standard recipe file for the Continuing Survey of Food Intake by Individuals (CSFII) 1994-1996, 1998 survey (USDA, 2000).

The NHANES 2003-2004 and 2005-2006 food consumption survey data were collected from individuals and households via 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) throughout all 4 seasons of the year. Day 1 data were collected in person, and Day 2 data were collected by telephone in the following 3 to 10 days, on different days of the week, to achieve the desired degree of statistical independence.

Consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of L-carnitine by the U.S. population. Estimates for the daily intake of L-carnitine represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2005-2006 data; these average amounts comprised the distribution from which mean and percentile intake estimates were produced. Mean and percentile estimates were generated incorporating survey weights in order to provide representative intakes for the entire U.S. population. All-person intake refers to the estimated intake of L-carnitine averaged over all individuals surveyed, regardless of whether they consumed food products containing L-carnitine, and therefore includes “zero” consumers (those who reported no intake of food products containing L-carnitine during the 2 survey days). All-user intake refers to the estimated intake of L-carnitine by those individuals consuming food products containing L-carnitine, hence the “all-user” designation. Individuals were considered users if they consumed 1 or more food products containing L-carnitine on either Day 1 or Day 2 of the survey.

Mean or percentile intake estimates based on small sample sizes or with high variability relative to the mean [assessed using the coefficient of variation (CV)] may be less statistically reliable than estimates based on adequate sample sizes or low variability relative to the mean (LSRO, 1995). Data presented herein for the estimated daily intake of L-carnitine follow the guidelines proposed by the Human Nutrition Information Service/National Center for Health Statistics Analytic Working Group for evaluating the reliability of statistical estimates adopted in the “Third Report on Nutrition Monitoring in the United States”, whereby an estimated mean may be unreliable if the CV is equal to or greater than 30% (LSRO, 1995). The CV is the ratio of the estimated standard error of the mean to the estimated mean, expressed as a percentage (LSRO, 1995). Therefore, for the estimated intakes of L-carnitine presented herein, values were considered statistically unreliable if the CV was equal to or greater than 30%. These values were not considered when assessing the relative contribution of specific food-uses to total L-carnitine consumption.

Calculations for the mean and 90th percentile all-person and all-user intakes, and percent of the population consuming were performed for each of the individual identified food-uses of L-carnitine. Similar calculations were used to determine the estimated total intake of L-carnitine from all identified food-uses combined. In both cases, the per person and per kilogram body weight intakes were reported for the following population groups:

- infants, ages 0 to 2;
- children, ages 3 to 11;
- female teenagers, ages 12 to 19;
- male teenagers, ages 12 to 19;
- female adults, ages 20 and up;
- male adults, ages 20 and up; and,
- total population (all population and gender groups combined).

In addition to collecting information on the types and quantities of foods and supplements being consumed, NHANES 2003-2004 and 2005-2006 collected socioeconomic, physiological and demographic information from individual participants in the survey, such as sex, age, height and weight, and other variables useful in characterizing consumption. The inclusion of this information allows for further assessment of food intake based on consumption by specific population groups of interest within the total population. Sample weights were incorporated with NHANES 2005-2006 to compensate for the potential under-representation of intakes from specific population groups as a result of sample variability due to survey design, differential non-response rates, or other factors, such as deficiencies in the sampling frame (CDC, 2006, 2009; USDA, 2009). A full intake report is provided in Appendix G.

The estimated total intake of L-carnitine from all permitted food categories and proposed use levels of Carnipure™ crystalline and Carnipure™ tartrate in the U.S. by population group is summarized in Table D.4.2-1 (p.98), while Table D.4.2-2 (p.98) presents these data on a per kilogram body weight basis.

Approximately 93.2% of the total U.S. population was identified as potential consumers of L-carnitine from the permitted and proposed food uses of Carnipure™ crystalline or Carnipure™ tartrate (15,551 actual users identified). Consumption of these types of foods by the total U.S. population resulted in estimated mean all-person and all-user intakes of L-carnitine of 622 mg/person/day (9.9 mg/kg body weight/day) and 658 mg/person/day (10.5 mg/kg body weight/day), respectively. The 90th percentile all-person and all-user intakes of L-carnitine from all proposed and permitted food-uses of Carnipure™ crystalline or Carnipure™ tartrate by the total population were 1,361 mg/person/day (22.0 mg/kg body weight/day) and 1,398 mg/person/day (22.5 mg/kg body weight/day), respectively.

On an individual population basis, the greatest mean all-person and all-user intakes of L-carnitine on an absolute basis were determined to occur in male teenagers at 943 and 962 mg/person/day, respectively. Infants displayed the lowest mean all-person and all-user L-carnitine intake estimates with values of 173 and 234 mg/person/day, respectively. On a body weight basis, the highest estimate for the mean all-person intakes were observed to occur in children at 16.1 mg/kg body weight/day, while the highest estimated all-user intake was observed to occur in infants at 18.9 mg/kg body weight/day.

Table D.4.2- 1 Summary of the Estimated Daily Intake of L-carnitine from All Proposed Food Uses of Carnipure™ crystalline or Carnipure™ tartrate in the U.S. by Population Group (NHANES 2003-2004, 2005-2006)

Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption		All-User Consumption	
				Mean (mg)	90th Percentile (mg)	Mean (mg)	90th Percentile (mg)
Infants	0 to 2	67.1	1,284	173	434	234	495
Children	3 to 11	98.8	2,701	444	890	453	900
Female Teenagers	12 to 19	98.4	1,956	582	1,164	591	1,170
Male Teenagers	12 to 19	98.5	1,911	943	1,854	962	1,858
Female Adults	20 and up	95.3	4,079	544	1,236	573	1,246
Male Adults	20 and up	94.3	3,620	782	1,730	827	1,772
Total Population	All ages	93.2	15,551	622	1,361	658	1,398

When heavy consumers (90 ^{h} percentile) were assessed, all-person and all-user intakes of L-carnitine from all proposed and permitted food-uses of L-carnitine or L-carnitine L-tartrate were again determined to be greatest in male teenagers at 1,854 and 1,858 mg/person/day, respectively. The lowest 90th percentile all-person and all-user intakes occurred in infants at 434 and 495 mg/person/day, respectively, on an absolute basis. On a body weight basis, infants were determined to have the greatest all-person and all-user 90th percentile intakes of L-carnitine at 33.4 and 38.3 mg/kg body weight/day, respectively.

Table D.4.2- 2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of L-carnitine from All Proposed Food Uses of Carnipure™ crystalline or Carnipure™ tartrate in the U.S. by Population Group (NHANES 2003-2004, 2005-2006)

Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption		All-User Consumption	
				Mean (mg/kg bw)	90th Percentile (mg/kg bw)	Mean (mg/kg bw)	90th Percentile (mg/kg bw)
Infants	0 to 2	67.1	1,284	14.0	33.4	18.9	38.3
Children	3 to 11	98.8	2,701	16.1	31.0	16.5	31.1
Female Teenagers	12 to 19	98.4	1,956	9.8	20.3	9.9	20.6
Male Teenagers	12 to 19	98.5	1,911	14.2	27.6	14.4	27.8
Female Adults	20 and up	95.3	4,079	7.5	16.9	7.9	17.4
Male Adults	20 and up	94.3	3,620	9.1	19.6	9.7	19.9
Total Population	All ages	93.2	15,551	9.9	22.0	10.5	22.5

D.4.3 Theoretical Calculation of the Consumption of L-tartaric acid from Carnipure™ tartrate

Carnipure™ tartrate is composed of 68% L-carnitine and 32% L-tartaric acid. Estimated L-carnitine intake values summarized in Table D.4.2-2 (p.98) were used to calculate the exposure of L-tartaric acid from the proposed food-uses on a body weight basis. The estimated intake of L-tartaric acid on a body weight basis is summarized in Table D.4.3-1 (p.99).

Table D.4.3- 1 Summary of the Estimated Daily Per Kilogram Body Weight Intake of L-Tartaric Acid from All Proposed Food Uses of Carnipure™ tartrate in the U.S. by Population Group (NHANES 2003-2004, 2005-2006)

Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption		All-Users Consumption	
				Mean (mg/kg)	90th Percentile (mg/kg)	Mean (mg/kg)	90th Percentile (mg/kg)
Infant	0-2	67.1	1,284	6.6	15.7	8.9	18.0
Child	3-11	98.8	2,701	7.6	14.6	7.7	14.6
Female Teenager	12-19	98.4	1,956	4.6	9.6	4.7	9.7
Male Teenager	12-19	98.5	1,911	6.7	13.0	6.8	13.1
Female Adult	20 and Up	95.3	4,079	3.5	8.0	3.7	8.2
Male Adult	20 and Up	94.3	3,620	4.3	9.2	4.6	9.4
Total Population	All Ages	93.2	15,551	4.7	10.3	4.9	10.6

On an all-user basis, the mean intake of L-tartaric acid by the total population from all proposed food-uses of Carnipure™ tartrate was estimated to be 4.9 mg/kg body weight/day. The heavy consumer (90th percentile) all-user intake of L-tartaric acid by the total population from all proposed food-uses was determined to be 10.6 mg/kg body weight/day. Infants were determined to have the greatest mean and 90th percentile all-user intakes of L-tartaric acid at 8.9 and 18.0 mg/kg body weight/day, respectively.

D.4.4 Replacement of Other Foods in the Diet

It is not anticipated that the addition of L-carnitine to foods would replace, or partially replace, any nutrients in the products, although the addition of L-carnitine as L-carnitine L-tartrate to beverages could result in a reduction of other acidic substances, such as citric acid.

D.5 Information Relating to the Nutritive Substance in Other Countries

Please also see chapter A.5 International and Other National Standards (pp. 19).

General Summary

L-carnitine and L-carnitine L-tartrate are approved and/or accepted in most countries:

- the products are approved for infant nutrition;
- the products are approved for foods for particular nutritional uses (PARNUTS);
- the products have a positive EFSA (European Food Safety Authority) safety evaluation;
- the products have US self-affirmed GRAS status (Generally Recognised As Safe); and
- are approved and/or accepted in many countries such as USA, European Union, Japan, China, etc.

USA:

In the U.S., L-carnitine and L-carnitine L-tartrate are self-affirmed as Generally Recognized as Safe (GRAS) for use in a number of foods with the following use levels (Table D.5-1 (p.100)).

Table D.5- 1 Summary of the Individual Permitted and Proposed Food Uses for L-carnitine from Carnipure™ crystalline or Carnipure™ tartrate in the U.S.

Food Category	Food Use	Use level for Carnipure™ crystalline (mg/serving)	Use level for Carnipure™ tartrate (mg/serving)**
Beverages and beverage bases	Carbonated soft drinks	250	368
	Energy and sports drinks	500	736
	Fruit-flavored drinks (RTD* and powdered)	100	147.2
	Non-mi k-based meal replacement beverages	250	368
Coffee and teas	Herbal teas	250	368
	Instant coffee	250	368
Dairy product analogs	Imitation and soy mi ks	250	368
Grain products and pastas	Health and breakfast bars	250	368
	Meal replacement bars	250	368
Hard candy	Hard candy	50	73.6
Milk products	Flavored milks and milk-based drinks	250	368
	Mi k-based meal replacement beverages	250	368
	Yogurt	250	368
	Yogurt drinks	250	368
Processed fruits and fruit juices	Fruit juice (RTD and frozen concentrates)	100	147.2
	Nectars	50	73.6
Soft candy	Chocolate	50	73.6
	Soft candy	50	73.6

*RTD = ready-to-drink

* Calculated levels for Carnipure™ tartrate to provide respective amounts of L-carnitine.

European Union:

- **Food (fortified):** For substances other than vitamins and minerals no specific lists are fixed within Regulation (EC) 1925/2006 on the addition of vitamins and minerals and of certain other substances to foods (EC, 2006d). However, L-carnitine crystalline and L-carnitine L-tartrate are allowed to be used in fortified foods.
  Within the EU there is no specifically listed allowed dosage of L-carnitine in food products, but prodcuts containing/promoting 250 mg per day are generally accepted in most EU countries.
- **Food Supplements:** For substances other than vitamins and minerals no specific lists are fixed within the Directive 2002/46/EC and subsequent amendments on the approximation of the laws of the Member States relating to food supplements (EC, 2002). However recital 11 expressly states that substances which have been approved by the Scientific Committee on Food for use in the manufacture of foods for infants and young children and other foods for particular nutritional use (PARNUTS) can be also used in the manufacture of food supplements. In the EU there is no common agreement about the dosage of L-carnitine (E.g. a decree in Belgium lists L-carnitine for use as dietary supplement without any dose limitation, Italy recommends a maximum of 1000 mg. Spain confirmed that L-carnitine crystalline (2g) and L-carnitine L-tartrate (3g) can be used for dietary supplements. Also the Danish regulation follows the existing EFSA-Opinion from 2003 allowing 2 g L-carnitine respectively 3 g L-carntine L-tartrate in dietary supplements (notified in EU (2010/793/DK - Tris database).

China:

Carnipure™ crystalline and Carnipure™ tartrate are allowed to be used in foods as listed in Table D.5-2 (p.101).

Table D.5- 2: Permitted Food Categories and Use Levels in China

Food category	**use level of L-carnitine**
Milk powder (children)	50 – 150 mg/kg
Milk powder (adults)	300 - 400 mg/kg
Fruit/vegetable juice beverages	600 – 3000 mg/kg
Flavored beverages	600 – 3000 mg/kg
Milk beverages	600 – 3000 mg/kg
Solid beverages	6000 – 30000 mg/kg
Sport beverages	100 - 1000 mg/kg
Athletic nutrition food	1 – 4 g/day
Health food (including food supplements)	product-dependent

D.6 For Foods where Consumption has Changed in Recent Years, Information on Likely Current Food Consumption

Existing survey data are sufficient to enable estimates of likely intake of L-carnitine.

E. INFORMATION ON THE NUTRITIONAL IMPACT OF A NUTRITIVE SUBSTANCE OTHER THAN VITAMINS AND MINERALS

E.1 Nutritional Properties of the Nutritive Substance and Its Impact in the Diet

L-carnitine, an essential cofactor for fatty acid metabolism in humans, is involved in the transformation of free long-chain fatty acids (LCFA) into acylcarnitines and subsequent transport of these fatty acids from the cytosolic compartment into the mitochondrial matrix for β-oxidation and cellular energy production (Haeckel *et al*., 1990; Kelly, 1998). Considering that L-carnitine's function of transporting and transforming free LCFA into energy is a crucial component of normal lipid metabolism in humans, maintenance of carnitine status is, therefore, essential for optimal health and well-being.

Full compositional information on Carnipure™ crystalline and Carnipure™ tartrate is provided in Section B.2 (p.24). The nutritional composition of Carnipure™ crystalline and Carnipure™ tartrate is presented in Table E.1-1 (p.103).

Table E.1- 1 Nutritional Composition of Carnipure™ crystalline and Carnipure™ tartrate based on 100 g

	L-carnitine	**L-carnitine L-tartrate**
Energy (kcal)	0	96[a]
Energy (kJ)	0	416
Fat (g)	0	0
Saturated fat (g)	0	0
Total carbohydrates (g)	0	0
Proteins (g)	0 (non-protein nitrogen: 9g)	0 (non-protein nitrogen: 6g)
Sodium (%)	≤0.1	≤0.1

[a] Calculated value based on the assumption that all organic acids provide an equal amount of energy.

It should be noted that Carnipure™ crystalline is similar to naturally occurring L-carnitine, and handled in a similar manner by the body following ingestion. The absorption, distribution, metabolism, and excretion of L-carnitine are discussed in detail in Section C.1 (pp.33). In addition, L-carnitine L-tartrate is expected to readily dissociate into L-carnitine and L-tartaric acid in the gastrointestinal tract, and the bioavailability of L-carnitine from L-carnitine-L-tartrate has been shown to be similar to L-carnitine given as the free base. L-carnitine (from Carnipure™ crystalline or Carnipure™ tartrate) will act the same as the endogenously formed L-carnitine, and thus, its addition to the food products is not anticipated to impact the overall role of the food product in the diet or modify the bioavailability of other nutrients present in the diet.

E.2 Clinical Studies using L-carnitine Fortified Food Products

Most of the studies performed to test the effect of L-carnitine have used L-carnitine in the form of food supplements. Two studies (Wall et al., 2011 and Stephens et al., 2013) have been identified as using a solution (700ml) containing 80 g of orange-flavoured carbohydrate polymer containing 2.0 g of L-carnitine tartrate.

Beside a beneficial effect of L-carnitine on physical activity, these studies provide evidence that L-carnitine applied in a food matrix high in carbohydrates have a positive effect on the utilisation of carbohydrates and their deposition.

Key messages Wall et al., 2011:

- "Long-term L-carnitine supplementation coupled with the intake of carbohydrates increases muscular L-carnitine content in recreational athletes."
- "The randomized, double-blind, placebo-controlled study was the first study in healthy humans showing that muscle L-carnitine content can be influenced by dietary means and that L-carnitine plays a dual role in skeletal muscle fuel metabolism during exercise that is dependent on exercise intensity. During low intensity exercise increasing muscle L-carnitine content led to glycogen sparing and increased fatty acid oxidation. In high intensity exercise it led to a decrease in anaerobic energy production, including a decrease in muscle lactate accumulation. Furthermore, during a performance test, L-carnitine supplementation resulted in lower perceived exertion as well as increased work output."

Key messages Stephens et al., 2013:

- "A 20% increase in muscle carnitine content, achieved via 12 weeks of twice daily supplementation of a beverage containing 1.36 g of L-carnitine and 80 g of carbohydrate (in order to stimulate insulin mediated muscle carnitine transport), prevented an 18% increase in body fat mass associated with carbohydrate supplementation alone in healthy young men."
- "A novel finding of the present study was that this prevention of fat gain was associated with a greater energy expenditure and fat oxidation during low intensity physical activity, and an adaptive increase in expression of gene networks involved in muscle insulin signalling and fatty acid metabolism."

E.3 Data on Nutrient Profiles of Foods Containing the Nutritive Substance Ingredient

The nutrient profiles of foods containing Carnipure™ crystalline or Carnipure™ tartrate are comparable to similar products that do not contain the nutritive substance L-carnitine.

It is not anticipated that the addition of L-carnitine to foods would replace, or partially replace, any nutrients in the products, although the addition of L-carnitine as L-carnitine L-tartrate to beverages could result in a reduction of other acidic substances, such as citric acid (please refer to D.4.4 Replacements of Other Foods in the Diet (p.99).

L-carnitine is synthesized from two amino acids but is neither a protein nor an amino acid by itself. The energy supply provided by protein (17kJ/gram) does not apply.

L-carnitine is either eliminated via the kidney mostly as unchanged carnitine and acylcarnitine or readily filtered through the glomeruli. Ninety percent of filtered L-carnitine is reabsorbed. Unabsorbed oral L-carnitine undergoes bacterial degradation in the gastrointestinal tract to form trimethyl amine (TMA) and γ-butyrobetaine (GBB). TMA is subsequently absorbed and metabolized to form trimethylamine-N-oxide (TMAO), which is primarily excreted in the urine, whereas GBB is mainly excreted in the faeces (please refer to C.1.3 (pp.36)).

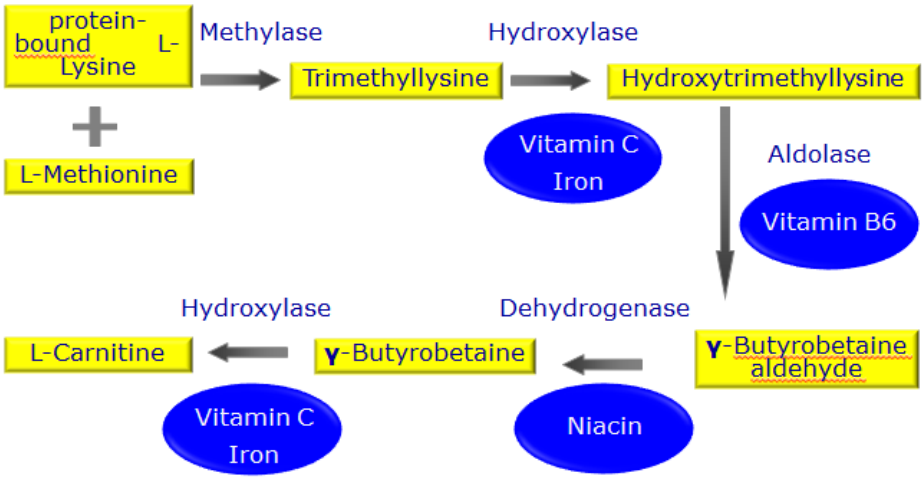
Only the use of L-carnitine L-tartrate contributes to a minor amount of additional calories due to the presence of L-tartrate (please refer to Table E.1-1 (p.103). With view to the maximum content of L-carnitine in a serving size this application addresses, (0.5 g L-carnitine (equal to 0.736 g of L-carnitine L-tartrate) L-carnitine L-tartrate contributes to an additional 0.71 kcal/3.1 kJ.

E.4 Information Related to the Nutritional Purpose of Adding the Nutritive Substance to Certain Food

An overview from Luppa (Luppa 2004) refers to the question of up to what extent endogenous L-carnitine biosynthesis of an adult is sufficient. Luppa assumes that the synthesis capacity is subject to significant individual variations. These variations include genetic requirements, gender, age as well as the availability of required amino acids and cofactors. A healthy adult with a moderate level of physical activity usually requires 50 to 100 mg L-carnitine in addition to L-carnitine provided by endogenous supply. This can be achieved by a well-balanced diet. For vegetarians or people following a specific diet either the intake of L-carnitine or/and the vitamin and mineral cofactors necessary for biosynthesis might be deficient. Fad diets, such as high protein and low carbohydrate diets can also contribute to increased excretion of L-carnitine (Pekala 2011).

Humans are able to synthesize L-carnitine from the essential amino acids lysine and methionine via a series of enzymatic reactions. Lysine residues destined for L-carnitine synthesis must be protein linked. This protein-bound lysine becomes available for L-carnitine biosynthesis following proteolytic release by lysosomal enzymes (Rebouche 1999).
Additional cofactors in latter steps of the L-carnitine biosynthesis pathway are vitamin C, niacin and vitamin B6 as well as iron (Figure E.4-1 (p.106)).

Figure E.4- 1 L-carnitine biosynthesis



It is apparent that a nutritional potpourri is required for L-carnitine biosynthesis and it follows accordingly that inadequate intake of essential micronutrients might be expected to jeopardize the L-carnitine status of an individual (Broquist et al. 1982).

For example, as previously discussed, vitamin C is a cofactor for two enzymes required for the biosynthesis of L-carnitine, ε-*N*-trimethyl-L-lysine hydroxylase and γ- butyrobetaine hydroxylase. Therefore, its presence and activity has an impact on carnitine biosynthesis. Since the oxidation of fatty acids in skeletal muscle is dependent on L-carnitine, this is a possible mechanism by which vitamin C affects fat oxidation. Rebouche and colleagues have shown that vitamin C deficient guinea pigs have decreased activity of butyrobetaine hydroxylase, the enzyme catalysing the last step of biosynthesis. (Rebouche 1991),

Preliminary data in humans also indicate that vitamin C status impacts fat oxidation. Human subjects with marginal vitamin C status oxidized 25% less fat per kg body weight during a 60-minute treadmill walk as compared to individuals with adequate vitamin C status. Moreover, fat oxidation during exercise was enhanced in these individuals by normalizing plasma vitamin C concentrations. (Johnston 2006).

Age is another factor which impacts endogenous biosynthesis. There is evidence to suggest that the enzymatic activity of hepatic γ-butyrobetaine hydroxylase is age-dependent. The activity of γ-butyrobetaine hydroxylase in infants was reported to be only 12% of the normal adult activity. By the age of 2.5 years the activity rises to 30% and by 15 years is within the standard deviation of the adult mean (Rebouche 1980). In elderly populations, L-carnitine biosynthesis has been found to decrease again (Leibovitz 1993).

The purpose of using L-carnitine as an ingredient in foods is to maintain the normal carnitine status of the body, particularly in those individuals consuming foods with minimal L-carnitine content and/or inadequate supply of micronutrients caused by certain forms of nutrition or changed eating habits.

E.4.1 L-carnitine Status in the Elderly

In addition to lower energy demands, elderly populations may also change their eating habits. For example, meat consumption typically decreases with age. As a result of changed eating habits, the dietary intake of both L-carnitine and the nutrients required for biosynthesis L-carnitine is reduced. Decreased endogenous synthesis has also been shown by Leibovitz and colleauges (Leibovitz 1993). Moreover, a decrease of L-carnitine in various body compartments with age has often been described in literature (Karlic 2002, Izgut-Uysal 2003). Taken together, decreased intake, biosynthesis and internal storage capacity can result in reduced energy metabolism. However, this reduction in energy metabolism due to lower L-carnitine levels can be restored by supplementation. Research has shown that two months of Carnipure™ tartrate administration (2 g/d) partly reversed age-related changes of oxidative metabolism in elderly females (Lohninger 2003).

Skeletal muscles contain a high proportion of mitochondria, since energy requirements are high during work. Thus it is no wonder that skeletal muscles constitute the main reservoir of L-carnitine in the body and have an L-carnitine concentration at least 200 times higher than blood plasma. Muscle mass and muscle strength tend to decrease with age (Pistone 2003). This can lead to a reduction in physical ability and may cause adverse metabolic effects. Analysis of muscle samples of healthy humans of different age showed a drastic reduction of L-carnitine and acyl-L-carnitine in the older subjects (Costell 1993, Müller 2002). Costell theorizes that an alteration of the L-carnitine carrier in the muscle cell membrane is the reason for muscle L-carnitine reduction with age (Costell 1993).

In healthy adults, Carnipure™ supplementation has been shown to stimulate *in vivo* long chain fatty acid metabolism (Müller 2002, Wutzke 2004) which supports another study conducted by Pistone. One month of supplemenation with L-carnitine in subjects aged from 70 to 92 resulted in a significant increase in total muscle mass, compared with placebo. In addition, the total fat mass was also reduced (Pistone 2003).

E.4.2 L-carnitine Status in Vegetarians

A convergence of several issues has led to an increase in vegetarian products and a general increase in vegetarian practices. Some may avoid meat and animal products for health reasons, while others may take ethical considerations into account. Food scares and food safety issues connected to meat and animal products also compound the desire and demand for vegetarian products. As such, there has been a broadening range of vegetarian products that are being brought into the mainstream market. One nutrient that is almost totally devoid in a vegetarian diet, however, is L-carnitne.

The results of a clinical study conducted in lacto-ovo-vegetarians demonstrate that a dose of L-carnitine as low as 990 mg/day results in significantly increased plasma free and total carnitine concentrations compared to baseline levels (Fokkema *et al.*, 2005). Since a vegetarian diet provides only small amounts of L-carnitine, and is potentially low in some of the substrates required for endogenous L-carnitine synthesis (*i.e.*, lysine and methionine) (Krajčovičová-Kudláčková *et al*., 2000), there is a possibility that consuming a strict vegetarian diet may result in L-carnitine deficiency in some individuals. L-carnitine deficiency is defined as "a state of carnitine concentration in the plasma or tissues that is below the requirement for the

normal function of the organism" (Famularo *et al.,* 1997). Clinically, L-carnitine deficiency is characterized by low plasma concentrations of free L-carnitine, and low red blood cell and/or tissue levels.

In 2011, a study was conducted by Stephens et al 2011 to test the hypothesis that muscle carnitine uptake is elevated in vegetarians compared with that in nonvegetarians to maintain a normal tissue carnitine content. In contrast to the hypothesis, the study found that healthy vegetarian volunteers had a reduced capacity to uptake carnitine into the skeletal muscle, which is the main store of carnitine within the body. Therefore vegetarians have lower muscle total carnitine and reduced capacity to transport carnitine into muscle than do omnivores. It is theorized that vegetarians have lower muscle carnitine stores in order to conserve carnitine for other tissues. Thus, as carnitine stores are lower, it may be beneficial for populations following a vegetarian lifestyle to seek exogenous sources of carnitine in food and beverage products. From the study, it was concluded that additional investigations are required whether reduced muscle carnitine content in vegetarian volunteers has an effect on physiologic functions, particularly because the muscle carnitine availability is rate limiting for fat oxidation and carbohydrate flux during exercise.

E.4.3 L-carnitine Status in Athletes

As public health experts have recommended decreasing intake of red meat, some athletes wish to consume a carbohydrate rich diet and omit meat. In these cases, L-carnitine from dietary intake may be insufficient. Moreover, some athletes may also choose to replete their protein with protein based supplements, which increases urinary excretion of carnitine. This may also contribute to compromised carnitine status in athletes. Researchers have observed that high performance athletes, such as triathletes, who follow an omnivorous diet, may still have a lower than normal plasma levels. In athletes this has been attributed to higher excretion of L-carnitine via the kidneys (Luppa 1996), and via perspiration (Suzuki 1976, Arenas 1991). This loss may be followed by a decrease of L-carnitine in the active muscles which may not always be rapidly replenished (Cerretelli 1990).

Moreover, studies show that as exercises intensifies, the conenctration of acyl-L-carnitine within the muscle increases and the availability of free L-carnitine decreases (Van Loon 2001, Le Blanc 2004, Nüesch 1999). Decreased availability of free L-carnitine in the mitochondria may lead to a decreased capacity of all energy generating pathways.

E.4.4 L-carnitine for Energy and Weight Management

As one of the components for an energy generating pathway, L-carnitine is essential for the conversion of fat into metabolic energy. The primary function of L-carnitine is to shuttle long chain fatty acids across the mitochondrial membrane for beta oxidation.

With regard to weight management, the goal of any weight management program is typically to decrease body weight, while maintaining lean body mass. In order to maintain lean body mass, body fat stores must be decreased. Outside of intensive medical interventions, the only way to decrease body fat stores is to use long chain fatty acids for energy. Two independent studies have shown that L-carnitine supplementation leads to an increase in fatty acid substrate utilization, as evidenced by labeled fatty acids ingestion with subsequent labeled carbon dioxide

exhalation measurement (Müller 2002; Wutzke 2004). From these independent studies, it is clear that carnitine supplementation can have a beneficial effect with regard to fatty acid oxidation and energy generation.

For weight management initiatives which include body weight reduction, several clinical trials have shown that L-carnitine supplementation in conjunction with dietary modification and/or exercise can be beneficial. In one clinical trial, obese participants followed a low fat diet alone or the same diet supplemented with a high fiber cookie containing L-carnitine. Those who received the cookie lost more body weight and body fat than those who only followed the low fat diet (Kaats 1992). Another study, which included 100 obese adult volunteers, looked at the impact of L-carnitine supplementation on body weight and BMI. The L-carnitine supplement group had a 25 percent greater loss in body weight and their BMI dropped by 1.5 points (Lurz 1998). A study published in 2013 also documents the positive effects of L-carnitine supplementation on body weight reduction. Overweight adults who received both motivation training and L-carnitine supplement showed a statistically significant decrease in body weight (Odo 2013).

F. INFORMATION RELATED TO POTENTIAL IMPACT ON CONSUMER UNDERSTANDING AND BEHAVIOUR

In accordance with Section 3.3.3 – Nutritive Substances of the Food Standards Australia New Zealand *Application Handbook* (FSANZ, 2013b) the following information on the nutritional impact of the nutritive substance is provided:

1. Information to demonstrate consumer awareness and understanding of the nutritive substances in the food(s).
2. Information on the actual/potential behaviour of consumers in response to the proposed foods.
3. Information to demonstrate that the consumption of food(s) containing the nutritive substance will not adversely affect any population groups (e.g. particular age or cultural groups).

Each point is addressed in the Section that follows with reference to Section D where appropriate.

F.1 Information to Demonstrate Consumer Awareness and Understanding of the Nutritive Substance in the Food(s)

See Section D (p.86) for a full discussion on the estimated exposure and the potential impact on consumers. One purpose of using Carnipure™ crystalline and Carnipure™ tartrate as an ingredient in foods is to provide assistance to healthy people as part of their weight management or weight loss regimes.

In February 2007, Lonza conducted an online survey in 4 different jurisdictions (*i.e.*, U.S., Germany, United Kingdom, and Japan) to evaluate consumer awareness of L-carnitine. The survey was conducted by the German consumer research organization GfK (Gesellschaft für Konsumforschung). Of the total surveyed population (2,024 individuals), approximately 29% were aware or had heard about L-carnitine, 13% had previously used products containing L-carnitine, and 5% were actually using L-carnitine. Detailed results are provided in Appendix K (Lonza, 2007).

In December 2012, the German consumer research organization GfK conducted a representative survey in regard to the consumer awareness of L-carnitine and its health benefits on behalf of Lonza again. The representative online survey was done among more than 1000 German consumers who reported to at least be occasional consumers of any kind of dietary supplements and/or functional foods. L-carnitine was known among 43% of the respondents. Of those, 39% could correctly relate L-carnitine supplementation to beneficial effects for weight management, 27% to exercise and 20% to energy.

Further the survey compared the general recognition of L-carnitine with that of other health ingredients, (outside of vitamins and minerals). Here L-carnitine figures among the best known:

- L-carnitine 43%
- Inulin (a prebiotic fiber) 22%
- CLA (Conjugated Linoleic Acid) 10%

The pressrelease (Lonza 2013a) as well as detailed information are provided in Appendix K (the final report exists in German only (Lonza 2012), only parts of the data presented is provided in English (Lonza, 2013b)).

Currently, consumers may obtain facts about Carnipure™ crystalline and Carnipure™ tartrate from Lonza's website (Lonza, 2013c) or from product´s webpage "Carnipure™-for-you" (Lonza, 2013d).

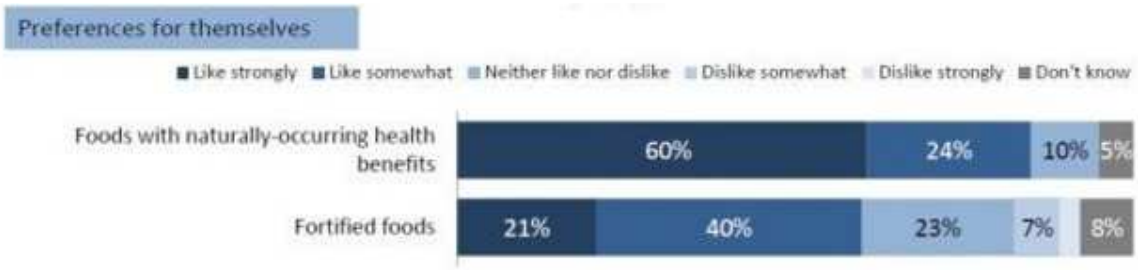
F.2 Information on Actual/Potential Behaviour of Consumers in Response to the Proposed Food(s)

Section D.1 to D.3 (pp.87) provide data about the actual/potential behaviour of consumers in response to the nutritive substance ingredient. The data demonstrate that L-carnitine in food is still a small market, even in countries where L-carnitine has been allowed to be used in food for many years. As indicated in section D.1/D.2 the majority of L-carnitine products are still be launched, sold and consumed in the category "food supplements".

It is anticipated that the proportion of consumers interested in consuming supplemental L-carnitine but do not prefer taking a food supplement be more likely to purchase an L-carnitine fortified food. Consumers taking already L-carnitine as a food supplement will most likely continue.

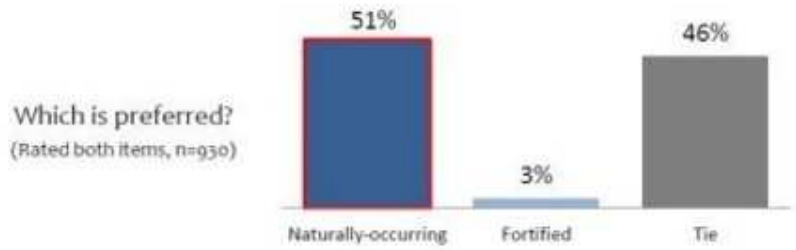
A survey done by Foodnavigator USA (Foodnavigator, 2013) shows the attitude of consumers concerning "foods with naturally-occuring health benefits" and "fortified foods. Consumers (n=1005) were asked how much would they like the idea of getting health promoting nutrients and food components from foods with naturally-occurring health benefits or fortified foods. Of those polled, 60% said they strongly like foods with naturally occuring benefits, while only one fifth said they strongly liked fortified foods (Figure F.2-1 (p.112)).

Figure F.2- 1 Favorite: Food with naturally-occurring health benefits or fortified foods?



Given the choice, slightly more consumers surveyed (51%) said foods with naturally occurring benefits are preferable to fortified foods while 46% were not fussed either way (Figure F.2-2 (p.112)).

Figure F.2- 2 Preference: "Health benefits" by natural food or fortified food?



The survey implies that the majority of consumers still prefer foods with naturally occuring health benefits. Therefore, independent of the addition of L-carnitine, it is the general attitude of consumers whether they are willing/open to consume fortified foods or not. Some do not prefer fortified foods at all, some choose fortified foods in specific circumstances and others are in turn truly fans of fortified foods. L-carnitine is no exception in the range of nutrients used to fortify food.
Finally far more than the majority of the products in the proposed food category will not include L-carnitine.

F.3 Information to Demonstrate that the Consumption of Food(s) Containing the Nutritive Substance Ingredient will not Adversely Affect Any Population Groups

Information on the safety of L-carnitine and L-carnitine L-tartrate in humans is discussed in detail in Section C (pp.32). It is not expected that L-carnitine (from Carnipure™ crystalline and Carnipure™ tartrate) will adversely affect any population groups given the long history of consumption of L-carnitine as a common component of the typical human diet, and the endogenous biosynthesis of L-carnitine as a normal body metabolite.

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Application A1102 – L-carnitine in foods

Further information required from the Applicant
(under s108 of the FSANZ Act)

Part A - Risk analysis using the best available scientific evidence

1 Has Lonza Ltd considered reports published after August 2013 in relation to TMAO and adverse health outcomes in humans? If so, please indicate which reports.
2 What conclusions about the safety of L-carnitine and its hemi-tartrate salt have you made from the additional information in these reports?

Executive Summary

This risk analysis provides some background information on L-carnitine, the concerns mainly raised by a publication from a research group from Cleveland in 2013, and an explanation of Lonza's internal knowledge management system for L-carnitine scientific information.

The discrepancy between the newly postulated role of trimethylamine N-oxide (TMAO) for atherosclerosis progression and long available data on the protective role of L-carnitine on cardiovascular diseases is discussed. Special emphasis is placed on the role of an enzyme, flavin-containing mono-oxygenase (FMO3), which is involved in the degradation of L-carnitine to TMAO.

In the next section, we present Lonza's initiatives in response to the Cleveland publications from 2013 and compare the resulting *in vitro* and mice data with the Cleveland data. In addition, own generated, confidential animal data, as well as very recent human data on endothelial function, are presented and discussed.

Because L-carnitine has many attributes in common with choline, the next section discusses data on choline and TMAO, and compares them with L-carnitine.

Very recent third party research in support of our own study findings is then discussed.

In conclusion, TMAO does not necessarily need to be considered a causative agent in human disease development and progression, but rather a marker of an underlying pathology. TMAO is not harmful but rather may protect against atherosclerosis, and L-carnitine consumption leading to increased TMAO levels may benefit those segments of the population who lack adequate levels of L-carnitine. This conclusion is supported by the outcome of other Regulatory initiatives around the world which are presented at the end of Part A.

Overview of scientific publications evaluated after 2013

Background: In April 2013, researchers from Cleveland first linked atherosclerosis and increased cardiac disease risks to trimethylamine N-oxide (TMAO), a degradation product of dietary quaternary ammonium compounds such as L-carnitine, betaine and choline (Koeth 2013). When these compounds are not completely absorbed in the intestine, gut microbiota can metabolize them to trimethylamine (TMA) which is then absorbed into the blood and further metabolized by liver flavin-containing mono-oxygenases (FMOs; FMO3 in particular) to TMAO (Bain 2005). TMA and TMAO presence in urine after administration of L-carnitine has first been described by Prof Strack in 1963 (Strack 1963).

In 2011, Cleveland researchers initially linked phosphatidylcholine derivatives and atherosclerosis. In a population of 2595 atherosclerotic patients, they showed that 3 metabolites of dietary phosphatidylcholine (choline, betaine, and TMAO) were independent predictors for the risk of a clinical vascular event. This correlation seems to be dependent on the microbial composition of the gut flora. Further, the apoE-/- mouse model, prone to develop atherosclerosis under high fat diet, showed also a correlation between TMAO levels, FMO, and atheroma (Wang 2011).

For more than 20 years, Lonza has maintained a large internal data base of scientific publications on L-carnitine, which not only contains final reports of all Lonza-initiated scientific studies, confidential expert opinions and other internal data, but also third party publications. All publications are electronically available and accessible to Lonza staff globally. We continuously monitor new 3rd party research activities from PubMed and other scientific data bases. Since 2013, special focus has been put on publications around L-carnitine and TMA(O). Besides the two publications referred to by FSANZ (Zheng 2016; Obeid 2016), additional publications by the Cleveland researchers (eg Tang 2014; Tang 2015; Koeth 2014; Hartiala 2014; Brown 2014; Shih 2015; Serban 2016), and many other publications (referred to below) have been studied.

A PubMed search on "**trimethylamine and atherosclerosis**", conducted in November 2016, found 70 results, most of which present TMAO as a candidate risk factor for cardiovascular disease and other adverse health outcomes. Dysregulated TMAO levels have been associated with renal disease, neurological disorders and cancer (Tang 2015; Chhibber-Goel 2016).

However, the relation between TMAO and chronic disease can be confounded by several factors, including kidney function, the gut microbiome, and flavin-containing monooxygenase 3 (FMO3) genotype. Thus, TMAO does not necessarily need to be considered a causative agent in human disease development and progression, but rather a marker of an underlying pathology. Importantly, dietary sources of TMAO have beneficial health effects and provide nutrients that have critical roles in many biological functions. Pre-emptive dietary strategies to restrict TMAO-generating nutrients as a means to improve human health warrant careful

consideration and may not be justified at this time. The following section discusses the most relevant publications on these topics.

To date, information on the potential role of **TMAO** on the pathophysiology of atherosclerosis comes from association studies (e.g. Wang 2011; Koeth 2013; Hartiala 2014). The speculated mechanism for the disease initiation by TMAO would be by foam cell formation as supported by an increase in CD36 (LDL-receptor) and SR-A1 receptors (Wang 2011). If this is true, TMAO would act as a reactive oxygen species (ROS) producer, leading to a decrease in nitric oxide and endothelial cell function, ultimately responsible for foam cell formation (Jairam 2012).
However, based on what is known on its function at the molecular level, it is unlikely that TMAO is the driver, nor the initiator of atherosclerosis. Indeed, TMAO has been shown to act as a chemical chaperone, possibly facilitating protein folding and tissue remodelling, a step required in the lesion regression (Mello 2003). In addition, TMAO has been reported to mitigate stress and reduce apoptosis in epithelial cells challenged with abnormal protein aggregation (Gong 2009). This means most likely TMAO is a marker but not a cause for increased CVD risk.

Several research groups found **FMO3** to play a crucial role in the regulation of circulating blood TMAO levels and in the promotion of atherosclerosis and cardiovascular disease in mice (Schugar 2015). Interestingly, recent studies suggest that FMO3 may also directly, and independently of TMAO levels, induce hyperglycemia, hyperlipidemia and atherosclerosis in mice (Shih 2015). Given that hepatic FMO3 expression is under the negative control of insulin, hepatic FMO3 gene expression is elevated in obese and insulin resistant mice and, to a lesser extent in obese and patients with diabetes (Miao 2015).

Interestingly, pollutants such as dioxin-like PCBs were found to increase FMO3 expression in mice, resulting in increased plasma TMAO levels (Petriello 2016). In addition, FMO3 activity can be influenced by hormones and food restriction (Cashman 2004).

The presence and absence of choline to total parenteral nutrition (TPN), bypassing the intestine, was studied in male Sprague Dawley rats. Compared with animals treated with a control diet, TPN plus choline in rats caused a 3-fold increase in hepatic microsomal FMO activity. Compared with control animals receiving standard chow, rats administered TPN plus choline for 5 days showed a decrease in urinary excretion of catecholamines (Cashman 2004).

A study investigating the association between FMO3 levels and risk of stroke showed that loss of function mutants in FMO3 play a major role. Heterozygote 158Glu/Lys (OR = 6.110, P b 0.001) and 308Glu/Gly (OR = 6.000, P = 0.006) genotypes increased the risk of stroke by 6 times in hypertensive subjects. This implies that decrease in FMO3 level may result in increased risk of stroke in the hypertensive subgroup. This is explained by decrease in catecholamine production, a known substrate for FMOs (Türkanoğlu Özçelik 2013). Catecholamines modulate heart rate and blood pressure and it has been reported that

decrease in their levels leads to high blood pressure, one of the risk factors for stroke. TMA - being also a substrate for FMO3- will accumulate and lead to decrease in TMAO levels in these heterozygote patients.

The homozygotes wild type 158Glu/Glu and 308Glu/Glu had 6.2-fold and 4.8-fold higher risk of ischemic stroke in obese subgroup. This is contradictory to the heterozygote results suggesting an effect on decreased FMO3 activity on ischemic stroke. However, the role of FMO3 can be different in hypertensive and obese patients. In obese individuals FMO3 has been shown to be over-expressed. Increased FMO3 activity would result in higher production of TMAO which would overcome the ROS formation by its anti-oxidant property. In this case, TMAO would have a protective effect and prevent the initiation of foam cell formation by ROS. This hypothesis is supported by the published data around the anti-oxidant role of TMAO (Wei 2008).

FMO3 is regulated by the transcription factor FXR and its ligands (e. g. bile acids). FXR activation has been shown to prevent vascular calcification which leads to plaque rupture, the last step in the thrombotic event (Miyazaki-Anzai 2010). Increased FXR levels result in increased FMO3 and TMAO levels (Türkanoğlu Özçelik 2013). This argues again against TMAO increased levels being the driver for CVD events at least when it comes to the final step of thrombosis.

Homologs of FMOs are also present in bacteria, for example in *Methylocella silvestris* where it is annotated as trimethylamine monoxygenase (TMM). In addition to the production of TMAO by mammalian hepatic FMO3, commensal or pathogenic bacteria could also potentially oxidize TMA to TMAO and thus contribute towards dysregulated TMAO levels in the human body (Chhibber-Goel 2016).

Another PubMed search on "**L-carnitine and atherosclerosis**" also conducted in November 2016 resulted in 148 hits, although the current link between L-carnitine and atherosclerosis is via TMAO (and should therefore result in a very similar number of hits as the previous "TMA and atherosclerosis" search). The big difference in the number of results can be explained by a different line of research, directly assessing the effect of L-carnitine on atherosclerosis.

There is extensive literature available on the **positive effects of L-carnitine on heart health** in general. A recent systematic review of 13 controlled trials in 3629 patients, involving 250 deaths, 220 cases of new heart failure and 38 recurrent heart attacks, found that L-carnitine was associated with a 27% reduction in all-cause mortality, a 65% reduction in ventricular arrhythmias and a 40% reduction in the development of angina as well as reductions in infarct size (DiNicolantonio 2013).

Another very recent systematic review and meta-analysis concluded that oral L-carnitine supplementation significantly reduces plasma levels lipoprotein a, which has atherogenic and thrombotic properties (Serban 2016). Surprisingly Dr Hazen who is part of the Cleveland

research group, also co-authored this systematic review article. They also reveal that “other studies have suggested beneficial effects of L-carnitine supplementation on inflammatory parameters, in the secondary prevention of CVD, in diabetes mellitus, on serum lipid profile in hemodialysis patients, for adults with end-stage kidney disease on hemodialysis or for patients in maintenance hemodialysis” (Serban 2016).

When 47 CAD patients were supplemented with either 1000 mg L-carnitine or placebo for 12 weeks, researchers found significant improvements in their lipid levels, which were attributed to the antioxidant ability of L-carnitine (Lee 2016).

Another mini review discussed the discrepancy of the observed relationship of TMAO and atherosclerosis on the one hand, and the beneficial properties of L-carnitine against metabolic diseases, including cardiovascular diseases on the other hand, concluding that more research is needed to better understand the root causes for the association between red meat consumption and cardiovascular risk (Ussher 2013).

In an older experiment, rabbits were fed on a hypercholesterolemic diet alone or in addition to propionyl-L-carnitine, and showed significant reduction in lipoprotein parameters, plaque thickness and macrophage-derived cells as compared to control (Spagnoli 1995).
In a different study, hypercholesterinemia was induced by feeding rabbits with 2% cholesterol-enriched diets for 28 days. L-carnitine deficiency was induced by D-carnitine infusions for 28 days. Four weeks treatment with L-carnitine prevented the progression of atherosclerotic lesions in these rabbits (Sayed-Ahmed 2001).

These findings are in stark contrast to the conclusions made by Hazen and Koeth on L-carnitine’s role on atherosclerosis.

Research by Lonza

In addition to literature monitoring, Lonza has initiated its **own studies** in *in vitro* systems and in mice.

We have performed experiments looking at the direct effect of TMAO on macrophage foam cell formation and on lesion progression in ApoE$^{-/-}$ mice expressing human cholesteryl ester transport protein (CETP), a key enzyme in reverse cholesterol transport (RCT) normally lacking in mice.

First, we showed that increasing TMAO levels up to 10-fold the Cmax as reported in humans, did not affect the foam cell formation *in vitro*, an obligatory step in the atherosclerosis disease progression (Bellamine 2014).

Second, we used an improved version of the ApoE$^{-/-}$ mouse model, expressing human CETP. CETP plays a major role in the cholesterol re-cycling in humans and its inhibition has been studied as a target for atherosclerosis treatment. Different doses of dietary L-carnitine

were used, referring to doses typically used in dietary supplements or functional foods in humans, leading to different levels of TMAO in an attempt to establish a dose-response curve (Collins 2014).

We found that TMAO levels inversely correlated with the lesion size. Significantly reduced aortic lesion size was observed at high levels of TMAO. These effects were independent of lipid changes. The doses of L-carnitine were chosen to correspond to the recommended dietary L-carnitine supplementation in humans, and lead to increased levels of TMAO which may provide protection against atherosclerosis development. The results of the Lonza-initiated studies are published by Collins 2014.

These observations suggest that TMAO may play a protective role in atherosclerosis development by reducing the lesion formation. When the lesions start developing, TMAO levels would be up-regulated by a compensatory mechanism (possibly by increasing FMO expression levels).

Given the difference in atherosclerotic lesion development in response to L-carnitine between that reported by Koeth (2013) and the present study, it is important to note several differences in how the studies were conducted.

There is a significant difference in the **dietary composition** between the two studies. The current study uses doses of L-carnitine (87 or 352 mg/kg for mice) that are, if converted, in the range used as dietary supplements in humans; and an atherogenic diet to assess lesion development. Koeth used an L-carnitine dose much higher, approximately 1700 mg/kg/day based on average mouse consumption of 1.3% L-carnitine as part of a normal chow diet. The difference in normal chow versus atherogenic diet alone can be seen in the level of aortic root lesion.

Furthermore, increased **lesions** above the baseline were observed by Koeth 2013 in only 3 out of 11 animals, making the difference significant. It is worthy to note that no dose-response was evaluated (Koeth, 2013)**.** Koeth observed lesion sizes of 1-5 x 10^5 μm^2, which is a low level of lesion development. The current study shows lesion sizes of 0.6-1 x 10^7 μm^2, a more advanced stage of lesion development.

While both studies used **ApoE knockout mice**, Koeth 2013 used female mice and the Lonza study used male mice that express human CETP.

Many rodent models have been developed to imitate several aspects of human CVD risk factors (SHR for hypertension, ApoE-/-, ApoB100 and ApoE-/- Leiden-CETP for atherosclerosis and ischemia-perfusion injury for myocardial infraction). In the apoE-/- where the APOE lipoprotein is absent, VLDL and LDL are not transported to the liver to be degraded, but rather accumulate in the circulatory system. LDL is then oxidized and is taken up by macrophages to initiate foam cell formation, the initial step in atherosclerosis (Jairam 2012).

For Lonza's study, a model with CETP expression was chosen because of its significant role in reverse cholesterol transport and atherosclerosis progression.

Furthermore, it has been reported that FMO is differentially regulated in **male and female** rodents (much higher levels in females) because of the androgen repression effect on FMO expression (Falls 1995, Falls 1997). This is not the case in humans (Bennett 2013). Since this model is prone to develop atherosclerosis under a fat-rich diet and given the differences in FMO gene regulations between humans and rodents, the observation reported by Koeth 2013 might not reflect the human situation. This and other models showed only limited success for atherosclerosis development as it takes place in humans given the differences in the lipid metabolisms mentioned above (de Haan 2009; Ng 2012).

In humans, FMO3 is involved in different pathways dependent on the substrates. Decreased FMO3 activity can lead to alteration of some substrates such as catecholamine production and an increase in blood pressure, a risk factor for CVD. However, increase in FMO3 activity can lead to an increase in TMAO production to overcome ROS formation and atherosclerosis initiation as seen in obese patients (Figure 2). Therefore, the correlation observed between high cardiovascular events and high levels of TMAO can be misleading when it comes to the implication of TMAO in the CVD risk. Althought a correlation between TMAO and CVD was determined; there is no evidence yet for the causality between TMAO level and CVD increased risk.

Further information required from the Applicant
(under s108 of the FSANZ Act)

Confidential unpublished information

Unpublished animal study data

See CCI section on Part A (1).

Non-confidential Summary:

Animal data are presented that show that supplementation with moderate amounts of L-carnitine, comparable to the doses used for human functional food products, only slightly increase TMAO levels in plasma.

Unpublished human study data

See CCI section on Part A (2).

Non-confidential Summary:

The results of a recently finished human study (ClinicalTrials.gov identification number NCT02635594) are presented here. Currently, a manuscript is in preparation for submission to a peer-reviewed journal. While for the whole study collective of healthy adults, L-carnitine was not found to have an effect on fasting or postprandial endothelial function, supplementation with L-carnitine led to a positive effect in two different subgroups: 1) on fasting endothelial function in the subgroup with impaired endothelial function at baseline, 2) on postprandial endothelial function in the subgroup where a high-fat meal had a negative effect.

Bioavailability of L-carnitine will be discussed in in detail in the Part B response, but it is clear that if there is a saturable uptake mechanism, smaller doses will lead to only smaller increases of TMA and TMAO, whereas high doses, where the majority of L-carnitine travels unabsorbed through the intestines, will lead to considerably higher TMAO increases.

TMAO and choline/phosphatidylcholine

Another dietary precursor of TMAO is **choline/phosphatidylcholine**. For instance, choline is required for the maintenance of cell membrane structural integrity, for supporting cholinergic neurotransmission, and for donating methyl groups in a number of biosynthetic reactions. Inadequate choline intake can result in non- alcoholic fatty liver disease (NAFLD) and/or haemorrhagic kidney necrosis (Chhibber-Goel 2016). A good overview of recent studies on the relationship of phosphatidylcholine and TMAO is provided in the review by Blesso 2015.

The European Food Safety Authority (EFSA) has recently established Dietary Reference Values for choline, published in September 2016 (EFSA 2016). In 2011, EFSA authorized three health claims on choline, on maintaining normal homocysteine metabolism, normal lipid metabolism and normal liver function (EFSA 2011). This shows that there seems to be no concern from choline intake than there is from L-carnitine intake, which is surprising as the TMAO level increases following foods rich in either precursor are very similar.

Among 46 different foods including fish and seafood, dairy, meat, fruits and vegetables, only the ingestion of fish and seafood led to significant increases in urinary TMAO content (Zhang 1999). There seems to be some correlation between plasma and urinary TMAO content, because the same observation has been confirmed in urine, and extended to plasma levels: a crossover feeding trial in 40 healthy young men was conducted with meals containing TMAO (fish), its dietary precursors, choline (eggs) and carnitine (beef), and a fruit control. Fish yielded higher circulating and urinary concentrations of TMAO (46-62 times; $p < 0.0001$) and TMA (8-14 times; $p < 0.0001$) than eggs, beef, or the fruit control. Circulating TMAO concentrations were increased within 15 min of fish consumption, suggesting that dietary TMAO is absorbed intact and independent of gut microbes. Consumption of fish yielded substantially greater increases in circulating TMAO than eggs or beef. The higher Firmicutes to Bacteroidetes enrichment and less gut microbiota diversity among men exhibiting a greater response to dietary TMAO precursor intake indicates that TMAO production is a function of individual differences in the gut microbiome (Cho 2016). These data suggest that diet and gut microbiota primarily determine TMAO levels, that L-carnitine levels do not always correlate with TMAO levels and that the beneficial effects of fish consumption on cardiovascular outcomes could be related to the elevated level of TMAO (500-fold) which also supports the conclusions based on the Lonza mice study.

In contrast, German researchers did not find associations of meat, egg, or fish consumption with TMAO, choline or betaine concentrations in 271 healthy adults from the general population with the exception of a positive correlation between dairy food consumption and TMAO concentrations (Rohrmann 2016). From these data it is rather unlikely that habitual diet is strongly linked to plasma TMAO levels.

In 122 women aged 60 and above, a recent observational study found that dietary habits and patterns influence plasma L-carnitine levels. Low intake of whole grain cereals and legumes,

for example, was associated with higher L-carnitine levels whereas interestingly, higher consumption of meat and fish did not significantly correlate with higher L-carnitine levels. Moreover, people with lower than the adequate intakes of choline (<425 mg/d) had higher concentrations of plasma L-carnitine. Plasma L-carnitine was inversely correlated with the intake of betaine, but did not show any statistically significant correlation with TMA or TMAO levels. However, the researchers found a correlation between TMAO concentration and elevated homocysteine levels, which is another marker of cardiovascular disease (Malinowska 2016).

After two weeks of eeucalroic control diet, nineteen healthy, non-obese males were provided with a hypercaloric (+ 1000 kcal/d), high fat diet (55% fat) for four weeks, which was found to significantly increase plasma TMAO levels (Boutagy 2015).

One year of daily administration of different L-carnitine concentrations (0, 1, 2 and 5 g/L) via drinking water in Fischer 344 rats did not lead to any adverse effects on the gastrointestinal or vascular system such as increase in formation of lesions in the colon or aorta, respectively (Empl 2015).

The impact of obesity, lifestyle-induced weight loss, and bariatric surgery on plasma levels of TMAO was investigated in 34 obese individuals. TMAO levels were not elevated in obese patients or reduced by lifestyle interventions but increased twofold after bariatric surgery. TMAO was not significantly correlated with visceral adipose tissue (p-value= 0.001). After surgery, TMAO plasma levels doubled from 4.4 to 10.5 µM (p-value< 0.001) regardless of surgical method (Trøseid 2016). These findings support our study in which high TMAO levels were indicative of a protective effect of TMAO on atherosclerosis and thereby, CVD risk. Bariatric surgery is also associated with a decreased risk of CVD, and the elevated TMAO levels after surgery may point to this protective effect.

Also the severity of related symptoms such as non-alcoholic fatty liver disease (NAFLD), diabetes or kidney disease seems to correlate with high TMAO levels (Dambrova 2016; Kim 2016; Ufnal 2015). Chen et al have shown though that there was an association but not a causal relationship between high TMAO levels and severity of NAFLD (Chen 2016).

Third party research supporting Lonza's findings

After presentation of Lonza's initial findings and conclusions at the Experimental Biology meeting in San Diego on 28 April, 2014 (Bellamine 2014), several scientists started research in the same direction.

According to Fukami 2015, L-carnitine consumption by hemodialysis patients, lead to increased TMAO blood levels, and correlated with a decrease in vascular injury markers. Mente 2015 found no significant association between carotid intimal medial thickness and L-Carnitine levels in a multiethnical population sample in Canada.

Kaysen reported that serum TMAO concentrations were levels markedly elevated. They directly correlated with biochemical markers of nutritional status and inversely correlated with markers of inflammation (hCRP). There was no significant association between serum TMAO concentrations and all-cause mortality, cardiovascular death, or hospitalizations in hemodialysis patients (Kaysen 2015).

In 339 patients with suspected coronary artery disease, plasma concentrations of TMAO were higher in the subgroup of patients with diabetes compared to euglycemic patients, as well as in patients with metabolic syndrome compared to patients without metabolic syndrome. Plasma concentrations of TMAO or choline increased significantly with decreasing renal function. They were associated with neither a history of myocardial infarction nor the angiographically assessed presence of coronary heart disease, nor incident cardiovascular events during 8 years of follow-up (Mueller 2015). The researchers concluded that plasma levels of TMAO are confounded by impaired kidney function and poor metabolic control but are not associated with the history, presence or incidence of symptoms or events of coronary heart disease.

In 220 subjects that participated in lifestyle intervention program, TMAO levels were positively associated with carotid intima-media thickness (cIMT). Although all of the other risk factors were mitigated by life-style intervention, TMAO levels were not changed but rather were associated with increased cIMT independently of established cardiovascular risk markers. In addition, cIMT decreased significantly ($p = 0.0056$) only in subjects in the tertile with the largest decrease of TMAO levels (>20%) (Randrianarisoa 2016). cIMT is used to detect the presence of atherosclerotic disease in humans and, more contentiously, to track the regression, arrest or progression of atherosclerosis. This is in agreement with our mice study that showed a reverse correlation between TMAO levels and atheroma lesions, suggesting that TMAO is up-regulated in response to the lesions and may be involved in the regression of these lesions.

Lonza's conclusions about the safety of L-carnitine and L-carnitine L-tartrate

Initial remark: L-carnitine L-tartrate is a salt form of L-carnitine, which in solution dissociates into L-carnitine and L-tartaric acid. Because there have been no safety concerns about the intake of L-tartaric acid since a positive evaluation by the EFSA in 2003, and another more recent risk assessment from the Norwegian Scientific Committee for Food Safety VKM in 2015, we have focused our conclusions on L-carnitine and assume that there will not be any unexpected, negative effects due to the uptake of L-tartaric acid.

From the literature review above and Lonza's own initiated studies, we can conclude that TMAO is not harmful but rather may protect against atherosclerosis, and that L-carnitine consumption leading to increased TMAO levels may benefit those segments of the population who lack adequate levels of L-carnitine. This conclusion is supported by the outcome of other Regulatory initiatives around the world.

Regulatory decisions on L-carnitine since 2013 on a global level

- In **Europe**, L-carnitine already represents an authorized nutrient according to "Commission Directive 1999/21/EC on dietary foods for special medical purposes". Also the new Regulation (EU) No 609/2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control, that came into force in summer 2016, lists L-carnitine. In addition, it is mandatory to add L-carnitine to infant formula.
- **France** and **Spain** have released national legislation on dietary supplements in 2015 and 2013, respectively.
- In **Italy**, the Ministry of Health has confirmed to maintain the upper dose of 1000 mg L-carnitine per day in its revision of June 2016 (http://www.salute.gov.it/imgs/c_17_paginearee_1268_listafile_itemname_4_file.pdf)
- In **Norway**, the Scientific Committee for Food Safety, VKM, have published a "Risk assessment of "other substances" –L-Carnitine and L-Carnitine-L-tartrate" in 2015 and concluded that a dose of 1500 mg of L-carnitine per day, which is equivalent to a dose of 2250 mg of L-carnitine-L-tartrate per day, is unlikely to cause adverse health effects in adolescents (14 to <18 years old) and adults (≥18 years), whereas intake at this level in children (10 to <14 years) may represent a risk of adverse health effects. The tartaric acid exposure from this dose of L-carnitine-L-tartrate is unlikely to cause adverse health effects. (VKM 2015)
- In its recent revision of special food law of February 2014, **Switzerland** has listed L-carnitine and L-carnitine L-tartrate in Annex 13 with an approved daily dose of 1000 mg (https://www.admin.ch/opc/de/classified-compilation/20050168/index.html)
- In **Brazil**, L-carnitine L-tartrate has been approved by ANVISA for the use in functional foods in 2014
- In **China**, the approved dose range of L-carnitine and L-carnitine L-tartrate of 600 – 3000 mg (based on L-carnitine) for beverages was extended to 100 – 3000 mg in June 2016. Lower dosages are expected to open up the market for many more companies or products

(http://www.nhfpc.gov.cn/sps/s7890/201606/125c3d8fa2034de3b7d52a82608709d2.shtml)

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Application A1102 – L-carnitine in foods

Further information required from the Applicant (under s108 of the FSANZ Act)

Part C - Delivering the stated purpose

5 Please provide any additional information or evidence that would support your general stated purpose for adding L-carnitine to certain foods i.e. to maintain the L-carnitine status in the body.
6 Please state the specific purpose(s) of the additional L-carnitine in foods for each of the 4 target groups you have identified.
7 Please provide any additional information or evidence that would support your stated purpose for adding L-carnitine to certain foods for each of the 4 target groups.

Stated purpose for adding L-carnitine to certain foods

As stated in Chapters A3 and D1/D2 of our original application, Lonza intends to market Carnipure® crystalline and Carnipure® tartrate as a food ingredient suitable for addition to various food categories, namely dairy products (excluding butter and butter fat), confectionary, cereal and cereal products, foods intended for particular nutritional uses, non-alcoholic beverages and sport gels. These categories were chosen, taking food consumption data, preferences of the respective target groups, technological feasibility and global market trends into account, which are further elaborated on below.

While most plants contain minimal amounts, reasonable amounts of L-carnitine can only be found in foods of animal origin. The addition of L-carnitine to other types of foods, such as dairy or beverages, will give consumers the opportunity to maintain L-carnitine levels from a wider variety of foods.

Because there is no consumer data on the preferred food carriers for L-carnitine across the world, we trust that general consumer / health ingredient data also hold true for L-carnitine. Consumer research from Australia confirmed the findings of similar studies in Europe, Canada and America, which found that food carriers for functional ingredients are preferred which *per se* are perceived as healthy (Williams 2008).

Demand for fortified or functional foods is being driven worldwide by an ageing society, increasing prevalence of lifestyle-related diseases and growing interest among consumers in health and wellbeing (Vella 2014). Socioeconomic evolution and food consumption trends have resulted in food increasingly being a “want” rather than a “need”, with a balance between indulgence and health desire. However, consumers will not compromise on taste or product quality for health products, and price is also an important determinant in repeat purchase. These factors, along with rising health care costs, are key reasons why opportunities exist for functional foods in general (Shortt 2016), and for L-carnitine, as specified below.

From a technological view, L-carnitine can be added to almost every type of food because it is heat and pH stable and water soluble, and does not contribute texture, colour or taste. No

additives, stabilizers or other ingredients are needed to stabilize foods fortified with L-carnitine (internal Lonza data and customer feedback as well as Rigault 2008).

Functional foods must demonstrate their effects in amounts that can normally be expected to be consumed in the diet (Cox 2008). L-carnitine dosages applied in single-ingredient dietary supplements around the globe are typically around 500-1000 mg, depending on the specific regulatory environment.

As specified in Chapter C 1.2, L-carnitine is taken up from the gut via the saturable OCTN2 transporter. Passive diffusion does not play a big role at lower dosages, but with increasing dose this pathway is gaining importance (Rebouche 2004). This means there is an inverse relationship between exogenous supplementation and bioavailability, i.e., fairly high oral doses from dietary supplements are not fully absorbed and thus a significant amount of L-carnitine remains in the gut. Since a food matrix in general delays the gut transit time which in the case of L-carnitine in turn can increase the portion that is taken up, addition of L-carnitine to foods that are naturally low in L-carnitine, can increase its bioavailability.

In addition to the benefits for bioavailability from a food matrix described above, the research group around Paul Greenhaff and Francis Stephens from the University Nottingham, UK, conducted several human clinical trials in an attempt to better understand the uptake mechanisms of L-carnitine from the plasma into the muscle (Stephens 2006; Stephens 2007; Wall 2011; Stephens 2011). Approximately 98% of L-carnitine in the human body is located in the skeletal muscles. Apparently, in combination with carbohydrates, L-carnitine uptake from plasma to the muscles can be improved.

In one of the few human studies that did not apply L-carnitine in dosage form but in a food matrix, Stephens (2013) achieved a 20% increase in muscle L-carnitine content after 12 weeks of twice daily supplementation of an orange-flavoured beverage containing 1.36 g of L-carnitine plus 80 g of carbohydrate in order to stimulate insulin-mediated muscle L-carnitine transport. At the same time, L-carnitine prevented an 18% increase in body fat mass associated with carbohydrate supplementation alone in healthy young men.

Most other human clinical trials involving L-carnitine have applied the ingredient as a supplement, mainly in capsule form and at high dosages. The intention has always been to ensure that the subjects were saturated with L-carnitine after the supplementation period. Only recently, studies with lower dosages became available (eg Samimi 2015; Odo 2013; Mahdavi 2015; Lee 2015; Biotesys unpublished 2016 [CCI]). From a science perspective, with the application of capsules, potential food matrix effects and other confounding factors can be excluded and intake can be better standardized. In addition, as human clinical trials represent large investments, the results shall be applicable as broadly as possible, and this can easily be achieved by providing pure L-carnitine.

Unpublished study data

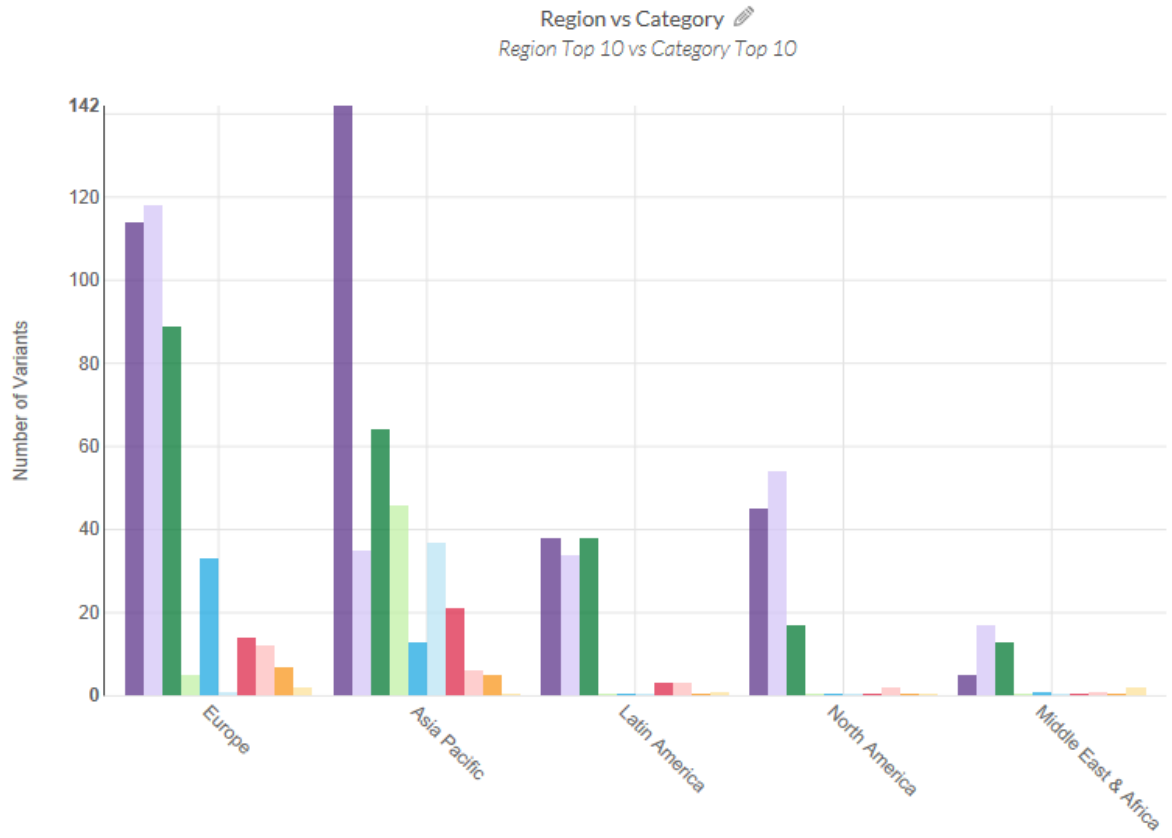
See CCI section on Part C (1).

Non-confidential study summary:

In this small human pilot trial, healthy adults were supplemented with several different single doses of L-carnitine, in random order, each separated by a wash-out period. Plasma levels after each intake occasion were analyzed during 24h. Already the lowest dose tested was found to maintain plasma L-carnitine levels, which in the placebo group (no dietary L-carnitine intake) was found to decrease.

With these results, there is a rationale for the addition of low dosages of L-carnitine to foods. Therefore we had proposed a dose of 250 mg per serving in table D.2.1. of Lonza's original application.

A new Mintel GNPD keyword search for "carnitin*" / new product launches between January 2014 and October 2016 per region and product category shows that the trend identified and described in Lonza's original application of a high number of new L-carnitine containing functional food product launches in the Asia Pacific is continuing, especially in the categories of beverages and dairy (see *Figure 2*; Mintel, www.gnpd.com). This also means that there is still a demand for L-carnitine in functional food form.



Highlight dataset (x) View Products

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Highlight trends over rows
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Below average — Above average

Sort by A-Z	Europe	Asia Pacific	Latin America	North America	Middle East & Africa	Total Sample
Other Beverages	114	142	38	45	5	344
Sports & Energy...	118	35	34	54	17	258
Healthcare	89	64	38	17	13	221
Hot Beverages	5	46	0	0	0	51
Snacks	33	13	0	0	1	47
Dairy	1	37	0	0	0	38
Juice Drinks	14	21	3	0	0	38
Water	12	6	3	2	1	24
Carbonated Soft...	7	5	0	0	0	12
Breakfast Cereals	2	0	1	0	2	5
Total Sample	404	386	119	118	41	1,068

Figure 2: Number of new product launches in the regions between January 2014 and October 2016, containing L-carnitine. Europe and Asia Pacific show highest numbers of new product in various drinks, dairy and snacks compared to the Americas, Middle East and Africa. [please note: "healthcare" in this table refers to dietary supplements] (Note: Figure 1 is CCI)

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Specific purpose(s) of the additional L-carnitine in foods for each of the four target groups

The introduction to Chapter E4 in the original application provided an overview of L-carnitine biosynthesis, as well as L-carnitine requirements beyond those met by endogenous synthesis.

Looking at the function of L-carnitine in the body and its central role in energy generating processes, it becomes clear that additional intake of this conditionally essential nutrient can be beneficial in situations where either energy demands increase, dietary intake is reduced, biosynthesis is reduced, or excretion is increased.

In an attempt to define distinct target groups who do have either increased energy demands, reduced dietary intake of L-carnitine, reduced biosynthesis or increased L-carnitine excretion, scientific literature on L-carnitine's effects for different healthy populations, life stages, lifestyle behaviours, and dietary habits was taken into consideration.

Combined with the main markets of L-carnitine outside of Australia, this has led to the identification of the four main target groups of athletes/sports nutrition; weight management; elderly and vegetarians.

With regard to L-carnitine's central role in energy metabolism, the benefit of L-carnitine-fortified foods for **athletes** is quite obvious. Athletes have higher energy demands than the average population, and a higher excretion of mainly carnitine esters via sweat and urine. Carnitine-fortified foods will help athletes replenish their L-carnitine stores (biochemical effect), and contribute to more efficient exercise recovery (consumer benefit).

There are many different dietary concepts for reducing body **weight**, with varying ratios of macronutrients (Strychar 2006; Johnston 2014)**.** Both low-fat and low-calorie diets come with recommendations to eat low fat proteins such as beans, lentils, fish and poultry, rather than meat, which is rich in L-carnitine. Therefore L-carnitine fortified foods of low energy density can help to maintain L-carnitine levels during dieting (biochemical effect), while at the same time help improve lipid profiles, body weight and BMI (body mass index) (consumer benefit). Even on a fat-reduced diet, L-carnitine can help with energy generation via its buffering function of Coenzyme A (biochemical effect).

For the **elderly,** typically, as people age, they take up less L-carnitine with their diet, and the endogenous synthesis is also reduced - this has been discussed in Lonza's original application. At a certain age, people start suffering from multiple age-related deficiencies in various organs, tissues or body compartments (heart, muscle, joints, brain etc). L-carnitine fortified foods can help restore L-carnitine levels in that age group (biochemical effect), which leads to multiple benefits related to energy metabolism (consumer benefit).

Vegetarians and especially vegans take up almost zero L-carnitine with their diet. Growth in plant-based milk imitates and other products for vegetarians can be noted, and interestingly these products are not only consumed by strict vegetarians but by a wider consumer base. While in vegetarian children, low dietary L-carnitine consumption may lead to severe growth retardation, adult vegetarians will be able to increase their L-carnitine levels (biochemical effect) and feel the benefit in certain situations with high energy demands such as after exercise (consumer benefit).

It is acknowledged that in order for L-carnitine to be used in a product to support a general level health claim (GLHC), the food-health effect would first need to be determined in accordance with Standard 1.2.7. It is noted that if a manufacturer chooses to explore a general level health claim then they will need to make a separate application to FSANZ or prepare a Scientific Literature Review (SLR) to meet the requirements of Schedule 6.

Stated purposes – additional evidence

From a market point of view, the order of priority of the four chosen target groups would be different to the order, in which they were presented in the original application. Sports nutrition and weight management are by far the most well-developed indications for L-carnitine for healthy people, followed by nutrition for the elderly and vegetarians/vegans. It is obvious though, that there is some overlap between the groups, eg elderly people who are exercising, people who exercise to lose weight etc.

This was also seen in an online consumer survey commissioned by Lonza at the beginning of 2016. An independent agency questioned 202 consumers in the UK aged between 18 and 35 who exercise at least three times a week about their attitudes and behaviour in terms of dietary supplement usage and, in particular, L-carnitine (Ingredient Communications 2016).

They found that 39% were current users of supplements containing L-carnitine, and that these respondents consumed L-carnitine predominantly for sports nutrition-related benefits such as exercise recovery, weight management, increased energy, muscle building and improved endurance, but also for cardiovascular health which would not be regarded as a main issue among that age group.

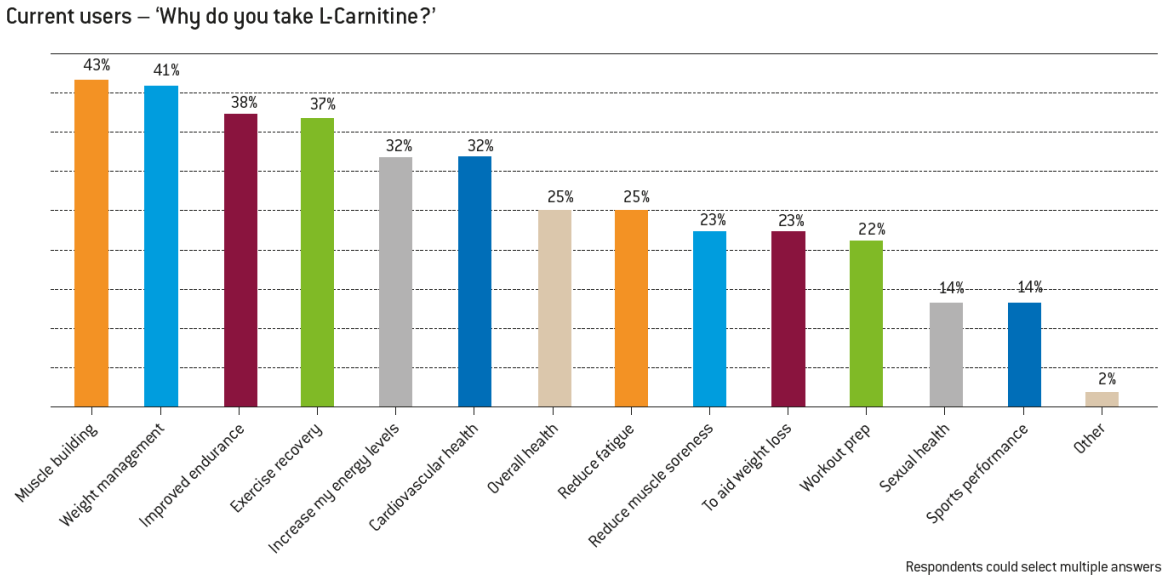

Figure 3: Expected health benefits and reasons why current users of L-carnitine products actually take these, as surveyed in the UK in spring 2016.

In the following, additional data is provided on the four target groups as distinct as possible.

Athletes

The Australian Institute of Sports (AIS) has developed an internationally very well-recognized classification system to rank sports foods and supplement ingredients into four groups (A, B, C, D) based on scientific evidence and other practical considerations that determine whether a product is safe, legal and effective in improving sports performance (AIS 2016). In 2011, based on the research conducted by the team of Prof Paul Greenhaff from Nottingham, UK, the AIS reclassified L-carnitine from a group C ingredient ("little meaningful proof of beneficial effects") to group B ("Deserving of further research and could be considered for provision to athletes under a research protocol or case-managed monitoring situation"). The Swiss Antidoping foundation followed shortly afterwards with the same reclassification. In June 2016 their supplement guide has moved under the responsibility of the Swiss Sports Nutrition Society, but the classification of L-carnitine remained group B (SSNS 2016).

Typically, health ingredients used in sports nutrition target direct performance effects. Although there is evidence that supplementation with L-carnitine to sports people can also support performance aspects, recent research has focussed on the beneficial effects of L-carnitine supplementation on recovery processes after exercise, which then indirectly may also have an effect on performance.

See CCI section on Part C (2).

Non-confidential Summary:

The results of a recently finished human study (ClinicalTrials.gov identification number NCT02635594) are presented here. Currently, a manuscript is in preparation for submission to a peer-reviewed journal. Supplementation with L-carnitine was found to result in less strength loss during the recovery period from strenuous exercise.

In addition, there are nine randomized double-blind placebo-controlled human intervention studies published in peer reviewed journals, all in support of L-carnitine for exercise recovery (Parandak 2014; Ho 2010; Spiering 2008; Spiering 2007; Kraemer 2006; Kraemer 2003; Volek 2002; Maggini 2000; Giamberardino 1996). The majority of studies were done in male volunteers of student age, but there are two studies that also included women of a different age group (Maggini 2000; Ho 2010). There were also variations in the duration of supplementation, ranging from 5 days to 4 weeks. Although the study subjects had various grades of training level, including untrained, healthy trained, recreationally active, weight trained subjects, all study participants, 104 in total, belong to a "healthy population". The results were conclusive in showing improved muscle oxygen consumption, less muscle soreness and reduced muscle damage, as well as improved antioxidative capacity. These data have triggered the development of various sport nutrition products including beverages, bars and gels with L-carnitine around the world. Some of them are already available in Australia.

As outlined in Chapter E 4.3, nutrition preferences of athletes vary. Bars, gels and other sports nutrition products fortified with L-carnitine may represent a viable option to exactly control the macronutrients in terms of protein/carbohydrate/fat ratio while at the same time adding other nutrients that may be beneficial in the athlete's specific situation. Consumption data from outside of Australia show that many athletes appreciate and make good use of such options (see Mintel GNPD).

Weight management

A very recent systematic review and meta-analysis on L-carnitine and weight management in adults based on 9 peer-reviewed human studies with a total (n) of 911 participants concluded that the L-carnitine-supplemented group lost significantly more body weight than the control group (Pooyandjoo 2016). Additional well-controlled human studies that have not been included in the Systematic Review article, with a supplementation of 250 mg – 4 g L-carnitine per day and a duration of 4 weeks to 6 months in various population subgroups of all ages (female and male, college students, adults, elderly, centenarians) have shown a significant reduction in body weight and BMI (Samimi 2015; Zhang 2014; Stephens 2013; Malaguarnera 2007; Pistone 2003). Both a human and a supportive animal study found L-carnitine supplementation to prevent an increase in body fat mass that occurred in the placebo group in the course of the 12 week study period (Stephens 2013; Bernard 2008).

Elderly

Chapter E 4.1 highlighted that eating habits and metabolic processes typically change with age. Older adults may not consume enough L-carnitine through the normal diet and may not synthesize adequate amounts due to decreased intake of precursors and cofactors.

Furtheron, chapter E 4.1 mainly focusses on muscle and oxidative metabolism in the elderly.

We have reviewed the potential benefit of L-carnitine fortification of foods with respect to benefits for the elderly. Additional information is provided on the relevant health issues associated with aging such as cardiovascular health, brain health, bone metabolism and age-related decrease in muscle mass. According to the Australian Institute of Health and Welfare AIHW, in 2016, 15% of the Australian population (3.7 million) were aged 65 and over. The proportion of older Australians is expected to grow—to 22% (8.7 million) by 2056 and to 24% (12.8 million) by 2096 (AIHW 2016).

Brain Health
On a functional level, memory loss has been associated with oxidative damage to lipids, proteins and DNA and by mitochondrial decay. L-carnitine has been linked to lipid oxidation and could have a protective effect in an aging brain. In animals, supplementation of L-carnitine reversed the age associated changes in lipid peroxidation and enzymatic antioxidant activity in various brain regions (Rani 2002). Lipid peroxidation was decreased while enzymatic antioxidant activity was increased with L-carnitine administration. Furthermore, L-carnitine restored levels of non-enzymatic antioxidants such as vitamins C and E and glutathione (Juliet 2005; Haripryia 2005). Repeat analyses showed similar results in other tissues, such as blood, liver and kidney (Kalaiselvi 1998; Kumaran 2004; Kumaran 2005).

In addition to reducing oxidative damage, animal studies have suggested that L-carnitine supplementation could provide functional support to the brain. One study found that L-carnitine suppressed seizures and impairments brain metabolism caused by hyperammonaemia in mice (Matsuoka 1991). This effect is attributed to an increase in the rate of urea production in the liver during L-carnitine supplementation, thereby decreasing the amount of circulating ammonia.

Cardiovascular Health
Hansford 1982 showed a decrease of L-carnitine in rat heart mitochondria with age. A decrease in myocardial free L-carnitine has been observed in both experimental animals and humans as a result of acute and chronic myocardial ischaemia (Shug 1978; Regitz 1990a; Regitz 1990b).

Although L-carnitine does not affect general or regional hemodynamics, there is evidence that it improves the stress tolerance of the heart by increasing the substrate availability required for energy production (Brevetti 1996).

Supplementation of 1000 mg L-carnitine to coronary artery disease (CAD) patients during 12 weeks was found to significantly reduce levels of inflammation compared to baseline and to those in the placebo group. It was concluded that L-carnitine may have the potential to reduce inflammation in CAD due to its antioxidant effects (Lee 2015). From the same study, the researchers highlighted L-carnitine's lipid-lowering effects in a separate publication (Lee 2016) which is in line with earlier findings that oral L-carnitine supplementation can help to maintain normal cholesterol levels in elderly people (Pistone 2003) and a recent systematic review and meta-analysis which suggests a significant Lp(a) lowering by oral L-carnitine supplementation (Serban 2016).

Bone Health

The bones are also impacted by the aging process. Due to a variety of factors, such as hormonal or other physiological changes, decreased bone health can lead to an increased risk of fracture and reduced quality of life (Clarke 2010). Benvenga 2004, Hooshmand 2008 and Orsal 2013 suggested that L-carnitine supplementation may help improve bone health. Hooshmand 2008 used an ovariectomized rat model to assess the impact of L-carnitine supplementation on bone health. This type of animal model can serve as a proxy for the hormonal changes which occur during aging. The authors demonstrated that L-carnitine supplementation increased bone mineral density and decreased markers of bone turnover. In a rat model with osteoporosis induced by ovariectomy and Mg-silicate, supplementation with L-carnitine was found to improve the healing of femur fractures (Aydin 2015). In a human study, it was shown that there was a trend toward a dose response relationship between L-carnitine and bone mineral density in patients with hyperthyroidism (Benvenga 2004).

Joint Health

In a randomized double-blind, placebo-controlled trial, 72 overweight or obese women with mild to moderate knee osteoarthritis (OA) were randomly allocated into 2 groups to receive 750 mg/d L-carnitine or placebo for 8 weeks. Supplementation with L-carnitine resulted in significant reductions in serum malondialdehyde, total cholesterol and LDL cholesterol. Pain intensity and patient global assessment of disease status were also significantly decreased (Mahdavi 2015). In addition, they found significantly decreased levels of IL-1β and MMP-1 (inflammatory mediators) as well as decreased scores for the visual analogue scale for pain compared to placebo (Mahdavi 2016).

Muscle Health

Sarcopenia, or the loss of muscle mass and strength, is another age associated impairment of growing attention which can affect quality of life (Crutz-Jentoft 2010). Strength decline in upper and lower limb muscles is typically 20-40% by the 7th decade and greater in older adults, and affects over 20% of 60- to 70-year-olds, approaching 50% in those over 75 years. It is well known that L-carnitine deficiency can lead to myopathy and weakness, as evidenced by patients with L-carnitine deficiency disorders. Crentsil 2010 has proposed a potential relationship between L-carnitine deficiency and geriatric frailty. This relationship is

based on the argument that mitochondrial dysfunction is directly related to L-carnitine deficiency and such dysfunction is a main contributor to frailty.

Supplementation of L-carnitine could help counteract L-carnitine deficiency, thereby delaying mitochondrial dysfunction and subsequent sequelae such as frailty and decreased quality of life. Pistone 2003 measured the impact of L-carnitine supplementation on activities of daily living in an elderly population and found that those in the L-carnitine group had improvements in both physical and mental fatigue.

Italian researchers recently recommended including 4-5 portions of meat per week for the elderly to maintain muscle mass and muscle strength which typically reduces at older age. They attributed the positive effects on protein metabolism seen by regular meat consumption to the biologically active components of meat such as L-carnitine (Rondanelli 2015).

Vegetarians

The number of Australian adults whose diet is all or almost vegetarian has risen to almost 2.1 million or 1.2% of the population in 2016 from 1.7 million people (or 9.7%) in 2012 (Roy Morgan Research 2016). Data from market researcher Euromonitor International has shown Australia's packaged vegan food market is currently worth almost $136 million, set to reach $215 million by 2020 (Euromonitor 2016). Australian Dietary Guidelines say appropriately planned vegetarian diets, including total vegetarian or vegan diets, are healthy and nutritionally adequate (NHMRC 2013). "Flexitarian" is a term for someone who actively chooses to restrain the intake of meat in their diet. Indeed, 30% of consumers globally claim that they are trying to limit the amount of meat they consume, and so could be classed as a flexitarian (Mintel 2015). In the UK, about 40% of consumers purchase meat-free products but only 6% claim to be vegetarians (Mintel 2014).

Several studies confirmed that humans ingesting a lacto-ovo- or a strict vegetarian diet over many years have decreased plasma L-carnitine concentrations (Sachan 1997; Vaz 2002; Lombard 1989; Delanghe 1989; Richter 1999). When omnivorous college students were provided with an L-carnitine free enteral diet, plasma total L-carnitine declined by 42% within the first 7 days (Chen 1998). Triathletes on a predominantly vegetarian diet were found to have the lowest plasma levels of L-carnitine among athletes of different disciplines (Föhrenbach 1993). Supplementation with L-carnitine for 6 weeks considerably increased plasma total and free L-carnitine levels, and also improved the ratio of acyl-L-carnitine to total L-carnitine, which is a means to express the supply of functionally active L-carnitine.

L-carnitine is a natural component of breast milk and cow’s milk. Manufacturers of soy-based infant formula routinely fortify their products with L-carnitine. A study relating breast milk L-carnitine concentrations to dietary habits of the mothers reported that the L-carnitine content of milk of lacto-ovo-vegetarian mothers was lower than that of omnivorous mothers at any time throughout the study (Barth 1985).

Animal studies provide support for these findings: supplementing the mother sow's diet, which normally is devoid of any animal products and thus devoid of L-carnitine, with this nutrient during pregnancy and lactation results in larger litters, increased weight of the piglets, faster growing during the suckling period and higher concentrations of L-carnitine in the milk (Ramanau 2004).

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Application A1102 – L-carnitine in foods

Further information required from the Applicant (under s108 of the FSANZ Act)

Part B - Absorption and Metabolism of L-carnitine

3 What data from human intervention studies can Lonza Ltd provide that shows the extent of intestinal absorption of L-carnitine and its hemi-tartrate salt from consumption of foods fortified with either of these two compounds?

4 What data from human intervention studies can Lonza Ltd provide that shows the extent of the change from baseline circulation concentration of TMAO after consumption of foods fortified with either of these two compounds?

What data from human intervention studies can Lonza Ltd provide that shows the extent of intestinal absorption of L-carnitine and its hemi-tartrate salt from consumption of foods fortified with either of these two compounds?

L-carnitine absorption data from human intervention studies using foods fortified with L-carnitine or L-carnitine L-tartrate are limited. However, a number of studies have assessed the absorption of natural dietary sources of L-carnitine, such as beef or milk (in the case of breast-fed vs formula-fed infants). Others have looked into the absorption, distribution and metabolism of L-carnitine provided in pharmacologically significant quantities, typically as a single bolus dose. The following section will provide insights in L-carnitine absorption and bioavailability from the most relevant data available.

The small intestine is the main site of L-carnitine absorption. Absorption of L-carnitine is characterized by slow mucosal uptake, prolonged mucosal retention and slow mucosal exit into the blood. In humans, the absorption half-life of L-carnitine typically is about 1.6 h and the time to reach maximum plasma concentration approximately 3 -5 h (Cao 2009; Reuter 2012). As reviewed by Evans 2003, uptake by human duodenal tissue occurs both by active and passive mechanisms.

Rizza et al studied the absorption of two different single oral doses of L-carnitine provided in tablet form. Intake of 2 g and 7 g L-carnitine was found to have estimated bioavailabilities of 13% and 21%, respectively (Rizza 1992).

The effects of physiologic and pharmacologic doses of L-carnitine consumption on intrajejunal concentrations and absorption appear to be different. When the effects of a hamburger meal providing approximately 55 mg total L-carnitine were compared with an enteral dose of about 4000 mg total L-carnitine, it was apparent that dietary L-carnitine (smaller doses) is primarily absorbed by active transport whereas higher dosages are largely assimilated by passive means (Li 1992).

A typical carnivorous diet is estimated to provide approximately 100-300 mg L-carnitine per day. For persons following a meat-reduced or vegetarian diet, it will be considerably less. Habitual dietary L-carnitine consumption plays a role in the overall bioavailability of L-carnitine from supplements. In 1991, Rebouche divided 12 healthy adult males into two groups, based on their estimated usual dietary L-carnitine intake. Subjects had to follow a

standardized diet either high or low in dietary sources of L-carnitine for five days. On day 5, each participant received a single oral bolus dose of an equal amount of radiolabelled L-carnitine dissolved in a fruit juice beverage. Urine and faecal excretions were collected during the 5 days after intake of L-carnitine. Intake and excretion for the two groups were significantly different. A high habitual L-carnitine consumption was associated with higher excretion of L-carnitine metabolites and vice versa, with a general bioavailability of L-carnitine from supplements of between 54-87% (Rebouche 1991).

In a very similar study setting, a tracer dose of [methyl-^{3}H] L-carnitine was administered orally with a meal to five healthy adult males, who had been adapted to a high-carnitine diet plus L-carnitine supplement (2 g/d) for 14 days. Maximum concentration of [methyl-3H] L-carnitine in serum occurred at 2.0 to 4.5 hours after administration of the tracer, indicating relatively slow absorption from the intestinal lumen. Total radioactive metabolites excreted in urine and feces ranged from 47% to 55% of the ingested tracer. Major metabolites found were [3H] trimethylamine N-oxide (8% to 49% of the administered dose; excreted primarily in urine) and [3H] gamma-butyrobetaine (0.44% to 45% of the administered dose; excreted primarily in feces). Urinary excretion of total L-carnitine was 16% to 23% of intake. Fecal excretion of total L-carnitine was negligible (less than 2% of total carnitine excretion) (Rebouche 1991).

The bioavailability and bioequivalence of three different oral dosage forms of L-carnitine, notably a solution, a chewable tablet and a tablet, was studied in 15 healthy male volunteers (Sahajwalla 1995). Supplements were provided every 12 h during 4 days and provided 2 g L-carnitine with each dose (4 g/day). With all three formulations, steady state was achieved by day 3 and maintained during day 4. No significant differences were found between the three formulations with regard to bioavailability, or other pharmacokinetic parameters.

Notably, all the studies mentioned above were done in small numbers of male subjects. Considering the large variability of individual microbiota, and advances in analytical methods in the last 25 years, these data can only serve as basic information. The following nevertheless may be regarded as valid conclusions (Rebouche 2004):

1) Dietary L-carnitine is absorbed by active and passive transfer across enterocyte membranes.
2) Bioavailability of dietary L-carnitine is dependent on the amount of L-carnitine in the meal.
3) Absorption of L-carnitine dietary supplements (0.5–6 g) is primarily passive.
4) Unabsorbed L-carnitine is mostly degraded by microorganisms in the large intestine.

L-carnitine doses typically applied in functional foods are much lower than the pharmacological doses studied above. Therefore it is worth looking at intervention studies applying lower dosages. Baker (1993) compared the effects of a single 500 mg L-carnitine supplement intake, with a single 2500 mg L-carnitine intake, and 10 days of 2500 mg L-carnitine supplementation, divided into three daily dosages, in groups of 6 healthy humans of all ages. While the smaller single dose did not have significant effects on plasma levels, both

high single and high chronic supplementation yielded significant increases in plasma L-carnitine.

See CCI section on Part B.

Non-confidential study summary:

In this small human pilot trial, healthy adults were supplemented with several different single doses of L-carnitine, in random order, each separated by a wash-out period. Plasma levels after each intake occasion were analyzed during 24h. Already the lowest dose tested was found to maintain plasma L-carnitine levels, which in the placebo group (no dietary L-carnitine intake) was found to decrease.

The study by Cao (2009) confirms the findings by Baker 1993 for the higher dose, as well as the findings by Eder (unpublished). A single oral dose of 2000 mg led to significant increases on plasma L-carnitine levels.

Breast milk, the "gold standard", represents the only natural source of L-carnitine for the newborn infant unless meat is introduced into the infant's diet. Most formulas based on cows' milk have a similar or even higher L-carnitine concentration than human milk. Nevertheless, L-carnitine blood concentrations in breast-fed newborns have been reported to be higher than those in formula-fed infants (Warshaw 1980). This has led to the speculation that the bioavailability of L-carnitine from breast milk is higher than that found in cow's milk**Error! Bookmark not defined.** (Schmidt-Sommerfeld 1985).

What data from human intervention studies can Lonza Ltd provide that shows the extent of the change from baseline circulation concentration of TMAO after consumption of foods fortified with either of these two compounds?

Lonza are not aware of human intervention studies including L-carnitine supplementation via fortified foods, looking at the TMAO response in plasma. Therefore the following section discusses both human studies that have looked into the effects of conventional foods in humans, or L-carnitine supplementation in animals. Some of these studies have already been presented in the response Part A questions

L-carnitine to TMAO

TMAO is found naturally in our diets in the preformed state (eg in fish), or can be generated from choline or L-carnitine. Of these dietary sources, preformed TMAO in fish has the greatest impact on circulating TMAO concentrations. For example, consumption of fish yielded approximately 50 times higher postprandial circulating TMAO concentrations compared with the consumption of eggs (abundant in choline) or beef (abundant in L-carnitine and choline). Populations with higher average fish intake also were found to have higher urinary TMAO excretion (Cho 2016).

As presented in the response Part A, Cho et al 2016 have concluded that the amount of TMAO produced following ingestion of L-carnitine depends on the composition of the gut microbiota, the activity of the enzyme flavin monooxygenase 3 (FMO3) and the composition of the diet. In addition, kidney function and genetics are other factors that can modulate circulating TMAO levels.

The diet of 122 elderly female volunteers was assessed and compared with BMI and various biomarkers such as serum glucose, total, HDL and LDL cholesterol, triacylglycerol, homocysteine, free choline, L-carnitine, TMA and TMAO (Malinowska 2016). Higher plasma concentrations of L-carnitine were associated with a more Western-style diet with high meat intake. Interestingly, the same study also showed that people consuming smaller than average amounts of dietary fiber, and also those consuming lower amounts of fermented dairy products, have higher concentrations of plasma TMA. One explanation for this observation is that such a diet favours the growth of TMA-producing bacteria in the gut. As the metabolism of L-carnitine to TMAO in humans comprises of two different steps, it is very likely that both reactions (L-carnitine to TMA by the microbiota; and TMA to TMAO by liver enzymes) can be separately influenced by different factors. The following section presents data on each of these steps.

L-carnitine to TMA

A recent *in vitro* study tested 79 isolates found in the human intestinal tract for choline consumption and their ability to produce TMA from choline under anaerobic conditions. All strains were inoculated with a diluted gut medium, supplemented with choline and incubated for 24 h at 37°C. Eight species were identified to produce TMA from choline. In a subsequent

test, none of these TMA-producing strains was found to generate TMA from L-carnitine under the same test conditions (Romano 2015).

TMA to TMAO

In an attempt to get better insights into inter-individual variability in the N-oxidation of TMA, in 1995 researchers compared data generated in a European population with those from a Jordanian population. Whereas from a British white population, the majority of individuals were found to excrete greater than 90% of the total TMA in the form of TMAO, prevalence of compromised ability to N-oxidize TMA may be higher in a Jordanian population (Hadidi 1995).

As mentioned in the responses to Part A, flavin monooxygenases (FMOs) are responsible for the N-Oxidation of TMA to produce TMAO. FMO activity measurements in hepatic tissue homogenates from mice indicated significantly higher enzymatic activity in females than in males, which suggests that gender-associated differences in TMAO accumulation are likely not derived from higher levels of microbiota-generated TMA, but rather higher FMO activity in females (Romano 2015). From a series of additional tests in mice inoculated with a low-complexity gut microbial community, the same researchers concluded that dietary choline is necessary for TMA production but does not impact the abundance of TMA-producing bacteria. Dietary choline was not found to be necessary for colonization of choline-consuming TMA-producing bacteria, and does not seem to provide these species with a major fitness advantage.

Considering the very similar chemical structure of L-carnitine, it is very likely that the same would hold true for L-carnitine, too.

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General requirements (3.1)

☑ 3.1.1 Form of application
☑ Application, abstracts and other key documents in English
☑ Executive Summary (separated from main application electronically and in hard copy)
☑ Relevant sections of Part 3 clearly identified
☑ Pages sequentially numbered
☑ Electronic copy (searchable)
☑1 hard copy
☑ Electronic and hard copy identical
☑ Hard copy capable of being laid flat
☑ All references provided (in electronic and hard copy)

☑ 3.1.2 Applicant details

☑ 3.1.3 Purpose of the application

☑ 3.1.4 Justification for the application
☑ Regulatory impact information
☑ Impact on international trade

☑ 3.1.5 Information to support the application
☑Data requirements

☑ 3.1.6 Assessment procedure
☑ General
☐ Major
☐ Minor
☐ High level health claim variation

☑ 3.1.7 Confidential Commercial Information
☑ Confidential material separated in both electronic and hard copy
☑ Formal request including reasons
☑ Non-confidential summary provided

☐ 3.1.8 Exclusive Capturable Commercial Benefit
☐ Justification provided

☑ 3.1.9 International and other national standards
☑ International standards
☑ Other national standards

☑ 3.1.10 Statutory Declaration

☑ 3.1.11 Checklist/s provided with application
☑ 3.1 Checklist
☑ Any other relevant checklists for Parts 3.3.3

Nutritive Substances (3.3.3)			
☑	A.1 Identification information	☑	C.2 Proposed maximum levels in food groups or foods
☑	A.2 Chemical and physical properties	☑	C.3 Likely level of consumption
☑	A.3 Impurity profile information	☑	C.4 Percentage of food group to use nutritive substance
☑	A.4 Manufacturing process	☑	C.5 Use in other countries (if available)
☑	A.5 Specification information	☑	C.6 Where consumption has changed, information on likely consumption
☑	A.6 Analytical detection method	☑	D.1 Nutritional purpose
☑	A.7 Proposed food label	☑	E.1 Need for nutritive substance
☑	B.1 Toxicokinetics and metabolism information	☑	E.2 Demonstrated potential deficit or health benefit
☑	B.2 Animal or human toxicity studies	☑	F.1 Consumer awareness and understanding
☑	B.3 Safety assessments from international agencies	☑	F.2 Actual or potential behaviour of consumers
☑	C.1 List of food groups or foods likely to contain the nutritive substance	☑	F.3 Demonstration of no adverse effects on any population groups