

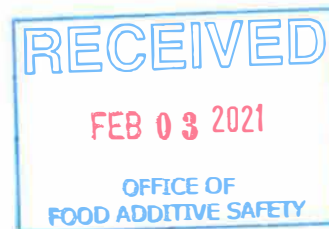


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By FedEx

January 29, 2021

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, MD 20740-3835



Re: GRAS Notice for L-Carnitine-L-Tartrate (LCLT)

Dear Sir or Madam:

We hereby submit the enclosed GRAS notice for the use of LCLT as a nutrient source of L-Carnitine in powdered non-exempt infant formula (IF) based on either goat's or cow's milk protein for term infants up to 12 months of age. LCLT will be added at a maximum level of 0.6 mg/100 mL of the liquid IF as prepared with water or 0.88 mg/100 kcal LCLT. The statutory basis of the GRAS conclusion is scientific procedures.

LCLT is not intended for use in products under the jurisdiction of the U.S. Department of Agriculture (USDA). The GRAS notice does not contain any designated confidential business information. In accordance with the Agency's guidelines, we have enclosed one original copy of the GRAS notice, and one complete electronic copy of the GRAS notice on a compact disk (CD).

The notified substance was also the subject of GRAS Notice No. 935, which we requested the agency to cease its review on November 19, 2020. We appreciate the detailed recommendations and guidance the Agency provided during our telephone conference on October 15, 2020. Ausnutria considered all of these comments when revising the updated GRAS notice and made corresponding modifications, when appropriate to provide further clarification.

For your easy reference, we have copied these comments below, followed by our brief response.

1.e Large sections of the safety narrative in Part 6 (pages 34-44 and 46-47) appear to be copied from a document published in 2018 by Food Standards Australia New Zealand (FSANZ) titled "Risks and technical assessment – Application A1102 L-carnitine in food." These sections should be rewritten and appropriately cited. Additionally, several Japanese studies are discussed in this section. Please provide copies of the original publications in Japanese as well as English translations verified to be complete and accurate.

Ausnutria response: The Part 6 of the amended GRAS notice has been rewritten and the sections are now appropriately cited with the full references to studies provided in footnotes on each page.

We are also attaching the original Japanese publications along with the English translations in **Attachment A**. These studies include:

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- a)e Kikumori, M., et al. (1988). "Chronic toxicity study of L-carnitine chloride in Beagles." Iyaku-hine Kenkyu 19(2): 261-281.e
- b)e Toshida, K. and Y. Wada (1988). "Acute toxicity studies of L-carnitine chloride in mice and rabbits." Iyaku-hin kenkyu 19(3): 446-453.e
- c)e Kudo, S., et al. (1988). "Acute toxicity studies of L-Carnitine chloride in 10- and 22-day-old and 5-week-old rats." Iyaku-hin Kenkyu 19(4): 689-699.e
- d)e Narita, N., et al. (1988). "Acute toxicity study of L-carnitine chloride in rats." Iyaku-hin Kenkyue 19(2): 191-196.e
- e)e Kikumori, M., et al. (1988). "Subacute toxicity study of l-carnitine chloride in beagles." Iyaku-hine Kenkyu 19(2): 244-260.e
- f)e Kudo, S., et al. (1988). "Chronic Toxicity study of l-carnitine chloride in rats." Iyaku-hin Kenkyue 19(2): 221-237.e
- g)e Yamate, J., et al. (1988). "Subacute Toxicity of L-Carnitine chloride in rats." Iyaku-hin Kenkyue 19(2): 197-220.e
- h)e Kikumori, M., et al. (1988). "Acute toxicity study of l-carnitine chloride in beagles." Iyaku-hine Kenkyu 19(2): 238-243.e
- i)e Toteno, I., et al. (1988). "Teratogenicity study of L-carnitine chloride in rabbits." Iyaku-hine Kenkyu 19(3): 510-521.e
- j)e Nakamura, A., et al. (1988). "Teratogenicity study of L-carnitine chloride in rats by oral administration." Iyaku-hin Kenkyu 19(3): 465-489.e
- k)e Itabashi, M., et al. (1988). "Reproduction studies of L-Carnitine Chloride in rats by oral administration prior to mating and in the early stage of gestation." Iyaku-hin Kenkyue 19(3): 454-464.e
- l)e Itabashi, M., et al. (1988). "Reproduction studies of L-carnitine chloride in rats by oral administration during the perinatal and lactating periods." Iyaku-hin Kenkyu 19(3): 490-509.e

2.e *The information provided in the notice under Part 4 Self-limiting Levels of Use (page 30) is not applicable. According to the GRAS Final Rule, self-limiting levels of use applies "in circumstances where the amount of the notified substance that can be added to human food or animal food is limited because the food containing levels of the notified substance above a particular level would become unpalatable or technologically impractical." Please revise this section and state whether Part 4 applies to the notified substance and if so, why*

Ausnutria response: Part 4 of the amended GRAS notice now states it does not apply to the notified substance.

3.e *The information provided in the notice under Part 5 Experience Based on Common Use in Food Before 1958 (page 31) is not applicable. According to the GRAS Final Rule, this section should*

discuss “the history of consumption of the substance for food use by a significant number of consumers (or animals in the case of animal food) prior to January 1, 1958 if a conclusion of GRAS status is based on common use of the substance in food prior to 1958.” Please revise this part and only discuss studies pertaining to consumption of the notified substance for food use by a significant number of consumers prior to 1958, if such publications exist. If no such studies exist, please state that Part 5 is not applicable to your GRAS conclusion.

Ausnutria response: Part 5 of the amended GRAS notice now states it is not applicable to the GRAS conclusion.

4. *With regard to the intended use of L-carnitine-L-tartrate, please specify the source of the protein base of the infant formula (e.g., cow milk, soy etc.).*

Ausnutria response: The source of protein is either goat’s or cow’s milk.

5. *The notifier refers to Food Chemicals Codex (FCC) specifications for L-carnitine-L-tartrate, as well as L-carnitine and L-tartaric acid. Please provide a complete citation to the edition of FCC referenced in your notice.*

Ausnutria response: We have amended the GRAS notice to reference the FCC 12th edition.

6. *Please clarify if the methods of analysis used for the specification parameters are validated for their intended purpose. If using published or compendial methods, please provide complete and appropriate citations.*

Ausnutria response: We have confirmed all the methods of analysis are validated for their intended purpose. We have also provided complete citations to published or compendial methods in Table 1 of the amended GRAS notice.

7. *Please confirm whether specifications (e.g., assays for L-carnitine and L-tartrate) are on an anhydrous basis. We note that FCC specifications for L-carnitine-L-tartrate include an infrared spectrophotometric identification test. Please confirm if the notifier’s specifications include this parameter.*

Ausnutria response: We hereby confirm the specifications are on an anhydrous basis. The specifications also include the infrared test. An internal method that is similar to USP 43 <197> Spectroscopic Identification test is used. The method uses USP 43 <197> sample preparation with potassium bromide. It is an FT-IR Potassium Bromide tablet method. Samples and controls are mixed homogeneously, and then placed in a tablet press to make a tablet. An IR spectra is then produced and compared to the IR spectra standard for LCLT provided by the Chinese standard GB 25550-2010.

8. *The notifier states that typical lead levels are <0.2 mg/kg; however, the results of the batch analyses are reported as <3 mg/kg. Please clarify the source of data used to determine the typical lead levels in L-carnitine-L-tartrate.*

Ausnutria response: In the amended GRAS notice, the lead specification is provided as ≤ 2 ppm, which is consistent with the FCC 12th monograph for LCLT. In Table 3 of the amended GRAS notice, we have also summarized the updated analytical results for 5 lots of LCLT meeting the lead specification of ≤ 2 ppm.

9.e *The specified limits for Salmonella and E. coli are listed as 'absent.' Please specify the sample size used in these tests.*

Ausnutria response: In Table 1 of the amended GRAS notice, the specified limits for *Salmonella* and *E. coli* are now both listed as "Absent in 10 g."

10.e *We note that the specifications for microorganisms do not include a limit for Cronobacter sakazakii. Limits for these organisms help to ensure that infant formula products that contain the notified substance are in compliance with 21 CFR 106.55. Please provide limits for Cronobacter sakazakii in L-carnitine-L-tartrate.*

Ausnutria response: In Table 1 of the amended GRAS notice, the specified limit for *Cronobacter sakazakii* is now listed as "Absent in 300 g."

11.e *We note that the analytical results for E. coli in five non-consecutive lots of LCLT are missing from Table 3. Please provide the analytical results for E. coli.*

Ausnutria response: These are now provided in Table 3 of the amended GRAS notice.

12.e *The notifier cites an FDA guidance document with regard to the estimated maximum formulae consumption of 900 mL/day. Please provide a complete citation to this guidance document. Further, please clarify whether 900 mL/day is representative of mean or an upper percentile estimate of dietary intake.*

Ausnutria response: According to the following reference, infants consume 1,200 mL (gram) of IF a day at the 95th percentile, and a representative weight for an infant aged <111 days old is 6.1 kg.

- EFSA Scientific Committee, et al. (2017). "Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age." EFSA Journal **15**(5).

13.e *With regard to exposure calculated on a body weight basis, please provide the source of the representative body weight of 6.3 kg used in your calculations and specify the ages that this estimate is representative of.*

Ausnutria response: Please see the response to question #12 above. We have updated the exposures and based them on a 6.1 kg bw basis.

14.e *On page 28, the notice includes a discussion of the minimum and maximum level of L-carnitine in infant formula recommended by an expert panel of the Life Sciences Research Office (LSRO). The notifier states that LSRO recommends a minimum L-carnitine content of 1.2 mg/100 kcal, "a level like that found in human milk", and a maximum L-carnitine content of 2.0 mg/100 kcal, "a value like the upper limit reported for human milk."*

- a. *On page 28, the notifier states, "The L-Carnitine content of IF as proposed by Ausnutria after having supplemented natural L-Carnitine levels from milk with LCLT is 2.1 mg L-Carnitine/100 ml of IF. This value roughly holds the middle between the concentration which is needed for optimal metabolism and which is maximally recommended." Please further explain this statement within the context of the minimum and maximum LSRO recommendations for L-carnitine in infant formula to support your proposed use level of LCLT.*

Ausnutria response: In the amended GRAS notice, the L-Carnitine content of IF as proposed by Ausnutria after having supplemented natural L-Carnitine levels from milk with LCLT is now 1.9 mg L-Carnitine/100 kcal of IF. This is discussed in more detail in Section 3.1.2.1 of the amended GRAS notice.

b.e We believe there is a typographical error on page 28 (see bullet above) where reference to "2.1 mg L-Carnitine/100 ml of IF" should be "2.1 mg L-Carnitine/100 kcal of IF." Please confirm the correct units.e

Ausnutria response: Yes there was a typographical error that has been corrected. Please see the response to question above. The updated value is 1.9 mg L-Carnitine/100 kcal of IF.

15.e Related to question #14 above, on page 33, the notifier states that "the total amount of L-Carnitine under these conditions in IF are calculated to be 2.1 mg/100 Kcal" and that "the total exposure to L-Carnitine is of a very similar value as found in human breast milk (see chapter 3.1.1),e ensuring that exposure to L-carnitine takes place at safe levels." According to EFSA (2014), "mean total carnitine concentrations have been reported to be in the range 0.9 mg-1.6 mg/100 kcal in human milk." Please further explain your statement that "the total exposure to L-Carnitine is of a very similar value as found in human breast milk" in relation to EFSA's data on human milk.e

Ausnutria response: We recognize the inconsistency in the statements and apologize for the confusion. We have removed this statement in the amended GRAS notice.

16.e In Part 4 of the notice, you note that infants maximally consume 1200 ml of infant formula per day. Please clarify whether this estimate of formula intake is representative of particular group, such as upper percentile consumers, and how it relates to the exposure estimates you provided based on a maximum intake of 900 mL of formula. (Please also see our Administrative Issue #2 above about Part 4.)e

Ausnutria response: We apologize for any confusion. We have amended the GRAS notice and are using 1,200 mL to calculate the estimated daily intake.

17.e Please briefly summarize the results of the article Wu, Q., Zhang, X., Zhao, Y., & Yang, X.e (2020). High L-Carnitine Ingestion Impairs Liver Function by Disordering Gut Bacteria Composition in Mice. Journal of Agricultural and Food Chemistry, 68(20), 5707-5714. Please also compare the approximate intake of L-carnitine in this study to your proposed intake levels and state whether you still think that the intake of L-carnitine is still safe at your proposed intake level in light of the results of this publication.e

Ausnutria response: The results of this 2020 study are summarized in Section 6.2.3.2 of the amended GRAS notice. While the authors concluded that a high intake of L-Carnitine (i.e., 4,500 mg/kg bw/day L-Carnitine) could induce a liver function decline by disordering the gut bacterial composition of mice resulting in an increased TMAO metabolism, the level tested is magnitudes higher than the L-Carnitine component from the LCLT's intended use (i.e., 0.8 mg/kg bw/day) and the safety thresholds established by EFSA for LCLT (50 mg/kg bw/day) and FSANZ for L-Carnitine of 50 mg/kg bw/day. Notably, the level tested in the study is also comparable to the LD₅₀ reported by an earlier

study in Crj:CD rats.^{1/} For the 10-day old rats, the observed LD₅₀ for L-Carnitine Chloride was 4,374 mg/kg bw/day (95% CI: 3,995-4,790 mg/kg bw/day) in males and 4,578 mg/kg bw/day (95% CI: 4,128-5,093 mg/kg bw/day). For the 22-day old rats, the LD₅₀ was determined to be 6,127 mg/kg bw/day in males (95% CI: 5,501-6,824 mg/kg bw/day) and 6,299 mg/kg bw/day in females (95% CI: 5,679-6,987 mg/kg bw/day). This is also in line with the LD₅₀ reported by other acute studies.^{2/} We, therefore, do not consider the study by Wu et.al 2020 as having any bearing on the safety assessment of LCLT at the proposed use levels.

18.e FSAZ (2018) discusses the results of a 13-week dietary study of L-carnitine-L-tartrate (LCLT) in rats in great detail (pp. 55-56). The notifier mentions this study in only one sentence on p. 38 even though this is the only subchronic study on the notified substance itself and not a study of a component of LCLT. Please discuss this study in more detail as it is an important part of the overall safety assessment of LCLT even though it is an unpublished study. Additionally, please provide the primary reference for this study, which can be found in FSAZ (2018).^e

Ausnutria response: This study is now discussed in detail in Section 6.2.2 of the amended GRAS notice. The reference of the study is:

- ^e LPT Laboratory of Pharmacology and Toxicology (2003). 90-day Subchronic Toxicity Study of LZ1780 by Repeated Oral Administration via the Diet to CD® Rats – According to OECD Guideline 408, Lonza.^e

19.e The notifier discusses three different publications by Kikumori et al., all from 1988. All three publications are simply referenced as “Kikumori et al. 1988”. Please specify on each page these studies are discussed, which of the three studies you are discussing and provide the references as 1988a, b, or c.^e

Ausnutria response: These three studies are now referenced as 1988a, 1988b, and 1988c, with the full references provided in the footnotes on each page where they are discussed.

20.e On page 43, the notifier states, “There were no treatment-related effects on electrocardiographic findings, ophthalmologic findings, or behavioral findings.” According to the original text in FSAZ’s publication, “There were no treatment-related effects on electrocardiographic findings, ophthalmologic findings, or otological findings” in this study. The study does not talk about examining the behavior of animals. Otology pertains to the anatomy and physiology of the ear and not to behavioral issues.^e Please explain where the “behavioral findings” come from in your statement.^e

Ausnutria response: We apologize for any confusion and we thank you for bringing this inconsistency to our attention. We have removed the reference to “behavioral findings” in the amended GRAS notice and limit the findings to those specifically reported in the FSAZ publication.

1/ Kudo, S., et al. (1988). "Acute toxicity studies of L-Carnitine chloride in 10- and 22-day-old and 5-week-old rats." *Iyakuhi Kenkyu* 19(4): 689-699.

2/ See e.g., Narita, N., et al. (1988). "Acute toxicity study of L-carnitine chloride in rats." *Iyakuhi Kenkyu* 19(2): 191-196; Toshida, K. and Y. Wada (1988). "Acute toxicity studies of L-carnitine chloride in mice and rabbits." *Iyakuhi kenkyu* 19(3): 446-453.

21. On page 44, the notifier states, "This high L-Carnitine concentration in drinking water is expected to lead to a L-Carnitine exposure of 1700 mg/ml (Collins, Drazul-Schrader et al. 2016)". Please note that the correct unit is 1,700 mg/kg bw and not 1,700 mg/ml. Please state whether you concur.

Ausnutria response: We agree and have corrected the error in the amended GRAS notice. Thank you for flagging this for us, and we apologize for the confusion.

22. On page 44, the notifier states that at the dose level of 1,700 mg/kg bw (Koeth et al., 2013) "L-carnitine promotes the induction of atherosclerosis in APOE -/- mice, due to the formation of TMAO in the intestines". To minimize this adverse effect, the notifier states that "the dose of 1700 mg/kg is ... more than 6 times the NOAEL of L-Carnitine in chronic rat studies". FDA notes that the notifier only discussed one chronic rat study with a duration of 1 year in detail and provided a NOAEL of 737 mg/kg bw/d for both sexes (Kudow et al., 1988). While a second chronic rat study by Koeth et al. (2013) was briefly mentioned, the notifier did not provide a NOAEL for this study, focusing only on whether the test substance increases the incidences of aberrant crypt foci (ACF) in the colon and atherosclerotic lesions in the aorta. All other rat studies were either subchronic or acute toxicity studies. Consequently, the dose of 1,700 mg/kg is only more than twice the chronic rat NOAEL of 737 mg/kg bw/d.

a) Please state whether you concur.

b) Based on the original statement, FDA assumes that the notifier compared 1,700 mg/kg bw to the NOAEL seen in the chronic dog study and not the rat study. Please confirm whether our assumption is correct.

c) FDA notes that comparing a toxic level to the lowest available NOAEL makes it seem as if there was a larger margin of safety (MOS) between a toxic dose and a safe dose when in fact the toxic dose of 1,700 mg/kg bw is only twice the highest (and only) chronic NOAEL in rats. FDA also notes that calculating the exact MOS between the intake of L-carnitine in infants resulting from the use of formula (i.e. 2.0 mg/kg bw/day) and the highest dose (i.e. 352 mg/kg bw/day) that showed no evidence of increased risk of atherosclerosis in 12-week and 1-year rat studies by Collins et al. (2016) and Empl et al. (2015), respectively, is more useful to support the safety of LCLT at the proposed intake levels and show an adequate MOS than calculating a MOS between the level that produced an effect to the lowest or highest NOAEL in a rat or dog study. Please state whether you concur, and if so, calculate the exact MOS between the proposed intake level and the highest dose showing no evidence of atherosclerosis.

Ausnutria response: We agree with each of the agency statements above and that for the purpose of the safety assessment, a toxic dose should not be compared to a safe dose. We agree the safety assessment should compare the proposed intake level with the safety threshold level and have done so in the amended GRAS notice. We also have addressed any toxicity studies that have shown adverse effects. We have rewritten Part 6 of the amended GRAS notice accordingly.

23. In support of the safety assessment, please briefly discuss the following reproductive and/or developmental toxicity and/or teratogenicity studies by:

a) Brandsch, C., & Eder, K. (2003). Reproductive performance of rats supplemented with L-carnitine. *Journal of animal physiology and animal nutrition*, 87(7-8), 301-308.

b)eltabashi M, Watanabe H, Yamate J, Takehara K, Tajima M and Wada Y (1988a) Reproductione studies of L-carnitine chloride in rats by oral administration prior to mating and in the early stage ofe gestation. *Iyaku hin Kenkyu* 19(3):454–464.e

c)eltabashi M, Yamashita K, Inoue T, Yamate J, Takehara K, Tajima M and Wada Y (1988b)e Reproduction studies of L-carnitine chloride in rats by oral administration during the perinatal ande lactating periods. *Iyaku hin Kenkyu* 19(3):490–509.e

d)e Nakamura A, Ueno H, Igarashi E, Fukasawa N, Katsumata Y, Tamura K, Suzuki T and Wada Ye (1988) Teratogenicity study of L-carnitine chloride in rats by oral administration. *Iyaku hin Kenkyue* 19(3):465–489e

e)e Toteno I, Furukawa S, Haguro S, Matsushima T, Awazu K, Morinaga T, Fujii S, Terada T ande Wada Y (1988) Teratogenicity study of L-carnitine chloride in rabbits. *Iyaku hin Kenkyu* 19(3):510–521e

Please clearly state the identity of the test substances, species and sexes of animals, the durations of the studies in terms of total number of days or weeks, the dose levels in mg/kg bw/day, and a summary of the findings. Please state whether a NOAEL in any of these studies can be established and if not, why not. If NOAELs can be established, please state the values using the unit of mg/kg bw/day. Please note, even though your product is for use in infants, reproductive and/or developmental toxicity and teratogenicity studies, if available, should be discussed to provide a complete toxicological profile of the notified substance and to establish safe levels of intake.

Ausnutria response: In line with the FDA’s recommendation above, the above studies are discussed in detail in Section 6.2.3.5 of the amended GRAS notice and we include in that discussion each of the parameters identified above by the agency.

24.e On page 46, the notifier states, “The skin of three New Zealand White rabbits was exposed toe 0.5 g LCLT for four hours. At 1, 24, 48 and 72 hours after removal no signs of either irritation ore corrosion were observed. The results therefore confirm that LCLT is non-irritant to the skin (FSANZe 2018).” FDA was unable to find this study in the FSANZ (2018) document. However, FDA found thee above statement almost word for word in EFSA, 2012. Please confirm that the original reference wase incorrect and that the reference for the above statement is EFSA, 2012.e

Ausnutria response: Because the intended use is only for oral intake, in the amended GRAS notice, we are now focusing on the safety studies involving the oral route only. We have removed the discussion of the skin test.

25.e On page 48, last paragraph, the notifier states, “Although human safety studies with L-carnitinee show safe levels around 3000 gram per adult per day, the studies...”. Please confirm that the 3000 ise a typo and the correct value is 3 g/p/day and not 3,000 g/p/d.

Ausnutria response: We hereby confirm the 3000 is a typo and the correct value is 3 g/p/day. We have revised the corresponding part in the amended GRAS notice.

26.e On pages 48 and 49, the discussion of subchronic and chronic toxicity of L-tartrate is limitee to two statements: “24 month toxicity studies performed with L-tartrate (Hunter et al., 1977) in rats dide not show evidence of carcinogenicitye” and “A 2 year safety study with L-tartrate in rats showed a NOAEL of around 3 grams L-tartrate per kg of BW (Hunter et al, 1977).” (Same study.)e

As you are performing a component-based safety evaluation and one of the two components is L-tartrate/L-tartaric acid, the safety of L-tartaric acid should be adequately discussed. Therefore, please discuss the Hunter et al. (1977) study in more detail and if available, other studies on the chronic and subchronic toxicity of L-tartaric acid as was done for the other component, L-carnitine.

Ausnutria response: We have discussed the Hunter et al. (1977) study in more detail in Section 6.2.4.3 of the amended GRAS notice. We also expanded our safety discussion of L-Tartaric acid in Section 6.2.4 of the amended GRAS notice.

27.e a) *On page 49, first paragraph (similar statement in the 6th paragraph as well), the notifier states, “vomiting induced by L-Carnitine probably inhibits the development of toxicity by L-Carnitine”.e FDA notes that in the 53-week study in dogs (Kikumori et al., 1988), vomiting at 800 and 1,600 mg L-carnitine chloride/kg bw/day did not prevent the development of toxicity at these dose levels.e Treatment-related linear erosion-like changes and hyperemia of the cardia or fundus of the stomache were observed during gross necropsy. Histopathological findings that correlated to the gross lesionse were edema and localized mucosal necrosis of the stomach. The development of these adversee effects was not inhibited by vomiting. Please state whether you concur and if not, clearly explain whye note.*

Ausnutria response: We concur, and have removed the statement in the amended GRAS notice.

b)*eFurther down the same page (4th paragraph), the notifier states, “Higher doses of L-Carnitinee (around 50 mg L-Carnitine/kg BW) cause gastrointestinal complaints including diarrhea, nausea ande cramping (Crill and Helms 2007). In dogs and human adults, similar symptoms are induced bye exposure to L-Carnitine as in infants. Importantly however at such dose levels no adverse effectse occur. Please explain whether the “at such dose levels” refer to the levels at which vomiting ande diarrhea were reported in dogs (i.e. 800 and 1,600 mg/kg bw/day) or refers to 50 mg/kg bw/day ate which you claim gastrointestinal disturbances in infants. If it refers to 800 and 1,600 mg/kg bw/day,e please see FDA’s comment for 27a). For future reference, please avoid using ambiguous expressionse and phrases such as the statement above.e*

Ausnutria response: We apologize for the confusion. We have rewritten Part 6 of the amended GRAS notice and the rewritten assessment no longer contains the confusing statement highlighted by FDA.

28.e*On page 49 in the section pertaining to infants, the notifier states, “However, data as obtained ine infants suggest that L-Carnitine (levocarnitine) at doses up to about 10-20 mg/kg BWare safe. Highere doses of L-Carnitine (around 50 mg L-Carnitine/kg BW) cause gastrointestinal complaints includinge diarrhea, nausea and cramping (Crill and Helms 2007).e FDA notes that the Crill and Helms (2007)e article simply states, “According to the available data and the fact that negative effects have been seene with doses of approximately 50 mg/kg/d..e” and does not specify what adverse effects were seen at 50 mg/kg bw/day. The Crill and Helms (2007) article provides the following reference for this statement: Sulkers EJ, Lafeber HN, Degenhart HJ, Przyrembel H, Schlotzer K, Sauer JP. Effects of high carnitine supplementation on substrate utilization in low-birth-weight infants receiving total parenteral nutrition. Am J Clin Nutr. 1990;52:889–894. According to the Sulkers et al. (1990) article, the only adverse effects seen at this dose level are “increased protein oxidation and decreased nitrogen balance”. The article does not mention diarrhea, nausea and cramping as adverse effects of carnitine administration at approximately 50 mg/kg bw/day. Please state whether you concur.*

Ausnutria response: We have rewritten Part 6 of the amended GRAS notice and have removed the highlighted statement that is confusing.

While the Crill and Helms (2007) article mentions that “When using larger doses or with the oral product, gastrointestinal symptoms, specifically diarrhea, nausea, and cramping, may appear.” This statement does not specify at what dose levels these gastrointestinal effects are observed (i.e. what “larger doses” exactly are). While the paper (Crill and Helms, 2007) had provided two references for this statement, one did not provide information on at what levels the above adverse effects are observed, and the other reference was unavailable (i.e. FDA could not locate the publication). Therefore, please provide primary references for your statement “Higher doses of L-Carnitine (around 50 mg L-Carnitine/kg BW) cause gastrointestinal complaints including diarrhea, nausea and cramping.” If the reference you intend to provide is not publicly available (i.e. the full publication is not on Google Scholar, Google, PubMed or on similar sites), please attach a copy of the publication.

Ausnutria response: We apologize for the confusion. We have rewritten Part 6 of the amended GRAS notice and have removed the highlighted statement.

29. On page 50, the notifier states, “In 2019, a multi-center, double-blind randomized, and controlled trial to examine the growth in infants consuming a cow milk-based IF or a goat milk-based IF was finalized. A third breast-fed arm was used as reference. The goat milk based IF contained 0.00412% (w/w) LCLT as the source of L-Carnitine ...”.

a) *FDA notes that a reference for this study was not provided, only a recipe of the formula in Appendix 6. Please provide the full reference for this 2019 study.*

Ausnutria response: the reference is provided in the amended GRAS notice and also provided below:

- *He T, Van Lee L, Woudstra F, Panzer F, Haandrikman A, Verkade H. Growth and safety of a goat milk based infant formula in newborns: A double-blind randomized controlled trial (unpublished).*

b) *FDA notes that this study is used by the notifier to support the safety of LCLT in infant formulae at the proposed intake levels. Therefore, please provide the daily estimated intake of LCLT (in mg/kg bw/day) based on the formula containing 0.00412% LCLT and compare this value to your proposed daily intake of LCLT.*

Ausnutria response: The daily estimated intake of LCLT is 1.28 mg/kg bw/day in the 2019 study. This is higher than the 1.18 mg/kg bw/day intended use level. Please see a more detailed discussion in Part 6 of the amended GRAS notice.

30. On page 50, the notifier states, “In 2015, we conducted a single-center, double-blind randomized, and controlled trial to examine the growth and nutritional status in infants consuming a cow milk-based IF or a goat milk-based IF (Kabrita Gold, Ausnutria B.V.). The latter IF contained 0.00687% (w/w) LCLT as the source of L-Carnitine...”. FDA notes that this study is used by the notifier to support the safety of LCLT in infant formula at the proposed intake levels. Therefore, please provide the daily estimated intake of LCLT (in mg/kg bw/day) based on the formula containing 0.00687% LCLT and compare this value to your proposed daily intake of LCLT.

Ausnutria response: The daily estimated intake of LCLT is 1.27 mg/kg bw/day in the 2015 study. This is higher than the 1.18 mg/kg bw/day intended use level. Please see more detailed discussion in Part 6 of the amended GRAS notice.

31.e On page 51, the notifier states, “The estimated maximum daily exposure to LCLT and its derivatives, L-Carnitine and L-tartaric acid, are well below the international recommendations and regulations for IF.” FDA notes that EFSA (2014) recommended a minimum L-carnitine content of 1.2e mg/100 kcal and did not specify an upper limit or range for infant formula. As the notifier’s use of LCLT in infant formula will result in a daily intake of 2.1 mg of L-carnitine/100 kcal, please explain this statement “the estimated daily exposure to... L-carnitine... are well below the international recommendations and regulations for IF.” FDA notes that the notifier’s proposed intake level is not below the minimum proposed by EFSA and as EFSA does not have a value for upper limit, the intake of 2.1 mg/100 kcal cannot be compared to an upper level. Please state whether you concur. Additionally, please state what other (i.e. other than EFSA) “international recommendations and regulations for IF” exists when it comes to the infant formula’s LCLT or L-carnitine content. Please provide full references. Moreover, please compare your proposed L-carnitine intake to the intakes specified in other “international recommendations and regulations” for infant formula.

Ausnutria response: We apologize for the confusion and have rewritten Part 6 of the amended GRAS notice. In Section 6.3 of the amended GRAS notice, we compared the intended use levels to safety threshold levels established by various regulatory bodies:

	EDI from Intended Use	Safety Threshold Thresholds
LCLT	<ul style="list-style-type: none"> LCLT intended use level: <u>1.18 mg/ kg bw/daye</u> 	<ul style="list-style-type: none"> EFSA Journal (2003)19, 1-13: L-carnitine-L-tartrate up to 3g/day in adults or <u>50 mg/kg bw/day</u> (when assuming a body weight of an adult of 60 kg).
L-Carnitine	<ul style="list-style-type: none"> L-Carnitine component from LCLT’s intended use: <u>0.8 mg/kg bw/daye</u> The L-Carnitine content of IF as proposed by Ausnutria after having supplemented natural L-Carnitine levels from milk with LCLT: 1.9 mg L-Carnitine/100 kcal Cumulative estimated daily L-Carnitine exposure from all sources of 2.54 mg/ kg bw/daye 	<ul style="list-style-type: none"> FSANZ, Approval Report – Application A1102 L-Carnitine in food (May 16, 2019) : intake of L-Carnitine up to 3 g/day or <u>50 mg/kg bw/day</u> (when assuming a body weight of an adult of 60 kg) is not associated with adverse effects. LSRO: recommended that L-Carnitine be added to term IF at a level of <u>1.2-2.0 mg/100 kcal</u>
L-Tartaric acide	<ul style="list-style-type: none"> L-Tartaric acid component from LCLT’s intended use: <u>0.38 mg/kg bw/daye</u> Cumulative estimated daily L-Tartaric acid from alle 	<ul style="list-style-type: none"> EFSA Journal 2020;18(3):6030: ADI for L-Tartaric acid of <u>240 mg/kg bw/day</u>.

	sources exposure of 18.1 mg L-Tartaric acid/ kg bw/day	
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We are confident that the amended GRAS notice addresses the questions FDA had regarding the administrative, chemistry and toxicology issues. We are committed to cooperating with the Agency and believe an open dialog is one of the most effective ways to accomplish that objective. If any questions arise in the course of your review, please contact us, preferably by telephone or e-mail, so that we can provide a prompt response.

Sincerely,



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GRAS Notification of L-Carnitine-L-Tartrate in Term Infant Formula

**Ausnutria B.V.
Zwolle, The Netherlands
January 2021**

Authors: Charlotte 't Hoen, MSc
Hiskias Keizer, PhD
Linde van Lee, PhD

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1. Part 1. SIGNED STATEMENTS AND CERTIFICATION

1.1 Basis for GRAS Conclusion

In accordance with 21 CFR §170 Subpart E consisting of §170.203 through 170.285, Ausnutria B.V. (Ausnutria) is submitting this GRAS Notice for its conclusion that L-Carnitine-L-Tartrate (LCLT) is Generally Recognized as Safe (GRAS) under the conditions of its intended use in powdered infant formula (IF) based on either goat's or cow's milk protein for term infants as a nutrient source for L-Carnitine. As such, its intended use is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act.

1.2 Name and Address of Organization

Ausnutria B.V.
Dokter van Deenweg 150
8025 BM Zwolle
The Netherlands
+31 (0) 88 11 63 600

1.3 Common Name of Notified Substance

The common or usual name of the substance of this GRAS notice is LCLT. Other names commonly used for LCLT are L carnitine L tartrate, L-Carnitine tartrate and carnitine tartrate.

1.4 Conditions of Intended Use

Ausnutria intends to use LCLT as a nutrient source of L-Carnitine in powdered non-exempt IF based on either goat's or cow's milk protein for term infants up to 12 months of age. LCLT will be added at a maximum level of 0.6 mg/100 mL of the liquid IF as prepared with water or 0.88 mg/100 kcal LCLT. ^{1/}

Ausnutria does not intend to add LCLT to any products that are under the jurisdiction of the U.S. Department of Agriculture (USDA).

1.5 Statutory Basis for GRAS Conclusion

This GRAS conclusion is based on scientific procedures in accordance with 21 CFR 170.30(a) and 170.30(b).

1.6 Claim of Exclusion from the Requirement for Premarket Approval

Ausnutria has concluded that LCLT is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on our conclusion that LCLT's intended use is GRAS.

^{1/} The caloric value of a standard infant formula is 68 kcal/100 ml, accordingly, 0.88 mg/100 kcal = 0.6 mg/100 mL ÷ 68 kcal/100 ml.

1.7 Availability of Data and Information

The data and information that serve as the basis for this GRAS Notice will be maintained at the office of Ausnutria (address below) and will be made available to FDA either in electronic format or on paper for review and copying upon request during customary business hours.

Ausnutria B.V.
Dokter van Deenweg
150 8025 BM
The Netherlands
+31 (0) 88 11 63 600

1.8 Data Exempt from Disclosure:

None of the data or information in Parts 2 through 7 of the GRAS Notice are exempt from disclosure under the Freedom of Information Act (FOIA), 5 U.S.C. 552.

1.9 Certification

Ausnutria certifies that, to the best of its knowledge, this GRAS conclusion is based on a complete, representative, and balanced dossier that includes all relevant information, available and obtainable by Ausnutria, including any favorable or unfavorable information, and pertinent to the evaluation of the safety and GRAS status of the intended use of LCLT in term IF.

1.10 Name and Position/Title of Responsible Person Who Signs Dossier

The name and title of the individual signing off on this GRAS Notice is:



ir. Leoniek Robroch
Manager Regulatory Affairs at Ausnutria B.V.
Dokter van Deenweg 150
8025 BM Zwolle
The Netherlands

2. Part 2. IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS, AND PHYSICAL OR TECHNICAL EFFECT

2.1 Chemical Identity and Composition

LCLT is a salt comprised of L-Carnitine (**Figure 1**) and L-Tartaric acid (**Figure 2**). LCLT can be used as a nutrient source of L-Carnitine and is also the most commonly used form of L-Carnitine (Kohl and Scholl 1991). ^{2/}

General descriptive properties of LCLT are summarized below:

Chemical name: L(-) Carnitine L(+) Tartrate

Synonym(s): β -hydroxy- γ -trimethyl aminobutyrate, L-Tartaric acid

Chemical formula: C₁₈H₃₆N₂O₁₂

Molecular weight: 472.49

CAS Reg. Number: 36687-82-8

LCLT is a stable, white crystalline, free-flowing salt, and has a pleasant citric taste. The substance is non-hygroscopic and, therefore, is the optimal form for all powdered and solid products including capsules, tablets, bars, etc. (Schmidbaur, Schier et al. 1998). ^{3/} The physiological properties of the L-Carnitine component are not altered by the tartrate “complexation” (Schmidbaur, Schier et al. 1998, Walter and Schaffhauser 2000). ^{4/} Aqueous solutions and solid state structure analysis have shown that LCLT completely dissociates into L-Carnitine and L-Tartaric acid in aqueous solution (Schmidbaur, Schier et al. 1998)

Generally speaking, LCLT is prepared by reacting L-Carnitine with L-Tartaric acid. L-Carnitine is a quaternary ammonium salt that occurs naturally in animal-based foods such as milk and red meat. L-Tartaric acid occurs naturally in fruits and wines. Unlike L-Carnitine or L-Tartaric acid, LCLT (**Figure 3**) does not occur naturally in foods.

^{2/} Kohl, W. E. and T. Scholl (1991). Preparations containing L-Carnitine. U. S. Patent, Lonza Ltd.

^{3/} Schmidbaur, H., et al. (1998). "The Solution and Solid State Structure of L-Carnitine L-Tartrate." *Zeitschrift für Naturforschung B* **53**(8): 788.

^{4/} Walter, P. and A. O. Schaffhauser (2000). "L-Carnitine, a 'Vitamin-like Substance' for functional food. Proceedings Of the symposium on L-carnitine, April 28 to May 1, 2000, Zermatt, Switzerland." *Ann Nutr Metab* **44**(2): 75-96.

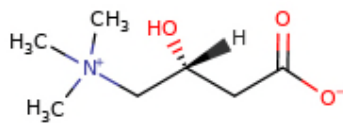


Figure 1. Chemical structure of L-Carnitine(ChemIDplus 2019a).

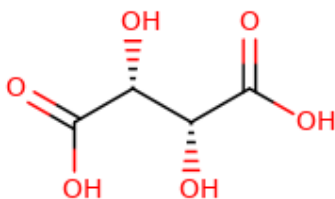


Figure 2. Chemical structure of L-Tartaric acid(ChemIDplus 2019b).

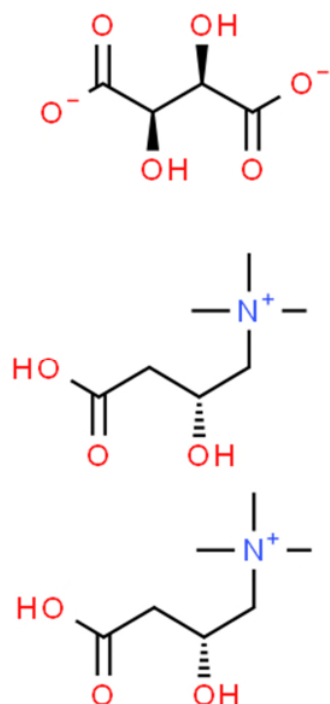


Figure 3. Chemical structure of LCLT(Chemspider 2019b).

2.2 Manufacturing Process

LCLT is produced by combining food grade L-Carnitine and L-Tartaric acid that meet the monographs of the Food Chemicals Codex (FCC) 12th Edition. The substances are first dissolved in deionized water. LCLT is then produced as a combination salt of crystalline free base L-Carnitine with L-Tartaric acid, and exists as a 2:1 ratio, being two molecules of L-Carnitine to one molecule of L-Tartaric acid (Walter and Schaffhauser 2000). The chemical reaction formula is described in **Figure 4**. The final LCLT molecule consists of 68% of L-Carnitine by weight and 32% of L-Tartaric acid.

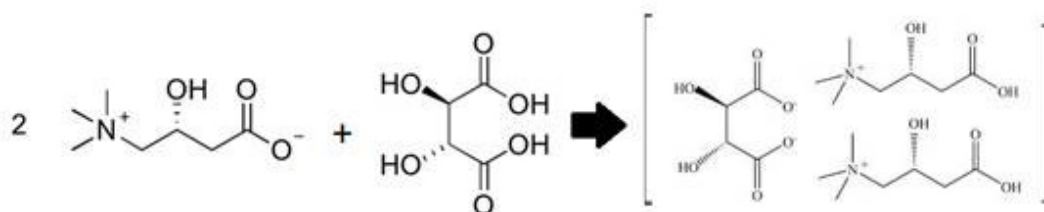


Figure 4. Chemical reaction formula for LCLT.

After the reaction, the compound is concentrated by vacuum drying. Food grade ethanol is added after which the crystallization step takes place and the product is cooled and centrifuged to obtain wet crystals. These wet crystals are then vacuum dried at 58-62 °C to obtain the dry crystals. These

dry crystals undergo sieving, metal detection, and are finally packaged. The reaction that takes place to form LCLT is a neutralization reaction, which is triggered by mixing an acid (i.e., L-Tartaric acid) with a base (i.e., L-Carnitine). The typical particle size of the LCLT is 20-80 mesh. No byproducts are formed.

The production process includes three Critical Control Points (CCPs) as shown in **Figure 5**.

CCP	Ascertain potential Hazard	CL *
CCP1-Loss on Drying	Ethanol residual	Control temperature, vaccum, time.
CCP2-Sieving	Foreign material	Check the filter integrity before and after filtration.
CCP3-Metal Detectiion	Metal foreign material	Fe2.5mm, Cu5.0mm, Sus4.5mm.

* CL = Critical Limits

Figure 5. The three CCPs during the manufacturing process of L-Carnitine-L-Tartrate

Based on monitoring data, the ethanol residual in the LCLT product complies with the “CMP/ICH/283/95 Impurities: Guideline for residual solvents” for pharmaceutical substances. For ethanol, a limit of 5,000 ppm (i.e., loss on drying $\leq 0.5\%$) is adopted. A flow chart of the production process can be found in **Figure 6**.

Flow chart:

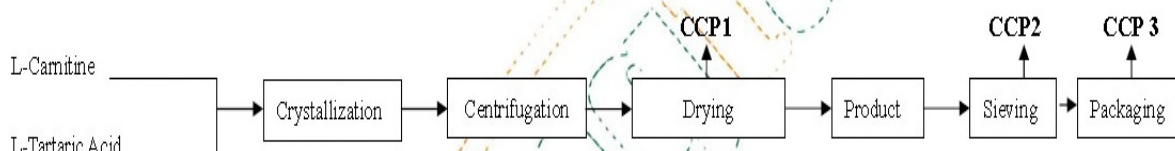


Figure 6. Flowchart of the Manufacturing Process of LCLT

2.3 Specifications and Batch Analysis

Table 1. Specifications of LCLT

Parameter	Limit	Test Methods
Appearance	Crystalline powder	Visual method
Identification	Pass	Internal FT-IR chemical method <u>5/</u>
Melting point	169-175 °C	USP43 <741> Melting range or temperature
Assay L-Carnitine	67.2-69.2%	Internal titration method <u>6/</u>
Assay L-Tartaric acid	30.8-32.8%	Internal titration method <u>7/</u>
Specific rotation	-11.0 to -9.5°	USP 43 <781> Optical Rotation
pH	3.0-4.5	USP 43 <791> pH
Loss on drying	≤ 0.5%	USP 43 <731> Loss on drying
Residue on ignition	≤ 0.2%	USP 43 <281> Residue on ignition
Arsenic	≤ 1 ppm	Ch. P 20200822
Chloride	≤ 0.4%	USP 43 <221> Chloride and sulfate
Lead	≤ 2 ppm	USP 43 <852> Atomic Absorption Spectroscopy

5/ This internal method is similar to USP 43 <197> Spectroscopic Identification test and uses USP 43 <197> sample preparation with potassium bromide. It is an FT-IR Potassium Bromide tablet method. Samples and controls are mixed homogeneously, and then placed in a tablet press to make a tablet. An IR spectra is then produced and compared to the IR spectra standard for LCLT provided by the Chinese standard GB 25550-2010.

6/ The internal method can be summarized as follows: accurately weigh 0.2 g of LCLT in a 250 ml conical flask with stopper, add 20 ml of acetic acid, use ultrasonic dissolving. Add a drop of Crystal violet IS, titrate with Perchloric acid VS (0.1 mol/L) VS to a pure blue end point. Also perform a blank determination. Each 1ml of 0.1M perchloric acid VS is equivalent to 0.01612 g of C₇H₁₅NO₃. The content of C₇H₁₅NO₃ should be in the range of 67.2%~69.2%.

7/ The internal method can be summarized as follows: accurately weigh 0.4 g of LCLT in a 250 ml conical flask with stopper, add 50 ml of water, use ultrasonic dissolving. Add a drop of phenolphthalein TS, titrate with Sodium hydroxide (0.1 mol/L) VS to a pale pink end point that lasts for 30 seconds without fading. Each 1 ml of 0.1 M Sodium hydroxide VS is equivalent to 0.007504 g tartaric acid. The content of tartaric acid should be in the range of 30.8%~32.8%.

Parameter	Limit	Test Methods
Cadmium	≤ 1 ppm	USP 43 <852> Atomic Absorption Spectroscopy
Mercury	≤ 0.1 ppm	USP 43 <852> Atomic Absorption Spectroscopy
Total plate count	< 1000 cfu/g	USP 43 <61> Microbiological test for non-sterilized products: Microbiological count test for microorganisms
Yeast & molds	< 100 cfu/g	USP 43 <61> Microbiological test for non-sterilized products: Microbiological count test for microorganisms
<i>E. Coli</i>	Absent in 10 g	USP 43 <62> Microbiological testing in non-sterilized products: specific microbiological testing
<i>Salmonella</i>	Absent in 10 g	USP 43 <62> Microbiological testing in non-sterilized products: specific microbiological testing
<i>Cronobacter sakazakii</i>	Absent in 300 g	ISO/TS 22964:2006

All test methods used to establish the specifications have been validated for their intended purpose and assays are on anhydrous basis. In addition, LCLT meets the specification of LCLT as listed in the first supplement of FCC 12th edition (**Table 2**).

We note that the FCC specifications include a spectrophotometric identification test which is not included in the current LCLT specifications. Instead, the product identification is tested by an internal chemical method, which is similar to USP 43 <197> Spectroscopic Identification test. Specifically, it is an FT-IR Potassium Bromide tablet method. Samples and controls are mixed homogeneously, and then placed in a tablet press to make a tablet. An IR spectra is then produced and compared to the IR spectra standard for LCLT provided by the Chinese standard GB 25550-2010. This specific method, like all other test methods, has been validated for its intended purpose.

Table 2. First supplement FCC12 Specifications for LCLT.

Parameter	FCC specifications
Melting point	169-175 °C
Identification	Spectrophotometric identification test
Specific rotation	-11.0 to -9.5°
pH	3.0-4.5
Loss on drying	≤ 0.5%
Residue on ignition	≤ 0.5%
Assay L-Carnitine	67.2-69.2%
Assay L-Tartaric acid	30.8-32.8%
Arsenic	≤ 1 mg/kg
Lead	≤ 2 mg/kg

Results of analyses demonstrate that five non-consecutive batches of LCLT meet the designated specifications, as shown in **Table 3**.

Table 3. Analytical Results for 5 Nonconsecutive Lots of LCLT.

Parameter	specifications	Results of Batch Numbers				
		1010022018 0527	10100220180 630	10100220108 0820	10100220190 231	101002201 90414
Appearance	Crystalline powder	Pass	Pass	Pass	Pass	Pass
Identification	pass	Pass	Pass	Pass	Pass	Pass
Melting point	169-175 °C	170-171.5 °C	170-171.5 °C	169-170.5 °C	169.5-171 °C	170-171.5
Assay L-Carnitine	67.2-69.2%	68.1%	67.96%	68.14%	67.74%	68.16%
Assay L-tartaric acid	30.8-32.8%	31.74%	31.68%	31.76%	31.79%	31.84%
Specific rotation	-11.0 to -9.5°	-10.2°	-10.08°	-10.05°	-10.30°	-10.04°
pH	3.0-4.5	3.64	3.66	3.63	3.60	3.64
Loss on drying	≤ 0.5%	0.3%	0.24%	0.28%	0.16%	0.32%
Residue on ignition	≤ 0.5%	0.04%	0.04%	0.04%	0.07%	0.05%
Arsenic	≤ 1 ppm	< 1 ppm	< 1 ppm	< 1 ppm	<1 ppm	<1 ppm
Chloride	≤ 0.4%	< 0.4%	< 0.4%	< 0.4%	< 0.4%	< 0.4%
Lead	≤ 2 ppm	< 2 ppm	< 2 ppm	< 2 ppm	< 2 ppm	< 2 ppm
Cadmium	≤ 1 ppm	< 1 ppm	< 1 ppm	< 1 ppm	< 1 ppm	< 1 ppm
Mercury	≤ 0.1 ppm	< 0.1 ppm	< 0.1 ppm	< 0.1 ppm	< 0.1 ppm	< 0.1 ppm
Total plate count	< 1000 cfu/g	30 cfu/g	30 cfu/g	30 cfu/g	30 cfu/g	20 cfu/g
Yeast & molds	< 100 cfu/g	10 cfu/g	10 cfu/g	10 cfu/g	10 cfu/g	10 cfu/g

Parameter	specifications	Results of Batch Numbers				
		1010022018 0527	10100220180 630	10100220108 0820	10100220190 231	101002201 90414
<i>E.Coli</i>	Absent in 10 g	Negative	Negative	Negative	Negative	Negative
<i>Salmonella</i>	Absent in 10 g	Negative	Negative	Negative	Negative	Negative
<i>Cronobacter sakazakii</i>	Absent in 300 g	Negative	Negative	Negative	Negative	Negative

2.4 Physical or Technical Effect

Within this GRAS notice LCLT is intended to be added only to term IF as a nutrient source of L-Carnitine. In aqueous solution, LCLT dissociates into L-Carnitine and L-Tartaric acid (Schmidbaur, Schier et al. 1998). Therefore, when LCLT is added to infant formulas which is prepared with water before serving, it enters the human gastrointestinal tract as dissolved L-Carnitine and L-Tartaric acid.

2.5 Regulatory Status of L-Carnitine-L-Tartrate, L-Carnitine, and L-Tartaric Acid

2.5.1 Regulatory Status of L-Carnitine-L-Tartrate

2.5.1.1 U.S. Regulatory History

In 2002, Lonza announced that its LCLT (Carnipure™ tartrate) product is GRAS when used as a functional food based on an independent GRAS determination (Eschenmoser 2002). ^{8/} Based on this determination, LCLT has been available for use in many food products in the U.S. LCLT is also listed in the National Foods Association (NNFA) and the Council for Responsible Nutrition (CRN) lists of ingredients that were used in dietary supplements prior to 1994 (pre-DSHEA).

2.5.1.2 European Regulatory History

On the European Union level, the European Food Safety Authority (EFSA) Scientific Committee evaluated the use of LCLT as a source of L-Carnitine in foods for nutritional uses (PARNUTS), including IF. When evaluating the petitioner’s proposed use level of LCLT in soy-based IF at 1.2 mg L-Carnitine/100 kcal, the EFSA noted LCLT readily dissociates into L-Carnitine and L-Tartaric acid in the gastrointestinal tract. ^{9/} EFSA also noted human tolerance of LCLT up to 3 g/day has been established in adults with respect to gastrointestinal symptoms, haematology and clinical chemistry, including markers of liver and kidney function. Further, the Acceptable Daily Intake for tartaric acid is of 0 – 30 mg/kg bodyweight. EFSA concluded that “L-carnitine-L-tartrate is not of concern from the safety point of view as a source of L-carnitine for use in foods for particular nutritional uses, provided the

^{8/} Eschenmoser (2002). Lonza's L-CARNIPURE® L-Carnitine Crystalline and L-Carnitine L-Tartrate are Generally Recognized as Safe (GRAS). New Hope Network. Boulder CO.

^{9/} EFSA Journal (2003)19, 1-13.

Acceptable Daily Intake for tartaric acid from all sources in the diet is not regularly exceeded.” ^{10/}

In Spain, the report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) of 2012 proposes a maximum daily amount of L-Carnitine of 2,000 mg for adults when using LCLT hydro chloride as sources, and an amount of 3,000 mg when using LCLT as a source in food supplements. The Royal Decree 867/2008 (BOE, 2008) regulates the inclusion of certain substances in the basic composition of IFs in Spain and includes the allowance of LCLT.

2.5.1.3 Other Regulatory History

The Codex Alimentarius Commission (CAC) lists LCLT as an allowed source of L-Carnitine in its advisory list of nutrient compounds for use in foods for special dietary uses intended for infants and young children (CAC/GL 10-1979), with no limit on its use levels. L-Carnitine is considered an essential component of IF (CODEX STAN 72-1981).

The Food Standards Australia New Zealand (FSANZ) has assessed an application to permit the use of LCLT as a source of L-Carnitine in a variety of food products and partially approved the request based on its finding that the current scientific evidence does not support that trimethylamine N-oxide (TMAO), a metabolite of L-carnitine, plays a causal role in initiating or promoting adverse cardiovascular effects. ^{11/} Further, FSANZ noted intake of L-Carnitine up to 3 g/day is not associated with adverse effects.

In Canada, L-Carnitine and acetyl-L-Carnitine are permitted novel food ingredients that can be used in a specific class of supplemented foods after obtainment of a Temporary Marketing Authorization Letter from Health Canada on a case-by-case basis (Food Standards Australia New Zealand 2018). Health Canada, in a monograph dated December 18, 2018, lists permitted uses of L-Carnitine. In this monograph, LCLT and L-Carnitine fumarate are listed as source materials for L-Carnitine (Health Canada 2018).

In China, LCLT is an approved source of L-Carnitine for use in special dietary foods, including IF (GB 14880-2012). L-Carnitine is considered an optional component of IF (GB 10765).

In Japan, LCLT is also approved for use in food by the Japanese Ministry of Health, Labor and Welfare. Both L-Carnitine and LCLT can be used in foods and dietary supplements with a maximum daily intake up to 1 g/day or 20 mg/kg bw/day.

2.5.2 Regulatory Status of L-Carnitine

There is no specific regulation permitting the use of L-Carnitine in IF in the US. FDA regulations on the nutrient requirements of IF (21 CFR 107.100(a)) currently do not require the addition of L-Carnitine. The specific function of L-Carnitine is as a “nutrient supplement”

^{10/} See *id.*

^{11/} FSANZ, Approval Report – Application A1102 L-Carnitine in food (May 16, 2019).

according to FDA regulation 21 CFR 170.3(o)(20). To the best of our knowledge, L-Carnitine has not been authorized as GRAS by the FDA through a regulation or GRAS notification as a nutrient and/or dietary supplement.

However, it is reported L- Carnitine has been added to soy-based IF products since 1986 and to cow's milk-based products since the mid-1990's (International Formula Council 2011). The basis for this is presumably the review and recommendations made by the Life Sciences Research Organization (LSRO) where they noted the need for L-Carnitine in infant nutrition and recommended that L-Carnitine be added to term IF at a level of 1.2-2.0 mg/100 kcal (Klein and Heird 2005). ^{12/}

2.5.3 Regulatory Status of L-Tartaric Acid

L-Tartaric acid is affirmed as GRAS under 21 CFR 184.1099 for use in food generally in accordance with 21 CFR 184.1(b)(1). This regulation does not explicitly permit the use of L-tartaric acid in IF. However, L-Tartaric acid is present in IF through the use of Choline Bitartrate (**Figure 7**). Choline Bitartrate, listed as GRAS by FDA under 21 CFR 182.5250 with no use limitations other than good manufacturing practice, is like LCLT regarding water solubility and dissociates in aqueous solution into its individual components, Choline and L-Tartaric acid.

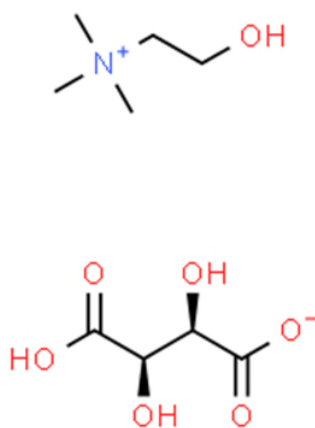


Figure 7. chemical structure of Choline Bitartrate (Chemspider 2019a).

In 2020, the EFSA Panel on Food Additives and Flavorings (FAF) provided a scientific opinion on the use of L-Tartaric acid and other -tartrates salt when used as food additives. ^{13/} The EFSA Panel developed a new ADI for L-Tartaric acid of 240 mg/kg bw/day. The ADI

^{12/} Klein, C. J. and W. C. Heird (2005). Summary and Comparison of Recommendations for Nutrient Contents of Low-Birth-Weight Infant Formulas Bethesda, Life Sciences Research Office, Inc. : 25.

^{13/} EFSA Journal 2020;18(3):6030.

was developed based on a chronic study in rats with no indication for carcinogenicity of monosodium L(+) tartrate reported at the highest dose tested (3,100 mg/kg bw per day).

In summary, the various safety thresholds established by reputable expert panels of regulatory agencies for LCLT or its components L-Carnitine and L-Tartaric acid can be summarized below:

- EFSA Journal (2003)19, 1-13: LCLT up to 3 g/day in adults or **50 mg/kg bw/day** (when assuming a body weight of an adult of 60 kg).
- EFSA Journal 2020;18(3):6030: ADI for L-Tartaric acid of **240 mg/kg bw/day**.
- LSRO: recommended that L-Carnitine be added to term IF at a level of **1.2-2.0 mg/100 kcal**.
- FSANZ, Approval Report – Application A1102 L-Carnitine in food (May 16, 2019) : intake of L-Carnitine up to 3 g/day or **50 mg/kg bw/day** (when assuming a body weight of an adult of 60 kg) is not associated with adverse effects.

2.6 Stability

2.6.1 Stability Data for *L-Carnitine-L-Tartrate*

Both a long-term (36-month) and an accelerated (6-month) stability study were conducted on the LCLT. For the long-term stability study samples were stored at a temperature of 25 ± 2 °C and a relative humidity of $60 \pm 5\%$ during the test period. Over the course of the study, three batches of LCLT were tested for assay (%), specific rotation (°), and water (%) at 0, 3, 6, 9, 12, 18, 24, and 36 months. The data of this study show that LCLT was stable over the entire duration of the study. A summary of the results of this stability study is presented in **Table 4**.

For the accelerated (6-month) stability study, samples were stored at a temperature of 40 ± 2 °C and a relative humidity of $75 \pm 5\%$. Over the course of this study, three batches of LCLT were tested for assay (%), specific rotation (°), and water (%) at 0, 1, 2, 3, 4, 5, and 6 months. The data of this study show that LCLT was stable over the entire duration of the study. A summary of the results of this accelerated stability study is presented in **Table 5**.

Table 4. LCLT Long-Term Stability Data.

1. Long - term Stability Test

1.1 Condition T: 25 ± 2° C RH: 60 ± 5%

1.2 Test result

Batch No.	Item	Specification	Test Date							
			0 months	3 months	6 months	9 months	12 months	18 months	24 months	36 months
20050504	Assay,% *	98.0~102.0	99.68	99.71	99.67	99.77	99.62	99.70	99.64	99.63
	Specific Rotation°	-9.5 ~ -11.0	-10.61	-10.73	-10.62	-10.58	-10.67	-10.72	-10.66	-10.65
	Water,%	≤ 0.5	0.14	0.11	0.13	0.15	0.12	0.16	0.17	0.14
20050507	Assay,% *	98.0~102.0	99.39	99.34	99.35	99.32	99.42	99.46	99.26	99.29
	Specific Rotation°	-9.5 ~ -11.0	-9.78	-9.75	-9.52	-9.68	-9.84	-9.62	-9.76	-9.65
	Water,%	≤ 0.5	0.21	0.25	0.19	0.24	0.23	0.22	0.20	0.21
20050509	Assay,% *	98.0~102.0	99.72	99.70	99.78	99.74	99.69	99.87	99.75	99.69
	Specific Rotation°	-9.5 ~ -11.0	-10.11	-10.25	-10.14	-10.24	-10.17	-10.20	-10.23	-10.19
	Water,%	≤ 0.5	0.26	0.24	0.28	0.27	0.25	0.23	0.26	0.25

* Assay of L-carnitine L-tartrate

Table 5. LCLT Accelerated Stability Data.

2. Accelerate Test

2.1 Condition T: $40 \pm 2^\circ \text{C}$ RH: $75 \pm 5\%$

2.2 Test result

* Assay of L-carnitine L-tartrate

Item Batch No.		Specification	Test Date						
			0 months	1 months	2 months	3 months	4 months	5 months	6 months
20050504	Assay,% *	98.0 ~ 102.0	99.68	99.80	99.62	99.75	99.58	99.66	99.72
	Specific Rotation°	-9.5 ~ -11.0	-10.61	-10.63	-10.56	-10.68	-10.49	-10.63	-10.68
	Water,%	≤ 0.5	0.14	0.20	0.18	0.15	0.19	0.18	0.16
20050507	Assay,% *	98.0 ~ 102.0	99.39	99.47	99.31	99.45	99.40	99.44	99.46
	Specific Rotation°	-9.5 ~ -11.0	-9.78	-9.83	-9.76	-9.81	-9.70	-9.73	-9.83
	Water,%	≤ 0.5	0.21	0.24	0.21	0.22	0.23	0.20	0.19
20050509	Assay,% *	98.0 ~ 102.0	99.52	99.53	99.55	99.50	99.64	99.46	99.48
	Specific Rotation°	-9.5 ~ -11.0	-10.11	-10.17	-10.26	-10.19	-10.21	-10.01	-10.18
	Water,%	≤ 0.5	0.26	0.25	0.28	0.25	0.27	0.24	0.23

3. Part 3. DIETARY EXPOSURE

3.1 Estimate of Dietary Exposure to the Substance

3.1.1 *Intended Use*

Ausnutria intends to use LCLT as a nutrient source of L-Carnitine in powdered IF formula for full term infants based on cow's or goat's milk protein achieving the recommended L-Carnitine level as proposed by international regulating bodies (Raiten, Safety et al. 1998, European Food Safety Authority 2014, FAO/WHO 2016). ^{14/} Not all L-Carnitine present in IF necessarily originates from added LCLT, as some L-Carnitine is naturally occurring in the milk source used. Ausnutria intends to supplement its IF, based on either cow's milk or goat's milk protein, with a maximum of 0.6 mg per 100 ml IF.

3.1.2 *Estimated Daily Intake from Infant Formula*

LCLT does not occur naturally in foods. For the purpose of this assessment, we assume the only source of LCLT for term infants up to 12 months old would be from the IF with LCLT added.

In FDA's guidance titled "Preparation of Food Contact Notifications for Food Contact Substances in Contact with Infant Formula and/or Human Milk: Guidance for Industry" (2019), ^{15/} FDA provided a default value for both infant body weight (6.3 kg-bw/infant) and infant food consumption (900g formula/infant/day) that were determined based on the 2-day 2005-2010 National Health and Nutrition Examination Survey (NHANES) food consumption survey. These values resulted in a consumption-to-mass ratio of 140 grams per kilogram body weight per day (140 g/kg bw/d), or 0.14 kg/kg bw/d. The agency further recommend calculating the estimated daily intake (EDI) of the Food Contact Substance (FCS) for infants by multiplying the migration of the substance to infant food (in parts per billion (ppb) or micrograms per kilogram ($\mu\text{g}/\text{kg}$)) by 0.14 kg/kg bw/d. While the guidance is for the exposure assessment of the FCS, the same consumption-to-mass ratio of 0.14 kg/kg bw/d can also be adopted here for the EDI calculation.

We also note in the GRAS Notice 855, which the agency favorably reviewed in 2020, the notifier referenced a 90th percentile infant formula intake of 207 mL/kg bw/day or 0.2 kg/kg bw/day from public literature. ^{16/} It is also reported that infants consume 1,200 ml (gram) of IF a day at the 95th percentile (Efsa Scientific Committee, Hardy et al. 2017). Assuming 6.1 kg as a representative weight for an infant aged <111 days old (Efsa Scientific Committee, Hardy et al. 2017), this also translates to around 0.2 kg/kg bw/day, which is higher than the FDA default consumption-to-mass ratio, and equivalent to the 90th percentile intake reported in public

^{14/} Raiten, D. J., et al. (1998). Assessment of Nutrient Requirements for Infant Formulas: LSRO Report ; Prepared for the Center for Food Safety and Applied Nutrition, Food and Drug Administration, Department of Health and Human Services, Washington, DC 20204 und Contract No. 223-92-2185, American Inst. of Nutrition.

^{15/} FDA, Preparation of Food Contact Notifications for Food Contact Substances in Contact with Infant Formula and/or Human Milk: Guidance for Industry" (2019), *available at:* <https://www.fda.gov/media/124714/download>

^{16/} Fomon SJ. 1993. Energy intake by normal infants. In Nutrition of Normal Infants, p. 104–111. Baltimore, MD: Mosby.

literature. ^{17/} For the purpose of conservativeness, in this GRAS notice, we will use the 1,200 mL and 6.1 kg reported by EFSA as the IF intake and infant body weight to calculate the dietary exposure.

With a maximum intake of 1,200 ml IF/day, an infant's total LCLT intake is $12 \times 0.6 \text{ mg} = 7.2 \text{ mg}$ LCLT per day. Assuming 6.1 kg as a representative weight for an infant aged <111 days old, this results in a total daily LCLT exposure of 1.18 mg/kg bw/day. ^{18/} Because the final LCLT molecule consists of 68% of L-Carnitine by weight and 32% of L-Tartaric acid, the intended use would result in a daily L-Carnitine intake of 0.8 mg/kg bw/day and a daily L-Tartaric acid intake of 0.38 mg/kg bw/day. ^{19/} These estimated daily intake or EDI from the intended use can be further summarized below:

- LCLT intended use level: **1.18 mg/kg bw/day**
- L-Carnitine component from LCLT's intended use: **0.8 mg/kg bw/day**
- L-Tartaric acid component from LCLT's intended use: **0.38 mg/kg bw/day**

Further, because many infant formulas may also contain both L-Carnitine and L-Tartaric acid from sources other than the addition of LCLT, the cumulative estimated daily intake of infants of both L-Carnitine and L-Tartaric acid will be discussed in more detail below.

3.1.2.1 Cumulative Estimated Daily Intake of L-Carnitine

Ausnutria intends to add up to 0.6 mg LCLT per 100 ml IF (0.88 mg LCLT/100 kcal). This equals to an amount of 0.408 mg L-Carnitine/100 ml IF. ^{20/} Ausnutria's formula also contains 0.885 ($\pm 10\%$) mg L-Carnitine/100 ml IF from a natural source. This results in a total concentration of L-Carnitine in Ausnutria's infant formula of around 1.29 mg/100 ml (1.9 mg/100 kcal). ^{21/}

It is reported that infants eat 1,200 ml (gram) of IF a day at the 95th percentile (Efsa Scientific Committee, Hardy et al. 2017). With a maximum intake of 1,200 ml IF/day, an infant's total L-Carnitine intake is $12 \times 1.29 \text{ mg} = 15.5 \text{ mg}$ L-Carnitine per day. Assuming 6.1 kg as a representative weight for an infant aged <111 days old (Efsa Scientific Committee, Hardy et al. 2017), this results in an estimated cumulative total daily carnitine exposure of 2.54 mg/kg bw/day ^{22/}

^{17/} $0.2 \text{ mg/kg bw/day} = 1.2 \text{ mg/day} \div 6.1 \text{ kg bw}$.

^{18/} $1.18 \text{ mg/kg bw/day} = 7.2 \text{ mg LCLT/day} \div 6.1 \text{ kg bw}$.

^{19/} $0.8 \text{ mg/kg bw/day} = 1.18 \text{ mg/kg bw/day} * 68\%$; $0.38 \text{ mg/kg bw/day} = 1.18 \text{ mg/kg bw/day} * 32\%$.

^{20/} $408 \text{ ug L-Carnitine} / 100 \text{ ml IF} = 0.6 \text{ mg LCLT} / 100 \text{ ml IF} * 68\%$.

^{21/} $1.29 \text{ mg/100 ml} = 408 \text{ ug} / 100 \text{ ml} + 885 \text{ ug} / 100 \text{ ml}$.

^{22/} $2.54 \text{ mg/kg bw/day} = 15.5 \text{ mg L-Carnitine/day} \div 6.1 \text{ kg bw}$.

3.1.2.2 Cumulative Estimated Daily Intake of L-Tartaric acid

L-Tartaric acid occurs in many fruits, free or combined with potassium, calcium or magnesium (Tartrates Informatics Inc 1974). Ausnutria intends to add 0.6 mg LCLT per 100 ml IF. This equals to an amount of 0.192 mg of L-Tartaric acid per 100 ml formula. ^{23/} Formulas often also contain L-Tartaric acid added as counterion for Choline in Choline Bitartrate. Breast milk contains about 6 mg free Choline/100 ml (Zeisel, Char et al. 1986). If 6 mg Choline is added to IFs in the form of Choline Bitartrate, then 15 mg of Choline Bitartrate needs to be added to 100 ml of formula. ^{24/} As 15 mg of Choline Bitartrate yields 6 mg of Choline and 9 mg of L-Tartaric acid, IF may contain up to 9 mg of L-Tartaric acid per 100 ml of formula when Choline is added as Choline Bitartrate.

The estimated daily intake of L-Tartaric acid from the addition of LCLT (i.e., 0.192 mg/100 mL) is therefore insubstantial when compared to that from the potential use of Choline Bitartrate (i.e., 9 mg/100 mL). When the amount of L-Tartaric acid coming from LCLT is added to the amount of L-Tartaric acid possibly already present in IF due to the use of Choline Bitartrate as Choline source, then the total L-Tartaric acid concentration is 9 mg + 0.192 mg = 9.19 mg L-Tartaric acid per 100 ml formula.

Again, assuming maximally 1,200 gram of IF a day for infants, their maximal total L-Tartaric acid intake is $12 \times 9.19 \text{ mg} = 110.3 \text{ mg}$ L-Tartaric acid per day. If 6.1 kg is taken as a representative weight for an infant aged <111 days this results in an estimated cumulative total daily exposure of 18.1 mg L-Tartaric acid/kg bw/day. ^{25/}

3.1.2.3 Dietary Recommendations for L-Carnitine in IF

Carnitine deficiency is assessed by measuring free and total L-Carnitine concentrations in serum. As reviewed by Crill and Helms (2007), ^{26/} infants fed un-supplemented soy-based formula, which contains little or no L-Carnitine, had lower serum L-Carnitine concentration compared to infants fed carnitine-supplemented soy-based formula suggesting infants lack capacity to synthesize sufficient L-Carnitine. Because of the critical role of L-Carnitine in lipid metabolism and the decreased rate of L-Carnitine biosynthesis in infants, L-Carnitine has now been considered to be a necessary addition to infant formula with a minimum amount corresponding to breast milk (Crill and Helms 2007, European Food Safety Authority 2014).

FDA commissioned an Expert Panel of the Life Sciences Research Office (LSRO) for recommendations on IF. This was published in the comprehensive review "Assessment of Nutrient Requirements for Infant Formulas" in 1998. The Expert Panel recommended L-Carnitine content of IFs of 1.2-2.0 mg/100 kcal based on levels found in human milk (Raiten, Safety et al. 1998). The LSRO Expert Panel was unaware of any studies in which a no-observed-adverse-effect

^{23/} 192 ug L-Tartaric acid /100 ml IF = 0.6 mg LCLT /100 ml IF * 32%.

^{24/} 15 mg of Choline Bitartrate = 6 mg Choline + 9 mg L-Tartaric acid.

^{25/} 18.1 mg/kw bw/day = 110.3 mg L-Tartaric acid/day ÷ 6.1 kg bw.

^{26/} Crill, C. M. and R. A. Helms (2007). "The Use of Carnitine in Pediatric Nutrition." Nutrition in Clinical Practice **22**(2): 204-213.

level (NOAEL) or Lowest Observed Adverse Effect Level had been identified for L-Carnitine exposure in infants. Consequently, in the absence of such data the Expert Panel concluded that the maximum should be set at a level comparable to the upper ranges of L-Carnitine concentrations reported for human milk (Raiten, Safety et al. 1998). In 2002, LSRO (Klein 2002) recommended the addition of L-Carnitine to preterm IF: The Expert Panel recommended that the minimum concentration of L-Carnitine in preterm IF shall be 2.0 mg/100 kcal. The Expert Panel recommended that the maximum concentration of L-Carnitine in preterm IF shall be 5.9 mg/100 kcal (= 4 mg L-Carnitine/100 ml).

The L-Carnitine content of IF as proposed by Ausnutria after having supplemented natural L-Carnitine levels from milk with LCLT is 1.9 mg L-Carnitine/100 kcal (1.29 mg / 100 ml) of IF. Notably, this level is within the range of 1.2-2.0 mg/100 kcal of L-Carnitine recommended by LSRO in term IF.

3.1.3 Estimated Dietary Exposure to L-Carnitine and L-Tartaric acid via Other Food

L-Carnitine and L-Tartaric acid are also naturally occurring in many food products that can be consumed by infants after weaning. A dietary assessment for infants aged 0-12 months is difficult, partly due to the unestablished dietary habits for solid foods and deviating portion sizes. For this reason, published data on dietary intake is limited for this age category.

Dietary sources rich in L-Carnitine include meat, poultry, fish, and milk. At the age of 7-8 months; meat, poultry, and fish may be introduced to the infant's diet, whereas regular cow's milk is advised from 12 months onwards (Centers for Disease Control and prevention 2018). An average portion size of meat at ages from 9 through 11 months was estimated to be 0.8 oz (~22.6 g) (Fox, Reidy et al. 2006). ^{27/} Assuming an average L-Carnitine content of 109 mg per 100 g meat (National Institutes of Health and Office of dietary supplements 2017), the maximum daily intake would be 24.6 mg L-Carnitine. For an infant weighing 6.1 kg (Efsa Scientific Committee, Hardy et al. 2017), this leads to an exposure of 4.1 mg L-Carnitine/kg bw/day.

In apples, the estimated average L-Tartaric acid content has been established to be 1.75 mg per ml (Khosravi, Rastakhiz et al. 2015), ^{28/} whereas the average content of grapes varies from 3.61 to 3.82 mg per ml (on average 3.72 mg per ml) depending on environmental exposures (Liu, Wu et al. 2006). ^{29/} According to estimated fruit juice intake from the Feeding Infants and Toddlers Study (FITS) population (Fox, Reidy et al. 2006, Kay, Welker et al. 2018), ^{30/} the average L-Tartaric acid content was estimated to be 155 mg/portion for grape juice and 73 mg/portion for

^{27/} Fox, M., et al. (2006). "Average Portions of Foods Commonly Eaten by Infants and Toddlers in the United States." *Journal of the American Dietetic Association* **106**: S66-76.

^{28/} Khosravi F., et al. (2015) Determination of Organic Acids in Fruit juices by UPLC *International Journal of Life Sciences* **9**: 41-4.

^{29/} Liu HF, Wu BH, Fan PG, Li SH, Li LS. Sugar and acid concentrations in 98 grape cultivars analyzed by principal component analysis. *Journal of the Science of Food and Agriculture* 2006; **86**: 1526-36.

^{30/} Kay MC, Welker EB, Jacquier EF, Story MT. Beverage Consumption Patterns among Infants and Young Children (0(-)47.9 Months): Data from the Feeding Infants and Toddlers Study, 2016. *Nutrients* 2018; **10**.

apple juice. Grape juice and apple juice were chosen as these were reported in the FITS study population (Fox, Reidy et al. 2006), and these estimations will give a probable range of L-Tartaric acid that US infants may be exposed to in real-life settings.

4. Part 4. SELF-LIMITING LEVELS OF USE

There is no self-limiting level of use. The LCLT is intended for use in IF by Ausnutria and the levels for use will be specified in a new infant formula premarket notification that will be submitted to FDA prior to marketing the product in the United States.

5. Part 5. EXPERIENCE BASED ON COMMON USE IN FOOD BEFORE 1958

This part is not applicable to this GRAS dossier.

6. Part 6. NARRATIVE AND SAFETY INFORMATION

Ausnutria will be adding LCLT at a level of 0.6 mg/100 mL to IF as a nutrient source for L-Carnitine. In aqueous solution, LCLT dissociates into L-Carnitine and L-Tartaric acid. As L-Carnitine and L-Tartaric acid coming from LCLT in solution are identical to L-Carnitine and L-Tartaric acid in solution from other dietary sources, and the two substances are not known to mutually interact at a functional level, the safety assessments for LCLT components L-Tartaric acid and L-Carnitine also apply to the LCLT when used in IFs.

6.1 GRAS Criteria

The objective of this safety assessment is to ascertain whether the intended use of LCLT as a nutrient source of L-Carnitine in IF, with defined use levels, meets the GRAS safety criteria for reasonable certainty of no harm.

6.2 Safety Studies Review

Below, we review the metabolism, animal, and human safety studies that support our safety determination for the intended use of LCLT in more details.

6.2.1 *Absorption, Distribution, Metabolism and Excretion of LCLT*

LCLT will be present as a powder in powdered IFs. Before the powdered IFs are prepared for consumption, they are mixed with water and mildly heated to body temperature. Under these conditions, LCLT dissolves completely and forms only L-Carnitine and L-Tartaric acid components in aqueous solution as demonstrated by optical rotation and conductivity measurements and ion chromatography (Schmidbaur, Schier et al. 1998). The absorption, distribution, metabolism and excretion of the LCLT components L-Carnitine and L-Tartaric acid will be discussed separately below.

6.2.1.1 *Absorption, Distribution, Metabolism and Excretion of L-Carnitine*

The pharmacokinetics of L-Carnitine resulting from dosing LCLT and from L-Carnitine itself are practically identical (Eder, Felgner et al. 2005). ^{31/} The pharmacokinetics of L-Carnitine has been well documented by public literature (Reuter and Evans 2012). ^{32/}

L-Carnitine behaves like a water-soluble bioactive food ingredient and its plasma concentrations are largely regulated by absorption, excretion and metabolism. Dietary L-carnitine is actively and passively absorbed across enterocyte membranes. The bioavailability of L-Carnitine from a normal Western diet has been estimated to be 54–87% (Rebouche and Chenard 1991, Rebouche

^{31/} Eder, K., et al. (2005). "Free and total carnitine concentrations in pig plasma after oral ingestion of various L-carnitine compounds." *Int J Vitam Nutr Res* **75**(1): 3-9.

^{32/} Reuter, S. E. and A. M. Evans (2012). "Carnitine and acylcarnitines: pharmacokinetic, pharmacological and clinical aspects." *Clin Pharmacokinet* **51**(9): 553-572.

2004), ^{33/} whereas bioavailability at higher oral doses, achievable from dietary supplements, has been estimated at 14–18%. Passive absorption of L-Carnitine is seen from dietary supplements (Rebouche 2004). Time to maximum plasma concentration after oral administration of L-Carnitine at doses of 0.5 to 6 g is reported to be 3–5 hours (Reuter and Evans 2012).

The kinetics of plasma L-Carnitine is performed by either one large and slow-turnover (muscle), or relatively small and with rapid-turnover (liver, kidney and other tissues). It is reported that approximately 97% of total body L-Carnitine is present in muscle, with only ~0.1% in plasma. The mean turnover time of L-Carnitine in skeletal muscle has been reported to be 105 hours (Reuter and Evans 2012).

L-Carnitine not absorbed following oral ingestion is converted to trimethylamine (TMA) by intestinal microbiota. TMA is then absorbed and metabolized to trimethylamine-N-oxide (TMAO; abbreviated as TMNO in some publications) in the liver by flavin monooxygenase. Gut microbiota can also convert L-Carnitine to gamma- butyrobetaine which is primarily excreted in feces.

Efficient renal reabsorption of L-Carnitine occurs at normal circulating concentrations. Efficiency of renal reabsorption decreases and urinary clearance increases after high-dose intravenous or oral administration of L-Carnitine, resulting in rapid decline of circulating L-Carnitine concentrations to baseline (Rebouche 2004). Generally, the renal clearance of L-Carnitine (1-3 mL/min) is considerably less than glomerular filtration rate, suggesting extensive (98-99%) tubular reabsorption. TMAO is excreted in urine (Taesuwan, Cho et al. 2017). ^{34/}

6.2.1.2 Absorption, Distribution, Metabolism and Excretion of L-Tartaric acid

Just like L-Carnitine, L-Tartaric acid behaves like a water-soluble food ingredient which is sparsely taken up and metabolized by humans. The metabolic fate of L-Tartaric acid in humans has been well documented by public literature including the publication by Chadwick, Vince et al. (1978). ^{35/} About 18% of the L-Tartaric acid is absorbed upon ingestion by human. Most of this absorbed fraction is then excreted unchanged in the urine. The rest, or about 22.2% of the absorbed, is metabolized in the tissues into CO₂. The unabsorbed fraction ends up in the intestines, where the majority is metabolized by intestinal bacteria, and the rest (about 6.1%) gets excreted as feces.

^{33/} Rebouche, C. J. and C. A. Chenard (1991). "Metabolic fate of dietary carnitine in human adults: identification and quantification of urinary and fecal metabolites." *J Nutr* **121**(4): 539-546; Rebouche, C. J. (2004). "Kinetics, pharmacokinetics, and regulation of L-carnitine and acetyl-L-carnitine metabolism." *Ann N Y Acad Sci* **1033**: 30-41.

^{34/} Taesuwan, S., et al. (2017). "The metabolic fate of isotopically labeled trimethylamine-N-oxide (TMAO) in humans." *J Nutr Biochem* **45**: 77-82.

^{35/} Chadwick, V. S., et al. (1978). "The metabolism of tartrate in man and the rat." *Clin Sci Mol Med* **54**(3): 273-281.

6.2.2 Animal Safety Studies of LCLT

- (LPT Laboratory of Pharmacology and Toxicology 2003) 36/

100 Rats, aged 41 to 43 days old at enrollment, were treated with 0, 0.25%, 1.25% and 5.0% w/w LCLT in the diet for 90 days. 20 rats per group were included for the two lowest doses (0.25% and 1.25%) and 30 rats per group for the control group and 5.0% group, resulting in a total of 100 rats. Distribution of sex was equal. The rats were sacrificed after a 90 day intervention period, whereas 10 rats in the control and 5.0% group were sacrificed after a 4-week recovery period after the 90-day intervention. Rats were provided with *ad libitum* food and water and were individually housed under standard laboratory environmental conditions. Rats were daily inspected, weekly measured on body weight, food consumption, and quantitative water consumption in weeks 6 and 12. Ophthalmoscopic measurements, urine and blood samples were collected from overnight fasted rats in the week prior to end of intervention. Rats were killed under light anesthesia by exsanguination and a gross necropsy was performed.

Daily and weekly observation revealed soft feces and increased water and food consumption in all animals undergoing the highest LCLT dose of 5.0%, whereas these changes reversed rapidly during the recovery period. Non-statistically significant decreases in body weight were also observed. No effect of intervention was observed in the other treatment groups. Group mean absolute and relative organ weights of seminal vesicles were lower than the control males. This was considered related to treatment, although there was no associated histopathology. Moreover, this phenomenon was not apparent at necropsy of males in the recovery cohort, indicating a reversible effect. No other effect of LCLT on organ weight was observed. No effect on hematology, clinical chemistry, urinary electrolytes, ophthalmologic findings, gross necropsy findings or histopathological findings were observed.

The study concluded that the NOAEL corresponds to the mean intake for the 5.0% group, or 3,934 mg/kg bw/day for males, and 5,042 mg/kg bw/day for females. Notably, having reviewed the data of this study, FSANZ concurred with the NOAEL values developed. (Food Standards Australia New Zealand 2018).

6.2.3 Animal Safety Studies of L-Carnitine and its Salts

6.2.3.1 Acute toxicity studies

- (Narita, Yamate et al. 1988) 37/

Five week old Crj:CD rats were treated with L-Carnitine Chloride dissolved in water in a volume of 20 ml/kg bw/day at dose levels of 0, 5,390, 6,200, 7,130, 8,200, 9,430, 10,845 and 12,470 mg/kg bw/day). The animals were observed during administration, after 30 minutes, and after 1, 3, 6 hours, and then twice a day for 15 days. In male rats, the calculated acute oral LD₅₀ (95%

36/ LPT Laboratory of Pharmacology and Toxicology (2003). 90-day Subchronic Toxicity Study of LZ1780 by Repeated Oral Administration via the Diet to CD® Rats – According to OECD Guideline 408, Lonza.

37/ Narita, N., et al. (1988). "Acute toxicity study of L-carnitine chloride in rats." *Iyakuhi Kenkyu* **19**(2): 191-196.

confidence limit) for L-Carnitine Chloride was determined to be 6,900 (6,330–7,521) mg/kg bw/day and 6,890 (6,380–7,441) mg/kg bw/day in female rats.

- (Kudo, Watanabe et al. 1988a) 38/

Another acute study using 10-day or 22-day old Crj:CD rats that were treated with L-Carnitine Chloride dissolved in water by gavage. Dose levels studied were 0, 3,228, 3,993, 4,792, 5,750, 6,900 and 8,280 mg/kg bw/day. For the 10-day old rats, the observed LD₅₀ was 4,374 mg/kg bw/day (95% CI: 3,995-4,790 mg/kg bw/day) in males and 4,578 mg/kg bw/day (95% CI: 4,128-5,093 mg/kg bw/day) in females. For the 22-day old rats, the LD₅₀ was determined to be 6,127 mg/kg bw/day in males (95% CI: 5,501-6,824 mg/kg bw/day) and 6,299 mg/kg bw/day in females (95% CI: 5,679-6,987 mg/kg bw/day).

- (Toshida and Wada 1988). 39/

Slc:ddY mice were five weeks old at the time of dosing after one week of husbandry. Animals were kept with five animals per cage under standardized temperature, humidity, and light-time. Animals were fed with commercial solid feed and had *ad libitum* access to water. L-Carnitine Chloride was dissolved in water and then dosed by oral gavage using a volume of 2 ml/kg bw/day. Doses equaled to 6,000, 7,200, 8,640, 10,400 and 12,400 mg L-Carnitine/kg bw/day. After administration, mice were observed for 4 to 6 hours, and then daily for up to day 14 or 21. Based on the mortality data, the estimated acute oral LD₅₀ of L-Carnitine Chloride was determined to be 8,200 mg/kg bw/day in the male mice and 8,000 mg/kg bw/day in the female mice.

- (Toshida and Wada 1988). 40/

Japanese white rabbits were treated with L-Carnitine Chloride at 15 weeks of age after approximately 3 weeks of preliminary husbandry. Animals were dosed by oral gavage of solutions of L-Carnitine Chloride in water at dose levels of 3,610, 4,330, 5,200, 6,240 or 7,490 mg/kg bw/day. Rabbits were individually housed under standardized conditions, were fed solid feed and had free access to water. After treatment, rabbits were observed for 4 through 13 hours and then daily up to 14 days. The acute oral LD₅₀ of L-Carnitine Chloride in the male rabbits was determined to be 5,400 mg/kg bw/day whereas in the female rabbits, it was determined to be 6,000 mg/kg bw/day.

38/ Kudo, S., et al. (1988a). "Acute toxicity studies of L-Carnitine chloride in 10- and 22-day-old and 5-week-old rats." *Iyakuin Kenkyu* **19**(4): 689-699.

39/ Toshida, K. and Y. Wada (1988). "Acute toxicity studies of L-carnitine chloride in mice and rabbits." *Iyakuin kenkyu* **19**(3): 446-453.

40/ *See id.*

- (Kikumori, Kida et al. 1988a) 41/

Both male and female seven-month old beagle dogs (n=6 per group) were treated with L-Carnitine Chloride in gelatin capsules. In a preliminary study, all dogs vomited at an administration of a single dose of 1,000 mg/kg BW, or twice 800 mg/kg bw/day at 2- or 4-hour intervals. 42/ Therefore, the dosing regimen selected for the definitive study was a single oral dose of 800 mg/kg bw/day or two oral doses of 800 mg/kg bw/day with a two-hour interval. After administration, all dogs were maintained on study for 14 days before being sacrificed and necropsied. None of the treated dogs died, and no treatment-related adverse effects were discovered at necropsy, and histopathological assessment. The authors concluded that the acute oral LD50 of L-Carnitine Chloride in beagle dogs was at least 1,600 mg/kg bw/day.

6.2.3.2 Sub-chronic Safety Studies

- (Wu, Zhang et al. 2020) 43/

20 mice were separated into two groups with one control and the other with 3% L-Carnitine in drinking water for 12 weeks. This translates to about 4,500 mg/kg bw/day L-Carnitine. 44/ The authors selected 3% as the dose because preliminary experiments with 1%, 1.5%, 2%, 2.5%, and 3% of L-Carnitine in drinking water indicated that the health of mice given 3% L-Carnitine drinking water was negatively impacted. The mice were then executed and blood samples were collected to analyze for clinical index assay of the livers including ALT and AST. Gut microbiota analysis and histopathological observation of liver tissues were also conducted. The study reported significant increases in hepatic injury indexes (ALT and AST) in high L-Carnitine mice when compared to the control group, as well as corresponding changes in gut microbiota (e.g., *Anaerobiospirillum*, *Akkermansia_muciniphila*. and *Helicobacter*).

The authors concluded that a high intake of L-Carnitine (i.e., 4,500 mg/kg bw/day L-Carnitine) could induce a liver function decline by disordering the gut bacterial composition of mice resulting in an increased TMAO metabolism.

The study involved administration of L-Carnitine at levels magnitudes much higher than the 0.8 mg/kg bw/day covered by this GRAS dossier. More importantly, other studies evaluating TMAO in rat and mice models have explored whether increased TMAO plasma levels may be protective in atherosclerosis. As explained in section 6.2.3.4, below, these studies show that at very high

41/ Kikumori, M., et al. (1988a). "Subacute toxicity study of l-carnitine chloride in beagles." *Iyakuin Kenkyu* **19**(2): 244-260.

42/ The LD50 value was not determined in an earlier acute study for the oral route because of vomiting. Kikumori, M., et al. (1988b). "Acute toxicity study of l-carnitine chloride in beagles." *Iyakuin Kenkyu* **19**(2): 238-243.

43/ Wu, Qiu, et al. "High l-Carnitine Ingestion Impairs Liver Function by Disordering Gut Bacteria Composition in Mice." *Journal of Agricultural and Food Chemistry* **68.20** (2020): 5707-5714.

44/ According to EFSA Guidance titled "Guidance on Default assumptions used by the EFSA Scientific Panels and Committee, and EFSA Units in the absence of actual measured data" available at: <https://www.efsa.europa.eu/sites/default/files/consultation/110707a.pdf>, 1 mg/L test compound in drinking water for mice in subchronic studies translates to 0.15 mg/kg bw/day. As such, 3% or 30,000 mg/L translates to 4,500 mg/kg bw/day.

levels (i.e., 1,700 mg/kg bw/day), L-Carnitine may induce atherosclerosis in APOE^{-/-} mice while at lower exposure levels such as 351.9 mg/kg bw/day, no adverse cardiovascular effects were noted in both rats and mice.

- (Yamate, Shinoda et al. 1988) 45/

In another rat study divided in two parts used Crj:CD rats that were individually housed under standard laboratory environmental conditions and provided with food and water *ad libitum*. Doses of L-Carnitine Chloride were based on a preliminary 14-day dose-range finding study. Study I used dose levels of 0, 100, 450, 1,500 and 2,000 mg/kg bw/day administered by gavage for 91 days in 15 rats per sex/group. Study II set doses of 0, 1,500 and 5,000 mg/kg bw/day for 91 days and administered in 10 rats per sex/group by gavage.

During the study period, survival status, clinical observations, food consumption and water intake were reported on a weekly basis. Ophthalmologic findings were reported on day 45 and at the end of the study. Blood samples and 24-hour urine was collected prior to termination for urinalysis and from fasted, anesthetized rats. All rats were subject to necropsy, and fresh organ weights were recorded. Treatment-related deaths only occurred in the 5,000 mg/kg bw/day group, with mortality rates of 17/25 males and 18/25 females. Clinical signs of loose feces were observed only in groups dosed with $\geq 1,500$ mg/kg bw/day and were treatment-related. Suppression of food consumption and bodyweight gain was observed in the 1,500 mg/kg bw/day group compared to the control group values, but this observation reversed during the recovery period. Water intake was increased during the dosing phase in both sexes of the 1,500 and 5,000 mg/kg bw/day group, but this effect was also reversible during the recovery phase for the 1,500 mg/kg bw/day group. Treatment had no effect on ophthalmological findings.

Urinalyses showed treatment-related increase in specific gravity, potassium and chloride in males treated with 1,500 mg/kg bw/day. Decreases were observed in sodium and in pH. Females in the same dose group also showed an increase in chloride and a decrease in pH. These effects were reversible during the recovery period. Hematological tests showed that rats in the 1,500 mg/kg bw/day group had significant decreases in group mean total WBC and lymphocyte count, which were reversible. Total blood protein, albumin, total cholesterol, triglycerides, creatinine and lactate dehydrogenase were increased only in females of the 1,500 mg/kg bw/day group. Increased phospholipids were observed in ≥ 450 mg/kg bw/day groups. Compared to sex-matched controls, both sexes in the 1,500 mg/kg bw/day group had significantly decreased group mean serum sodium and chloride. At the end of the recovery period, group mean serum creatinine of the 1,500 mg/kg bw/day females was lower than that of female controls, although lactate dehydrogenase was higher.

The NOAEL for this study was determined to be 450 mg/kg bw/day, based on the decreased food consumption and bodyweight gain observed at 1,500 mg/kg bw/day.

45/ Yamate, J., et al. (1988). "Subacute Toxicity of L-Carnitine chloride in rats." Iyakuhin Kenkyu **19**(2): 197-220.

6.2.3.3 Chronic safety studies

- (Kudo, Watanabe et al. 1988b) 46/

Rats, 30/sex/group, were assigned to groups dosed by daily oral gavage at levels of L-Carnitine Chloride of 0, 100, 272, 737 and 2,000 mg/kg bw/day for 12 months. The effects of the compound on the rats were evaluated in comparison to controls receiving distilled water. The following parameters were administered daily; clinical observations, bodyweight, food consumption and water intake. Ophthalmological examination and collection of urine were performed on day 182 and at end of intervention. Fasted blood samples were analyzed for hematology and biochemistry. All rats were subject to detailed gross necropsy. Fresh organ weights were recorded and histopathology examination was performed.

None of the rats in the 100 and 272 mg/kg groups showed any treatment-related abnormalities. Treatment-related mortality rates for females and males in the 2,000 mg/kg bw/day group were 28.6% and 35.3%, respectively and these were significantly higher than that of the control group. The rats in the 2,000 mg/kg bw/day group also had higher incidences of abnormal respiratory sounds, soft feces and, in females, 'loss of vigor'. Group mean water intake of only male rats in the 2,000 mg/kg bw/day group was significantly increased, relative to that of male controls, in most weeks,

In males, decreases in body weight, liver, heart, thymus, prostate gland, cecum and epididymis weight were observed in the 2,000 mg/kg bw/day group, whereas in females decreases in body weight at necropsy, heart, thymus weight and increase in cecum weight was observed. In addition, the increased cecum weight in males was also observed in the 737 mg/kg bw/day group. Relative organ weights in males were increased for kidneys, heart, lungs, adrenal glands, salivary glands, testes, epididymites and brain in the 2,000 mg/kg bw/day group, whereas it was increased for liver, kidneys, heart, lungs, adrenal glands, salivary glands, cecum and brain in female counterparts. Increase in relative cecum weight was also observed in the males receiving 737 mg/kg bw/day.

Further, poor body condition, stained fur, congestion of liver and lungs, pulmonary emphysema, distension of stomach and cecum, and prostatic atrophy was observed in male rats treated with 2,000 mg/kg bw/day. Female rats from the same treatment group showed significant increases in incidence of gross lesions of rough or stained fur, hepatic congestion, and reddish-brown foci in lungs and pituitary glands.

Histopathological examinations on male and female rats of the group receiving 2,000 mg/kg bw/day revealed congestion in the liver, kidneys, lungs, adrenal glands and pituitary gland, pulmonary edema, dilatation of the splenic sinuses, atrophy of red and white pulps of the spleen, atrophy of hematopoietic tissue in the bone marrow, and hyperplasia of transitional epithelium in the urinary bladder.

46/ Kudo, S., et al. (1988b). "Chronic Toxicity study of l-carnitine chloride in rats." Iyakuin Kenkyu **19**(2): 221-237.

The authors of the study concluded that the NOAEL for L-Carnitine Chloride identified in this study was 272 mg/kg bw/day.

- (Kikumori, Kida et al. 1988c) 47/

Beagle dogs (n=25 per sex) aged ~6 months old were treated orally via gelatin capsules with doses of 0, 50, 200, 800 and 1,600 L-Carnitine Chloride mg/kg bw/day. Dogs were individually housed under standard laboratory environmental conditions. For the 1,600 mg/kg bw/day group, the daily dose was split into two capsules administered 4 to 6 hours apart, because prior experience had shown that a bolus dose of 1,600 mg/kg bw/day was likely to induce vomiting. Effects of treatment on general condition, food and water consumption, bodyweight were assessed, as well as effects on blood hematology and blood and urine biochemistry. At the end of the study gross necropsy was conducted, tissues weights were recorded, and tissues were preserved for histopathological evaluation.

All dogs survived treatment to scheduled termination and no signs of toxicity occurred. There were no treatment-related effects on electrocardiographic findings, ophthalmologic findings, or otological findings. The most common clinical observations throughout the intervention period was related to the gastrointestinal tract. Diarrhea was common in all dogs in the 1,600 mg/kg bw/day group and in three of each sex in the 800 mg/kg bw/day group. Diarrhea in these groups tended to worsen over the course of the study into watery diarrhea. Vomiting frequency was increased in dogs dosed with ≥ 800 mg/kg bw/day, particularly within 30 minutes of dose administration. Despite the presence of gastrointestinal clinical signs, male dogs treated with ≥ 800 mg/kg bw/day showed greater bodyweight gain than controls over the course of the study. Females in the 1,600 mg/kg bw/day and 200 mg/kg bw/day group showed somewhat lower weight gain than female controls in the first 7 or 8 weeks on study, but then caught up with female controls. Food intake showed no noteworthy changes over treatment period. Water intake by males in the 1,600 mg/kg bw/day group was slightly higher, whereas no dose-related changes were found in the other treatment groups. In females, water intake was increased for those that received >800 mg/kg bw/day, followed by higher mean urine volume. There were no variations in organ weights that showed any dose-response relationship. Dogs of both sexes dosed with ≥ 800 mg/kg bw/day tended to have more acidic urine than dogs in lower dose groups or control groups. Treatment had no apparent effects in urine sediment findings.

There were no treatment-related differences in group mean hematology data of treated dogs, relative to sex-matched controls. Group mean total serum cholesterol was statistically significant increased and showed an apparent dose-response relationship, in male dogs treated with ≥ 800 mg/kg bw/day, when compared to male controls. However, the authors considered the levels of total serum cholesterol found were not adverse.

Gross necropsy findings showed mild blood congestion and associated linear 'erosion-like changes' of the cardia or fundus of the stomach, present in most or all dogs of both sexes in the

47/ Kikumori, M., et al. (1988c). "Chronic toxicity study of L-carnitine chloride in Beagles." Iyakuhin Kenkyu **19**(2): 261-281.

1,600 mg/kg bw/day group, and females in the 800 mg/kg bw/day group. Other histopathological lesions showed no clear dose response relationship and were incidental.

Based on adverse effects on the gastrointestinal tract at ≥ 800 mg/kg bw/day, the NOAEL of L-Carnitine Chloride in dogs was determined to be 200 mg/kg bw/day.

6.2.3.4 Toxicity Studies Related to Exposure to TMAO

In 2013, it was described that exposure to L-Carnitine promotes the induction of atherosclerosis in APOE^{-/-} mice, due to the formation of TMAO in the intestines (Koeth, Wang et al. 2013). ^{48/} There are potential safety concerns associated with this finding because the human plasma levels of TMAO appear to correlate with cardiovascular disease (Tang, Wang et al. 2013). ^{49/} However, Koeth et al. (2013) used a dose level of L-Carnitine of 1.3% in the drinking water. This L-Carnitine concentration in drinking water is expected to lead to a very high L-Carnitine exposure of 1,700 mg/kg bw/day (Collins, Drazul-Schrader et al. 2016). ^{50/}

At more moderate dose levels of L-Carnitine of 87 and 352 mg/kg bw/day, chosen to mimic relevant levels of human exposure (comparable to 500 and 2,000 mg L-Carnitine /day or 7 and 28 mg L-Carnitine/kg BW), Collins et al. (2016) observed that L-Carnitine protected against atherosclerosis in APOE^{-/-} CETP transgenic mice, a model which models the human situation more carefully regarding the handling of plasma cholesterol than the APOE^{-/-} mice as used by Koeth et al. (2013).

Similar levels of L-Carnitine (70.4, 140.8 and 351.9 mg/kg bw/day) showing protection against atherosclerosis in the study performed by Collins et al. (2016), were also tested in a chronic 12 month study in rats (Empl, Kammeyer et al. 2015). ^{51/} In this study the authors focused on potential cardiovascular and carcinogenic effects of L-Carnitine (Empl, Kammeyer et al. 2015) and its metabolites. L-Carnitine exposure resulted in a dose-related increase in plasma TMAO concentration. In the high dose L-Carnitine group, TMAO concentrations were observed of about 25 μ M, being about 10 times the levels as in controls. Despite these considerable levels of exposure to L-Carnitine and its gastrointestinal metabolite TMAO and the chronic character of exposure, no increase in cardiovascular lesions was observed in this study.

Having considered most information available for the relationship between cardiovascular disease and the exposure to TMAO, it seems possible that TMAO plasma levels correlate with cardiovascular disease in humans because increased TMAO plasma levels may be protective in

^{48/} Koeth, R. A., et al. (2013). "Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis." *Nature medicine* **19**(5): 576-585.

^{49/} Tang, W. H., et al. (2013). "Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk." *N Engl J Med* **368**(17): 1575-1584.

^{50/} Collins, H. L., et al. (2016). "L-Carnitine intake and high trimethylamine N-oxide plasma levels correlate with low aortic lesions in ApoE^(-/-) transgenic mice expressing CETP." *Atherosclerosis* **244**: 29-37.

^{51/} Empl, M. T., et al. (2015). "The influence of chronic L-carnitine supplementation on the formation of preneoplastic and atherosclerotic lesions in the colon and aorta of male F344 rats." *Arch Toxicol* **89**(11): 2079-2087.

atherosclerosis (Ufnal and Nowinski 2019). 52/ At high exposures to TMAO however, it may interfere with the reversed cholesterol transport (Koeth, Wang et al. 2013) and cause atherosclerosis in APOE^{-/-}-mice.

In conclusion, at very high levels (i.e., 1,700 mg/kg bw/day), L-Carnitine may induce atherosclerosis in APOE^{-/-} mice. However, at lower L-Carnitine exposure levels (e.g., 351.9 mg/kg bw/day), no adverse cardiovascular effects were noted in both rats and mice.

6.2.3.5 Teratogenicity, Reproductive, and Developmental Toxicity Studies

- (Brandsch and Eder 2003) 53/

Throughout pregnancy and lactational period (34 weeks), 30 rats of which 15 female Sprague Dawley rats aged 4 weeks at enrollment were dosed with 1 g/kg diet L-Carnitine and the other 15 female rats were fed the same diet without L-Carnitine (Brandsch and Eder 2003). 15 Sprague Dawley male rats aged 12 weeks or older were used for mating. All animals were housed individually with standardized temperature and humidity. The diet during pregnancy was standardized to be 18 g/day, whereas during the lactational period of 21 days the diet was provided *ad libitum*. The rats underwent two more reproduction cycles with a 3-week reproduction-free period, of which the diet during lactational period was standardized. Offspring received 20 g diet/day after separation.

Mean diet intake was 13.8 g/day during growth period and 18 g/day during pregnancy, resulting in 5.38 g/kg bw/day of L-Carnitine. 54/ During the first lactational period the mean dietary intake was higher in the intervention group (56.0 ± 5.5 g/day vs. 46.5 ± 8.7 g/day). There were no differences between the intervention group and controls regarding the number of pregnancies, total rat pups alive, stillborn pups, or pups' death during the lactational period. The mean weight of the litters was significantly higher from mothers fed the control diet as compared to those fed the intervention diet in the first parturition cycle. After weaning, the rats from the mothers fed the intervention diet showed greater mean weight gain (209 ± 13 g vs. 197 ± 9 g, $p < 0.05$) for the first cycle. It was concluded by the authors that L-Carnitine supplementation during the perinatal period showed no beneficial effect in rats (Brandsch and Eder 2003). The NOAEL for teratogenicity toxicity in female rats can be established as 5,380 mg/kg bw/day, the highest dietary concentration tested.

- (Itabashi, Watanabe et al. 1988). 55/

52/ Ufnal, M. and A. Nowinski (2019). "Is increased plasma TMAO a compensatory response to hydrostatic and osmotic stress in cardiovascular diseases?" *Medical hypotheses* **130**: 109271.

53/ Brandsch, C. and K. Eder (2003). "Reproductive performance of rats supplemented with L-carnitine." *Journal of Animal Physiology and Animal Nutrition* **87**(7-8): 301-308.

54/ $18 \text{ g diet / day} * 1.0014 \text{ g LCLT / kg diet} = 18.0252 \text{ mg LCLT / day}; 18.0252 \text{ mg LCLT} \div 0.33 \text{ kg bw} = 53.8 \text{ g /kg bw /day.}$

55/ Itabashi, M., et al. (1988a). "Reproduction studies of L-Carnitine Chloride in rats by oral administration prior to mating and in the early stage of gestation." *Iyakuin Kenkyu* **19**(3): 454-464.

In another rat study, both male and female Crj:CD rats were dosed with L-Carnitine Chloride (0, 100, 520 or 2,700 mg/kg bw/day) prior to mating. For female rats this treatment continued to the early state of gestation. Male rats were aged 6 weeks old and received intervention 2 weeks prior to mating and throughout the mating period. Female rats were aged 8 weeks old and received intervention 2 weeks prior to mating until 7 days after a confirmed copulation. Rats were individually housed with standardized temperature, humidity, and light, with unrestricted access to food and water. Rats were paired for mating according to their intervention group, and each group contains 25 males and 25 females.

Both female and male rats in the 2,700 mg/kg bw/day group showed loose bowels throughout the administration period, a transient decrease in food consumption, and a significant increase in water intake. While there was a significant increase in testes: body-weight ratio, this seemed to have resulted from the significant decrease in their body weight. Rats of both sexes in the 520 mg/kg bw/group showed a transient decrease in food consumption with a slight suppression of body-weight gain in males.

There were no adverse effects on the estrous cycle, mating, and fertility of rats in any of the treated groups. External, visceral and skeletal examinations of fetuses also did not reveal any treatment-related abnormalities. The authors concluded that a NOAEL of 520 mg/kg bw/day can be established for parent rats of both sexes and a NOAEL of 2,700 mg/kg bw/day can be established for the reproductive performance of both sexes and the development of their fetuses.

- (Itabashi, Yamashita et al. 1988). [56/](#)

In another rat study conducted by the same research group, Crj:CD rats were dosed with L-Carnitine Chloride (0, 100, 548 or 3,000 mg/kg bw/day). 25 female rats were used in each group, with one rat in the control group not impregnated. The L-Carnitine Chloride was administered once per day via gastric intubation. Administration began on day 17 of pregnancy and continued until day 21 after delivery.

The effects of L-Carnitine Chloride on the parent rats and reproductive performance of the F1 generation were evaluated. No treatment-related adverse effects were reported for parent rats for all groups. There were also no treatment-related changes in the live pups at birth, viability index at 4 days of age, lactation index at 21 days of age, and morphological and functional developments of F1 rats. Further, no treatment-related effects were found in the learning abilities, mating index, fertility, ovulation, and implantation, as well as in the growth and development of F2 fetuses. The study found that body weight gain was significantly suppressed in F1 females of the 548 and 3,000 mg/kg bw/day group. A closer look at the findings (summarized in Table 8 of the study) reveals that the body weight decrease was not dose-dependent. The lower body weight is associated with the 540 mg/kg bw/day group rather than the higher dose of 3,000 mg/kg bw/day group. Further,

[56/](#) Itabashi, M., et al. (1988b). "Reproduction studies of L-carnitine chloride in rats by oral administration during the perinatal and lactating periods." *Iyakuhin Kenkyu* **19**(3): 490-509.

the findings are not consistent across all days of age with no statistically significant difference reported for age of 63 days.

Based on these findings, the authors concluded the NOAEL for the reproduction of the parent rats was 3,000 mg/kg bw/day and the NOAEL for the development and growth of the F1 rats was 100 mg/kg bw/day. While we agree with the NOAEL of 3,000 mg/kg bw/day for the reproductive toxicity of parent rats, we do not view the body weight decrease, which is inconsistent and not dose-dependent, an adverse effect. Indeed, other than the absolute body weight, there were no other treatment-effects in the F1 live pups at birth, viability index at 4 days of age, lactation index at 21 days of age, and morphological and functional developments. As such, we view the NOAEL for the development and growth of the F1 rats to be 3,000 mg/kg bw/day, the highest dose tested.

- (Nakamura, Ueno et al. 1988) 57/

L-Carnitine Chloride was administered orally to pregnant Crj:CD rats at dose levels of 0, 100, 547.7, or 3,000 mg/kg bw/day from day 7 to day 17 of gestation. Each group used in this experiment contained 21-24 females used as a late gestation observation group and 12 females used as a natural delivery group. At day 20 of gestation, about two-thirds of the rats in each group were sacrificed and their fetuses were examined. F1 generation rats were also tested for learning ability at week 9 using a water T-maze test. The water T-maze test has a straight path 15 cm wide and 150 cm long and a water depth of 30 cm. Five trials of the water T-maze tests were conducted on the straight path on the first day and five trials on a water maze were conducted on the second and third days, examining the time required to reach the goal (swimming time) and the number of errors (number of times their body entered the maze) in each trial.

Some rats receiving 3,000 mg/kg bw/day were observed to have salivation and soft feces during the later part of the administration period. However, there were no treatment-related abnormalities reported in autopsy, organ weight (with the exception of the increment of lung weight), delivery and lactation. The NOAEL for general toxicity in these parental rats was determined to be 547.7 mg/kg bw/day. For the F1 rats, there were no abnormalities in the findings of Cesarean section, or external, visceral or skeletal observations. There were also no abnormalities in the mating, pregnancy rate, pregnancy, delivery, lactation and delivered F2. The NOAEL for the reproductive toxicity of parental rats and F1 was determined to be 3,000 mg/kg bw/day.

However, in the water T-maze test, according to the authors, increases of errors and prolongation of elapsed times were recognized in F1 female of the 547.7 and 3,000 mg/kg bw/day groups. A closer look at the raw data (Tables 15&16 of the study) reveals that the effects observed were not dose-dependent. Specifically, in Trial #9 of the T-maze test, the swim time in group receiving 547.7 mg/kg bw/day was longer than those receiving 3,000 mg/kg. Further, statistically significant differences were only observed for Trials #8 and 9, and not the other 8 trials. Other than the T-maze test, there were no abnormalities during lactation, or in the observations at weaning, the open-field test or the conditioned avoidance response test. As such, while the authors

57/ Nakamura, A., et al. (1988). "Teratogenicity study of L-carnitine chloride in rats by oral administration." *Iyakuhin Kenkyu* **19**(3): 465-489.

concluded the NOAEL for the development and behavior of F1 rats was 100 mg/kg bw/day based on the T-maze test, we disagree and believe the NOAEL should be 3,000 mg/kg bw/day instead.

- (Toteno, Furukawa et al. 1988) 58/

In a rabbit study, L-Carnitine Chloride was administered orally to pregnant Japanese white rabbits (13 or 14 females per group) at dose levels of 100, 316, and 1,000 mg/kg bw/day from day 6 to day 18 of pregnancy. The control group received distilled water. Rabbits were observed daily for changes in their general condition and for any signs of death, with autopsies conducted on any animal exhibiting signs of miscarriage or premature delivery. Food consumption and water intake were measured daily, and body weight was measured daily during the administration period and every two days during other periods. Cesarean sections were performed on all rabbits on day 29 of pregnancy and their fetuses were examined for external, visceral and skeletal abnormalities.

12 of 14 rabbits in the 1,000 mg/kg group exhibited diarrheic symptoms like loose bowels and muddy excretions between day 7 and day 19 of pregnancy. There were also significant decreases in food consumption or water intake in the 1,000 mg/kg bw/day. It was also found that body weight gain was slightly retarded in the 1,000 mg/kg bw/day group. No such adverse effects were observed for the other treatment groups. Also, there were no treatment-related effects and no external, visceral or skeletal abnormalities were observed in fetuses. The authors concluded that the NOAEL is 1,000 mg/kg bw/day for fetal development and the NOAEL is 316 mg/kg bw/day for the parent rat.

6.2.4 Animal Safety Studies of L-Tartaric acid

6.2.4.1 Acute Toxicity Studies

A single dose of 25% solution of disodium tartrate in doses of 12 (30 mice), 20 (35 mice), or 30 (51 mice) mmol/kg was administered by gavage to white mice of mixed strain and sex (Locke, Locke et al. 1942). 59/ Average weight was 22-23 g resulting in doses of 2,770, 4,616, or 6,924 mg/kg bw/day, respectively. The LD₁₀ was set at 4,385 mg/kg bw/day. In the same study of New Zealand White male rabbits dosed once with 25% disodium tartrate solution in water. At an average oral dose of 5,308 mg Tartaric acid/kg bw/day, three out of seven rabbits died, while all six rabbits survived an average dose of 3,693 mg Tartaric acid/kg bw/day (Locke, Locke et al. 1942).

Male rabbits were dosed with single doses of potassium sodium tartrate or tartaric acid of 26-300 mg Tartaric acid/kg bw/day (Underhill, Leonard et al. 1931). 60/ In parallel, fasting dogs were

58/ Toteno, I., et al. (1988). "Tertogenicity study of L-carnitine chloride in rabbits." *Iyakuin Kenkyu* **19**(3): 510-521.

59/ Locke, A., et al. (1942). "The comparative toxicity and cathartic efficiency of disodium tartrate and fumarate, and magnesium fumarate, for the mouse and rabbit." *Journal of the American Pharmaceutical Association* **31**(1): 12-14.

60/ Underhill, F. P., et al. (1931). "Studies on the metabolism of tartrates II. The behavior of tartrate in the organism of the rabbit, dog, rat and guinea pig. ." *Journal of Pharmacology and Experimental Therapeutics* **43**(2): 359-380.

given single doses between 100-2,000 mg/kg bw/day tartaric acid sourced from potassium sodium tartrate. At the highest dose of potassium sodium tartrate of 300 mg/kg bw/day as Tartaric acid, all rabbits exhibited diarrhea, whereas in dogs this was the case from the dose 400 mg/kg bw/day onwards. In all groups with rabbits, kidney function was decreased, whereas this was seen in dogs from 600 mg/kg bw/day onwards.

6.2.4.2 Short-Term and Sub-Chronic toxicity

Male CFY rats (n=20) received 2.73 g/kg bw/day of monosodium L-Tartaric acid or monosodium DL-Tartaric acid for 7 days by oral intubation (Down, Sacharin et al. 1977). 61/ At day 7, one rat per group was sacrificed, while the remainder was sacrificed at intermittent intervals over following 12 days. No adverse effects were observed for the weighted and macroscopically examined liver and kidneys from rats receiving the L-Tartaric acid. Rats receiving the DL-Tartaric acid showed changes in kidneys consistent with crystalluria. The main findings were small number of tubules comprising birefringent crystals and focal chronic and mixed inflammation of the interstitium.

In a 90-day intervention study with groups of 10 male and female F344/DuCrj rats, four doses of monopotassium DL-Tartaric acid corresponding to 0, 75, 150, 600 mg/kg bw/day were tested (Inoue, Morikawa et al. 2015). 62/ Results from microscopy indicated inflammatory cell infiltration, irregular dilation of the distal tubule lumen, and foreign body giant cells. Regeneration of renal tubules was observed in the renal cortex and/or medulla at doses of 150 mg/kg bw/day and higher. The severity of these lesions was dose-dependent. An increase in urinary protein and white blood cells was found at doses of 150 mg/kg bw/day. However, no renal function failure was observed from blood biochemical analysis. A NOAEL was identified at 60 mg/kg bw/day for males and 68 mg/kg bw/day for females, respectively (Down, Sacharin et al. 1977).

Sodium tartrate at doses of 0 or 7.7% diet was fed to male rabbits (n=15) for 150 days (Packman, Abbott et al. 1963). 63/ Estimated intake was 550 mg/kg bw/day. At regular intervals, blood and urine samples, and gross pathological examinations were performed, thereafter surviving animals were sacrificed at day 150. No adverse effects were observed that were treatment related.

A daily oral dose of 990 mg Tartaric acid / kg bw/day by two gelatin capsules for 90-114 days was administered to four dogs (Krop and Gold 1945). 64/ Throughout the study period, weight changes varied to -32% through 30%. In 3 dogs, urinal casts appeared after 19, 38, and 89 days. One dog showed increased blood non-protein nitrogen, creatinine and albumin after 88 days of

61/ Down, W. H., et al. (1977). "Renal and bone uptake of tartaric acid in rats: comparison of L(+) and DL-Forms." Toxicology **8**(3): 333-346.

62/ Inoue, K., et al. (2015). "Obstructive nephropathy induced with DL-potassium hydrogen tartrate in F344 rats." Journal of toxicologic pathology **28**(2): 89-97.

63/ Packman, E. W., et al. (1963). "Comparative subacute toxicity for rabbits of citric, fumaric, and tartaric acids." Toxicol Appl Pharmacol **5**: 163-167.

64/ Krop, S. and H. Gold (1945). "On the toxicity of hydroxyacetic acid after prolonged administration: Comparison with its sodium salt and citric and tartaric acids." Journal of the American Pharmaceutical Association **34**(3): 86-89.

treatment, hereafter the dog died at day 90. Advanced kidney tubular degeneration was observed by histopathological examination. No other adverse effects were observed (Krop and Gold 1945).

6.2.4.3 Chronic Toxicity and Carcinogenicity

Fitzhugh and Nelson (1947) fed Osborne-Mendel male and female rats (n=24) a diet containing either 0.1, 0.5, 0.8 or 1.2% Tartaric acid, while 48 rats received a control diet for two years. Doses were equivalent to 0, 50, 250, 400 and 600 mg/kg bw/day. ^{65/} Growth rate was similar to control in the first 52 weeks of the intervention. No effect of Tartaric acid was observed for mortality rate, and any microscopic pathologic examinations.

Another 2-year experiment feeding diets containing 0, 25,600, 42,240, 60,160 or 76,800 ppm monosodium L-Tartaric acid to 350 CFY rats aged 28 days old (Hunter, Batham et al. 1977). ^{66/} These doses equal to 0, 890, 1,620, 2,200 and 3,100 mg monosodium L(+)-Tartrate/ kg bw/day for males, and for females to 0, 1,190, 2,050, 3,030 and 4,100 mg monosodium L(+)-Tartrate/ kg bw/day. Rats were per five housed in cages with standardized temperature, humidity, and hours daylight. Rats receiving treatment diets showed no difference in appearance or behavior. Survival rate from 78 weeks was lower in the rats receiving 42,240, 60,160 or 76,800 ppm monosodium L-Tartaric acid than controls. Also, for urinary, hematological or clinical chemistry parameters, ophthalmic examinations, or histopathological examination, no treatment-related adverse events were observed. However, body weight was decreased at the three highest doses with 15-20%. Moreover, an increase in relative organ weights was observed in brain, heart, liver, kidney, uterus and gonad. It was thought that this organ weight increase was due to the decreased body weight.

6.2.4.4 Teratogenicity Studies

There were no reproductive toxicity studies available, but unpublished studies on developmental toxicity studies have been described in a recent EFSA report (Younes, Aquilina et al. 2020). ^{67/} These unpublished studies were performed by the Food and Drug Research Lab.

Mice

Pregnant CD-1 mice (n=20-22 per group) orally received daily 2.74, 12.7, 59.1 or 274 mg Tartaric acid/kg bw/day at day 6 to 15 of gestation. At regular intervals, body weight was measured and at day 17 of gestation mice underwent caesarean section. Up to 274 mg Tartaric acid /kg bw/day, no developmental toxicity was observed (Younes, Aquilina et al. 2020).

Rats

^{65/} Fitzhugh, O. G. and A. A. Nelson (1947). "The Comparative Chronic Toxicities of Fumaric, Tartaric, Oxalic, and Maleic Acids*." Journal of the American Pharmaceutical Association (Scientific ed.) **36**(7): 217-219.

^{66/} Hunter, B., et al. (1977). "Monosodium L(+) tartrate toxicity in two year dietary feeding to rats." Toxicology Journal **8**(2): 263-274.

^{67/} Younes, M., et al. (2020). "Re-evaluation of l(+)-tartaric acid (E 334), sodium tartrates (E 335), potassium tartrates (E 336), potassium sodium tartrate (E 337) and calcium tartrate (E 354) as food additives." EFSA Journal **18**(3): e06030.

Pregnant Wistar rats (n=19-24 per group) received daily doses of 0, 1.81, 8.41, 39.1 or 181 mg Tartaric acid/kg bw/day at day 6 to 15 of gestation. At regular intervals, body weight was measured and at day 20 of gestation the rats underwent caesarean section. In all treated groups, fetal weight was increased by 11-16%. The incidence of wavy ribs was higher in the treated groups, but without a dose-dependent relationship. No other treatment-related maternal or developmental toxicity was observed in any group (Younes, Aquilina et al. 2020).

Hamsters

Pregnant golden hamsters (n=20-23 per group) were treated with daily oral doses of 0, 2.25, 10.45, 48.35 or 225 mg Tartaric acid/kg bw/day at day 6 to 10 of gestation. Body weight was determined regularly, and caesarean section was performed at day 14 of gestation. No signs of maternal or developmental toxicity was observed at any doses (Younes, Aquilina et al. 2020).

Rabbits

Dutch-belted groups of rabbits (n=10-11) were inseminated and were treated via gavage with doses of 0, 2.15, 10, 46.4 or 215 mg Tartaric acid/kg bw/day at day 6 to 18 of gestation. A caesarean section was carried out at day 29 of gestation. As compared to the control group, the fetal weight was 7, 9, 9 and 15% lower at respective doses of 2.15, 10, 46.4, and 215 mg Tartaric acid/kg bw/day. No signs of maternal or developmental toxicity for any of the treatment groups (Younes, Aquilina et al. 2020).

6.2.5 Genotoxicity Studies

Mutagenicity of L-Carnitine Chloride was investigated using rec- assay, *Ames* test, and chromosomal aberration test in Chinese hamster V79 (lung fibroblast) cells (Hamai, Kojima et al. 1988). ^{68/} Based on the results, L-Carnitine Chloride is not considered genotoxic. Genotoxicity studies performed with L-Tartaric acid, including an *Ames* test and a chromosomal aberration test, neither of which showed any evidence of genotoxicity (Yamada and Honma 2018). ^{69/} L-Tartaric acid is considered not to be genotoxic (Yamada and Honma 2018). Formal carcinogenicity studies have not been performed with L-Carnitine or L-Tartaric acid. However, 12 month toxicity studies conducted with L-Carnitine (Kudo, Watanabe et al. 1988b) and 24 month toxicity studies performed with L-Tartaric acid in rats (Hunter, Batham et al. 1977) did not show any evidence of carcinogenicity.

As both L-Carnitine and L-Tartaric acid are not genotoxic and there was no evidence of carcinogenicity, and as LCLT completely dissociates into L-Carnitine and L-Tartaric acid in water (Schmidbaur, Schier et al. 1998), LCLT is not genotoxic and not carcinogenic. The lack of genotoxicity was also confirmed by the data of a (unpublished) bacterial gene mutation study performed with LCLT (IBR 1991).

^{68/} Hamai, Y., et al. (1988). "Studies on the mutagenicity of L-carnitine chloride." *Iyakuin Kenkyu* **19**(3): 522-528.

^{69/} Yamada, M. and M. Honma (2018). "Summarized data of genotoxicity tests for designated food additives in Japan." *Genes and Environment* **40**.

6.2.6 Clinical Studies with L-Carnitine-L-Tartrate

6.2.6.1 Human Adult Studies with LCLT

Tolerance of LCLT up to 4.4 g/day for three weeks has been established in adults regarding gastrointestinal symptoms, hematology and clinical chemistry, including markers of liver and kidney function. Doses of L-Carnitine exceeding 3 g/day (corresponding to 4.4 g LCLT) may produce a fishy body odor resulting from degradation to trimethylamine by intestinal bacteria (Odle, Adams et al. 2014). ^{70/} Note that there were no adverse findings in the following studies with LCLT with dosages at 4.4 g/day or above.

The safety of LCLT has been established by exploring the dose and acute and sub-chronic supplementation (Rubin, Volek et al. 2001, Abramowicz and Galloway 2005). ^{71/} Rubin et al. (2001) conducted a double-blind randomized cross-over study exploring the safety of 3 g LCLT supplemented to healthy American men for 3 weeks. Results showed no significant differences in markers of renal function, liver function, and hematology. Moreover, no symptoms of gastrointestinal distress were reported by any subject. In another randomized, double-blind, placebo-controlled, cross-over trial, healthy men and women (n=12) were exposed to 3 g LCLT in a period of 3 weeks and an acute supplementation of one day. Supplementation was reported to be well-tolerated by all participants included in the study (Abramowicz and Galloway 2005).

In a recent review, LCLT has extensively been studied in relation to its positive effect in muscle physiology in adults (Fielding, Riede et al. 2018). ^{72/} Separate randomized experiments not discussed in the latter review, supplemented adults with doses between 2 to 4.5-gram oral LCLT (Wutzke and Lorenz 2004, Galloway, Craig et al. 2011, Wall, Stephens et al. 2011, Novakova, Kummer et al. 2016, Shannon, Nixon et al. 2016, Shannon, Ghasemi et al. 2018). Overall, the supplementation was well tolerated, and no adverse events have been reported.

6.2.6.2 Human Infant Studies with LCLT

To further support the safety of the intended use of LCLT in IF, in 2015, we conducted a single-center, double-blind randomized, and controlled trial to examine the growth and nutritional status in infants consuming a cow milk-based IF or a goat milk-based IF (Kabrita Gold, Ausnutria B.V.) The latter IF contained 0.00687% (w/w) LCLT as the source of L-Carnitine (Appendix 1). No registration of IF volumes were performed, so assuming a mean intake of 1,200 ml/day, the daily estimated intake of LCLT would be 1.27 mg/kg bw/day at end of the intervention. ^{73/} The results

^{70/} Odle, J., et al. (2014). "Carnitine." *Advances in Nutrition* **5**(3): 289-290.

^{71/} Rubin, M. R., et al. (2001). "Safety measures of L-carnitine L-tartrate supplementation in healthy men." *J Strength Cond Res* **15**(4): 486-490; Abramowicz, W. N. and S. D. Galloway (2005). "Effects of acute versus chronic L-carnitine L-tartrate supplementation on metabolic responses to steady state exercise in males and females." *Int J Sport Nutr Exerc Metab* **15**(4): 386-400.

^{72/} Fielding, R., et al. (2018). "L-Carnitine Supplementation in Recovery after Exercise." *Nutrients* **10**(3).

^{73/} The calculation of 1.27 mg LCLT / kg bw/day can be summarized below: 0.00687% w/w=0.0000687 g LCLT / g IF powder; 1 ml reconstituted IF = 0.135 g IF powder; 1200 * 0.135=162 g IF powder; 162 g IF powder * 0.0000687 g LCLT = 0.0111294 g LCLT = 11.13 mg LCLT/day; 11.13 mg LCLT ÷ 8.75 kg bw = 1.27 mg LCLT / kg bw/day. Please note the calculation was conducted with the actual body weight (i.e., 8.75 kg as the average) as measured after 3 months of intervention.

indicated no difference in growth, selected plasma micronutrient status, urine and fecal parameters or adverse events regarding gastrointestinal symptoms between the two IFs (Xu, Wang et al. 2015). ^{74/} These data confirm that also in practice this formulation with LCLT does not cause any adverse or unexpected effects.

In 2019, a multi-center, double-blind randomized, and controlled trial to examine the growth in infants consuming a cow milk-based IF or a goat milk-based IF was completed (He, van Lee et al. Unpublished). ^{75/} A third breast-fed arm was used as reference. The goat milk based IF contained 0.00412% (w/w) as the source of L-Carnitine (Appendix 2). Mean daily IF intake ranged from 658 ml at 14 days after start of intervention to 849 ml at the end of the intervention, translating to a daily estimated intake of LCLT of 1.28 mg/ kg bw/day. ^{76/} The goat milk based IF showed a good safety and tolerability profile. Furthermore, it was demonstrated that the goat milk based IF supported normal physical growth in infants from birth. The total incidence of treatment emergent adverse events and treatment emergent serious adverse events was similar between the goat milk based IF and the cow based IF, whereas the total number of treatment emergent adverse events trended higher in the cow milk based IF. In addition, the assigned categories of causality, severity and the system of organ classes of the treatment emergent adverse events appeared to be also similar between the studied groups.

6.3 Conclusion

Several expert panels organized by reputable scientific and regulatory agencies including LSRO, EFSA, and FSANZ have reviewed the available safety data on the LCLT and the components of LCLT (i.e., L-Carnitine and L-Tartaric acid). These reports are publicly available. The calculated EDIs from the proposed use are below these safety threshold levels. The comparison between the intended use levels and the safety threshold levels established by various expert panels can be summarized below:

	EDI from Intended Use	Safety Thresholds Established by Expert Bodies
LCLT	<ul style="list-style-type: none"> LCLT intended use level: <u>1.18 mg/ kg bw/day</u> 	<ul style="list-style-type: none"> EFSA Journal (2003)19, 1-13: L-carnitine-L-tartrate up to 3g/day in adults or <u>50 mg/kg bw/day</u>

^{74/} Xu, M., et al. (2015). "Comparison of growth and nutritional status in infants receiving goat milk-based formula and cow milk-based formula: a randomized, double-blind study." *Food Nutr Res* **59**: 10.3402/fnr.v3459.28613.

^{75/} He T, van Lee L, Woudstra F, Panzer F, Haandrikman A, Verkade H. Growth and safety of a goat milk based infant formula in newborns: A double-blind randomized controlled trial (unpublished).

^{76/} The calculation of 1.28 mg LCLT / kg bw/day can be summarized below: 0.00412 %w/w = 0.0000412 g LCLT / g IF powder; 1 ml reconstituted IF = 0.135 g IF powder; 1200 * 0.135 = 162 g If powder; 162 g IF powder * 0.0000412 g LCLT = 0.0066744 g LCLT = 6.67 mg LCLT/day; 6.67 ÷ 5.2 kg = 1.28 mg LCLT /kg bw/day. Please note the calculation was conducted with the actual body weight (i.e., 5.2 kg as the average) as measured per visit during the study.

		(when assuming a body weight of an adult of 60 kg).
L-Carnitine	<ul style="list-style-type: none"> • L-Carnitine component from LCLT's intended use: <u>0.8 mg/kg bw/day</u> • Cumulative estimated daily L-Carnitine exposure of 2.54 mg/ kg bw/day • The L-Carnitine content of IF as proposed by Ausnutria after having supplemented natural L-Carnitine levels from milk with LCLT: 1.9 mg L-Carnitine/100 kcal 	<ul style="list-style-type: none"> • FSANZ, Approval Report – Application A1102 L-Carnitine in food (May 16, 2019) : intake of L-Carnitine up to 3 g/day or <u>50 mg/kg bw/day</u> (when assuming a body weight of an adult of 60 kg) is not associated with adverse effects. • LSRO: recommended that L-Carnitine be added to term IF at a level of <u>1.2-2.0 mg/100 kcal</u>
L-Tartaric acid	<ul style="list-style-type: none"> • L-Tartaric acid component from LCLT's intended use: <u>0.38 mg/kg bw/day</u> • Cumulative estimated daily L-Tartaric acid exposure of 18.1 mg L-Tartaric acid/ kg bw/day 	<ul style="list-style-type: none"> • EFSA Journal 2020;18(3):6030: ADI for L-Tartaric acid of <u>240 mg/kg bw/day</u>.

Further, based on the available animal and human safety studies we have reviewed, we are not aware of any data and information that are, or may appear to be, inconsistent with our conclusion of GRAS status. Indeed, in two recent human studies conducted in 2015 and 2019 with infants, LCLT at levels of 1.27 mg/kg bw/day and 1.28 mg/kg bw/day, which are both higher than the intended use level of 1.18 mg/ kg bw/day, were well tolerated.

In a more recent study published in 2020 the authors report a high intake of L-Carnitine (i.e., 4,500 mg/kg bw/day L-Carnitine) could induce a liver function decline by disordering the gut

bacterial composition of mice resulting in an increased TMAO metabolism. ^{77/} The level tested is magnitudes higher than the L-Carnitine component from the LCLT's intended use (i.e., 0.8 mg/kg bw/day) and the safety thresholds established by EFSA, FSANZ, and LSRO. Notably, the level tested in the study is also comparable to the LD₅₀ reported by an earlier study in Crj:CD rats. ^{78/} For the 10-day old rats, the observed LD₅₀ for L-Carnitine Chloride was 4,374 mg/kg bw/day (95% CI: 3,995-4,790 mg/kg bw/day) in males and 4,578 mg/kg bw/day (95% CI: 4,128-5,093 mg/kg bw/day). For the 22-day old rats, the LD₅₀ was determined to be 6,127 mg/kg bw/day in males (95% CI: 5,501-6,824 mg/kg bw/day) and 6,299 mg/kg bw/day in females (95% CI: 5,679-6,987 mg/kg bw/day). This is also in line with the LD₅₀ reported by other acute studies. ^{79/} We, therefore, are of the view that there is a consensus among experts qualified by scientific training and experience to evaluate the safety that there is reasonable certainty in the minds of competent scientists that the substance is not harmful under the conditions of its intended use.

In summary, we conclude that the LCLT's intended use can be considered GRAS through scientific procedures.

^{77/} Wu, Qiu, et al. "High l-Carnitine Ingestion Impairs Liver Function by Disordering Gut Bacteria Composition in Mice." *Journal of Agricultural and Food Chemistry* 68.20 (2020): 5707-5714.

^{78/} Kudo, S., et al. (1988a). "Acute toxicity studies of L-Carnitine chloride in 10- and 22-day-old and 5-week-old rats." *Iyaku hin Kenkyu* **19**(4): 689-699.

^{79/} See e.g., Narita, N., et al. (1988). "Acute toxicity study of L-carnitine chloride in rats." *Iyaku hin Kenkyu* **19**(2): 191-196; Toshida, K. and Y. Wada (1988). "Acute toxicity studies of L-carnitine chloride in mice and rabbits." *Iyaku hin kenkyu* **19**(3): 446-453.

7. Part 7. LIST OF SUPPORTING DATA AND INFORMATION IN THE GRAS NOTICE

7.1 List of Abbreviations

Acceptable Daily Intake	ADI
Spanish Agency for Food Safety and Nutrition	AESAN
Body Weight	BW
Codex Alimentarius Commission	CAC
Critical Control Points	CCPs
Critical Limits	CL
Council of Responsible Nutrition	CRN
European Food Safety Authority	EFSA
European Pharmacopoeia	EP
Food Agricultural Organization of the United Nations	FAO
Food Chemical Codex	FCC
US Food & Drug Administration	FDA
Feeding Infants and Toddlers Study	FITS
Freedom of Information Act	FOIA
Food Standards Australia New Zealand	FSANZ
Generally Recognized As Safe	GRAS
Infant Formula	IF
L-Carnitine-L-Tartaric acid	LCLT
Median Lethal Dose	LD50
Life Sciences Research Office	LSRO
National Health and Nutrition Examination Survey	NHANES
National Foods Association	NNFA
No-Observed-Adverse-Effect Level	NOAEL
Margin Of Safety	MOS
Organization for Economic Co-operation and Development	OECD
Foods for Particular Nutritional Uses	PARNUTS
Reference Daily Intake	RDI
Selected Committee on GRAS Substances	SCOGS
Safety Data Sheet	SDS
Trimethylamine	TMA
Trimethylamine N-oxide	TMAO
United States	U.S.
World Health Organization	WHO

7.2 References

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Appendix 1. Recipe Composition of Kabrita Gold Goat Based Infant Formula, China, 2011

Kabrita 1 GOLD MLO. HNC (PD10102 3)

Base Vronie Goat	73.750
Z-Lactose	20.319
GOS op glucosestroop	2.185
FOS	1.289
AA Powder	1.159

Natriumchloride Suprasel Fijn	0.14916
Zinkpremix	0.08833
IJzerpremix	0.08762
Vitamine C	0.05438
Kalium Chloride	0.03098
Magnesiumchloride	0.02132
Inositol	0.01970
Koperpremix 1.5%	0.01348
Mangaanpremix	0.01316
L-Carnitine L-tartrate	0.00687
Choline Bitartraat	0.00544
Vitamine A	0.00399
Vitamine E	0.00357
Calciumpantothenaat	0.00300
Vitamine D3 Cvs 100.000 IU/g	0.00258
Niacine	0.00148
Pre-premix foliumzuur 10%	0.00090
Natrium-Selenaat 1%	0.00089
Pre-premix biotine 1%	0.00089
Vitamine K1 5%	0.00082
Vitamine B1	0.00071
Vitamine B6	0.00017
Vitamine B2	0.00007
TCP Puremin Ca301	0.25000

Appendix 2. Recipe Composition of Goat Milk Based Infant Formula for Clinical Trial

Code	Description	Status	Weight
31038	Base HANNA goat	P	81.50000
31011	GOS on glucose syrup	P	10.52632
30074	Lactose edible (monohydrate)	P	4.53341
31037	ARASCO protein free (Vana-Sana)	P	1.73182
30880	DHASCO powder (Vana-Sana)	P	0.69273
30981	Tricalcium (di) fosfaat	P	0.32796
30160	Fe premix (3%)	P	0.19429
30690	Choline bitartrate	P	0.16557
30161	Zn premix (3%)	P	0.14393
30163	Mn premix (0.1%)	P	0.04735
30165	Taurine	P	0.03190
30162	Cu premix (1.5%)	P	0.02466
30153	Vitamin E (DL-alpha tocopheryl acetate 50%)	P	0.01745
30144	Inositol	P	0.00556
31070	Magnesium carbonate	P	0.01561
30071	Vitamin C (L-ascorbic acid)	P	0.01374
30154	Vitamin A (Retinyl acetate)	P	0.00481
30073	Niacinamide	P	0.00458
30963	KI premix (0.765%)	P	0.00278
30928	L-carnitine L-tartrate	P	0.00412
31127	Vitamin D3 Cws 100.000 IU/g (Cholecalciferol)	P	0.00260
30080	Calcium pantothenate (D-calcium pantothenate)	P	0.00246
31042	Biotin 1% (D-Biotin)	P	0.00157
30193	Vitamin B12 0.1 % (Cyanocobalamin)	P	0.00087
30072	Vitamin K1 5% (Phytomenadione)	P	0.00104
20059	Pre-premix folic acid 10% (Pteroylmonoglutamic aci	P	0.00095
30581	Sodium selenate 1%	P	0.00076
30069	Vitamin B1 (Thiamin hydrochloride)	P	0.00054
30192	Vitamin B2 (Riboflavin)	P	0.00038
30070	Vitamin B6 (Pyridoxine hydrochloride)	P	0.00026



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Via Electronic Mail

July 19, 2021

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Re: Response to FDA Questions for GRN 993

Dear Ms. Anderson,

On behalf of our client Ausnutria B.V. (Ausnutria), we hereby submit our responses to the FDA questions for GRAS Notice No. GRN 000993 (GRN 993) dated July 2, 2021. For your ease of reference, we first repeat the FDA question, followed by our response.

- ***FDA Question #1)*** *In the notice, the estimated dietary exposures use an upper percentile estimate of infant formula consumption (1,200 mL/day) and a representative bodyweight of 6.1 kg that are based on information in the publication "Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age" (EFSA, 2017). Please provide a discussion of the potential dietary exposure for all infants (ages up to 12 months) who may be exposed to LCLT from the intended use and confirm that the estimates provided are representative of the maximum level of exposure among all infants.*

Ausnutria Response:

Ausnutria intends to use LCLT as a nutrient source of L-Carnitine in powdered IF formula for full term infants. In **Section 3.1.2** of the GRN 993, Ausnutria discussed the estimated daily intake from infant formula. Ausnutria first noted that in FDA's guidance titled "Preparation of Food Contact Notifications for Food Contact Substances in Contact with Infant Formula and/or

Human Milk: Guidance for Industry” (2019), ^{1/} FDA provided a default value for both infant body weight (6.3 kg-bw/infant) and infant food consumption (900 g formula/infant/day) that were determined based on the 2-day 2005-2010 National Health and Nutrition Examination Survey (NHANES) food consumption survey. These values resulted in a consumption-to-mass ratio of 140 grams per kilogram body weight per day (140 g/kg bw/d), or 0.14 kg/kg bw/d.

Section 3.1.2 also provided information on the upper percentile estimate of infant formula consumption (1,200 mL/day) published by the European Food Safety Authority (EFSA) and a representative bodyweight of 6.1 kg. See, "Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age" (EFSA, 2017). The estimated intake translates to around 0.2 kg/kg bw/day, which is higher than the FDA default consumption-to-mass ratio at 0.14 kg/kg bw/d.

In response to the agency question, we also conducted a dietary exposure assessment to estimate infant formula consumption using NHANES 2015-2016. The data and methods used to conduct the intake assessment and results are summarized below.

In **Appendix A**, we list the corresponding food names from NHANES 20015-2016 for this assessment. This approach very conservatively assumes that the infant formula with LCLT added will have 100% market share of the entire infant formula category in the US. The average and 90th percentile *per user* intake of infant formula, using those foods as surrogates, among the infant population (≤ 12 months) can be summarized in **Table 1**.

Table 1. Infant Formula Consumption in the U.S. (NHANES 2015-2016)				
Age Group	Average Consumption	Average Consumption	90th Percentile	90th Percentile
Infant Population (≤ 12 months)	741.36 g/person	100.02 g/kg bw/day	1132.1 g/person	172.38 g/kg bw/day

As the above table indicates, the 90th percentile infant formula consumption, among the general infant population (≤ 12 months), is reported in NHANES 2015-2016 as 172.38 g/kg bw/day, or 0.17 kg/kg bw/day.

For the purpose of conservatism, Ausnutria adopted in **Section 3.1.2** the higher value of 0.2 kg/kg bw/day, which is higher than the default value of 0.14 kg/kg bw/d found in FDA’s guidance for all infants and also higher than the 0.17 kg/kg bw/d we estimated using NHANES 2015-2016. The data used in the intake assessment, therefore, are representative of the maximum level of exposures across all infants between 0 to 12 months.

^{1/} FDA, Preparation of Food Contact Notifications for Food Contact Substances in Contact with Infant Formula and/or Human Milk: Guidance for Industry” (2019), available at: <https://www.fda.gov/media/124714/download> (accessed on July 10, 2021).

- **FDA Question #2):** *On page 17, the notifier states that the method used to detect Cronobacter sakazakii is ISO/TS 22964:2006. We note that this method has been revised and replaced by ISO 22964:2017, which corresponds to Microbiology of the Food Chain – Horizontal Method for the Detection of Cronobacter spp. Please make a statement that corrects this reference.*

Ausnutria Response:

We hereby confirm the correct reference should be ISO 22964:2017 as noted by the agency. We apologize for the use of outdated reference.

- **FDA Question #3):** *Please clarify whether the provided specifications on page 17 for C. sakazakii in LCLT are performed using a single 300-gram sample or smaller samples (provide the sample size) totaling 300 grams. We note that the referenced ISO method states, “This document has been validated for test portions of 10 g ... A larger test portion than that initially validated may be used, if a validation/verification study has shown that there are no negative effects on the detection of Cronobacter spp.”. If a 300-gram sample is used, please clarify if the analytical method used to detect C. sakazakii has been validated for that particular purpose and confirm that those results will not give a false negative, or please provide results from analysis of three non-consecutive batches for C. sakazakii in a sample size of 10 grams of LCLT.*

Ausnutria Response:

The laboratory used a sample size of 300 grams. The lab has advised the method is validated for the larger sample size of 300 g and the results will not provide a false negative. However, we have been unable to get the validation testing from the laboratory. While we trust the information from the laboratory will address the agency question, Ausnutria is providing results from three non-consecutive batches for *C. sakazakii* in a sample size of 10 grams of LCLT. The laboratory analyzed 30 of 10 g samples (90 samples in total) from three different production lots and found no *C. sakazakii* in the three lots tested. We are attaching the results as **Appendix B**.

- **FDA Question #4):** *On page 36, we note that reference 38 is listed as “Kudo, S., et al. (1988a). “Acute toxicity studies of L-Carnitine chloride in 10- and 22-day-old and 5-week-old rats.” Iyaku hin Kenkyu 19(4): 689-699.” On page 39, we note that reference 46 is listed as “Kudo, S., et al. (1988b). “Chronic Toxicity study of l-carnitine chloride in rats.” Iyaku hin Kenkyu 19(2): 221-237.” We believe the last name of the first author is actually Kudow and not Kudo. Please confirm that “Kudow” is the first author of these references.*

Ausnutria Response:

We hereby confirm that “Kudow” (as opposed to “Kudo”) is indeed the first author of both references. It appears the typo comes from the English translations of the Japanese literature. We apologize for the confusion.

- **FDA Question #5):** *For the Collins et al. (2016) study mentioned on page 41, please provide its duration, the mode of administration (i.e., gavage, dietary, drinking water, or other), and the sex of animals (i.e., males, females, or both).*

Ausnutria Response:

In the Collins, H. L., et al. (2016) 2/, male mice transfected with human cholesteryl ester transfer protein (hCETP) were fed L-carnitine and/or methimazole, a flavin monooxygenase 3 (FMO3) inhibitor that prevents the formation of TMAO. L-carnitine and methimazole were dissolved in sterile water and administered once daily by oral gavage at specified doses based on body weight at a volume of 1 ml per 100 g of body weight. Following the 12 week treatment, L-carnitine and TMAO plasma levels, aortic lesion development, and lipid profiles were determined.

In the event the agency has any further questions regarding the design of the study, more details are also available [here](#).

- **FDA Question #6):** *For the Empl et al. (2015) study mentioned on page 41:*
 - a. Please provide the mode of administration and the sex of animals.*
 - b. The notifier states that this study “focused on potential cardiovascular and carcinogenic effects,” yet only the cardiovascular effects were discussed. Please briefly discuss whether carcinogenic effects or noncarcinogenic adverse effects were observed in this study.*
 - c. Please state the overall NOAEL (for carcinogenic and noncarcinogenic effects), if any.*

Ausnutria Response:

- a) In the Empl et al. (2015), 3/ the authors used male rats and the L-carnitine was administered to the animal through drinking water for a year at a level of 0, 1, 2, and 5 g/L.

2/ Collins, H. L., et al. (2016) ("L-Carnitine intake and high trimethylamine N-oxide plasma levels correlate with low aortic lesions in ApoE(-/-) transgenic mice expressing CETP." *Atherosclerosis* 244: 29-37), available at: <https://www.atherosclerosis-journal.com/action/showPdf?pii=S0021-9150%2815%2930192-1> (accessed on July 11, 2021).

3/ Empl, M. T., et al. (2015). "The influence of chronic L-carnitine supplementation on the formation of preneoplastic and atherosclerotic lesions in the colon and aorta of male F344 rats." *Arch Toxicol* 89(11): 2079-2087, available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7079849/> (accessed on July 11, 2021).

- b) Specifically, the authors investigated whether a 1-year administration of different L-carnitine concentrations (0, 1, 2 and 5 g/l) via drinking water leads to carcinogenic effects (i.e., the increased incidence of preneoplastic lesions—so-called aberrant crypt foci—in the colon of male Fischer 344 rats), as well as cardiovascular effects (i.e. the appearance of atherosclerotic lesions in the aorta of these animals). No significant difference between the test groups regarding the formation of lesions in the colon and aorta of the rats was observed, suggesting that, under the given experimental conditions, L-carnitine up to a concentration of 5 g/l in the drinking water does not have adverse carcinogenic or cardiovascular effects.
- c) The authors did not report an NOAEL but one can be calculated. Based on average water consumption of 18.3 ml/rat/day and an average body weight of 260 g/rat, the concentration of 5 g/l translates to 351.9 mg L-carnitine/kg bw/day, which is the highest level tested in the study and can be considered as the NOAEL. ^{4/}

In the event the agency has any further questions regarding the design and findings of the study, more details are available [here](#).

- **FDA Question #7):** For the Brandsch and Eder (2003) study described on page 42, the notifier states “Mean diet intake was 13.8 g/day during growth period and 18 g/day during pregnancy, resulting in 5.38 g/kg bw/day of L-Carnitine.” In the footnote, the following calculation is provided: “18 g diet / day * 1.0014 g LCLT / kg diet = 18.0252 mg LCLT /day; 18.0252 mg LCLT ÷ 0.33 kg bw =53.8 g /kg bw /day.”
 - a. In the calculations, we note that the test article is incorrectly listed as LCLT. Please confirm that the correct test article is L-carnitine.
 - b. Dividing 18.0252 by 0.33 is 54.6. Please explain how the value 53.8 was obtained for this calculation.
 - c. We note that the calculated value, regardless if it is 53.8 or 54.6 g/kg bw/day, is 10-times higher than the NOAEL of 5,380 mg/kg bw/day (5.38 g/kg bw/day) listed in the notice. We further note that FSANZ (2014) also reviewed this study and stated that the daily intake of L-carnitine was “approximately 50 mg/kg bw/day”. Please check what the correct unit should be when 18.0252 mg/day is divided by 0.33 kg bw. Please explain each individual step of the calculation and provide the value and the unit of the correct dose level in this study.

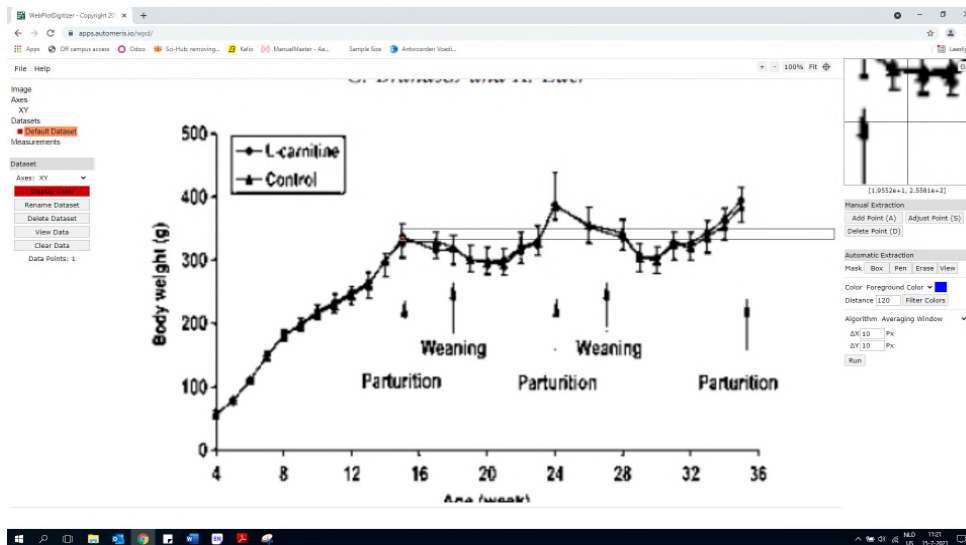
Ausnutria Response:

- a) In the Brandsch and Eder (2003) study, ^{5/} for which we are attaching a full copy of the study (**Appendix C**), the test article is L-carnitine as noted by the agency, not LCLT. We apologize for the confusion.

^{4/} 351.9 mg L-carnitine/kg bw/day = 18.3 ml/rat/day * 5 g/l * 1 L/1000 mL * 1000 mg/g ÷ 260 * 1,000 g/kg.

^{5/} **Appendix C:** Brandsch, C., and K. Eder. "Reproductive performance of rats supplemented with l-carnitine." *Journal of animal physiology and animal nutrition* 87.7-8 (2003): 301-308.

- b) With regard to the agency observation regarding our calculated level of 53.8 g LCLT/kg bw /day, there is an error in the reporting in that it should be **53.8 mg L-carnitine/kg bw/day**. Below we provide details on how we calculated the 53.8 mg/kg bw/day. While the study did not report body weight, Ausnutria calculated the body weight of the animals at 0.335 kg before 1st pregnancy from Figure 1 of the study using webplotdigitizer (as shown below).



As the agency noted, $18.0252 \text{ mg L-carnitine} \div 0.33 \text{ kg bw} = 54.6 \text{ mg /kg bw /day}$. The use of 0.335 kg bw, however, will result in the 53.8 mg/kg bw/day used in the GRAS notification.

$$53.8 \text{ mg/kg bw/day} = 18.0252 \text{ mg L-carnitine} \div 0.335 \text{ kg bw.}$$

We truncated the value as 0.33 kg bw (as opposed to 0.335 kg) in the written submission while we used 0.335 kg bw in the calculation. We apologize for the confusion.

- c) In the *Brandsch and Eder (2003)* study, the mean diet is 18 g/day for each female rat throughout all three pregnancies. Further, rats of the treatment group received the basal diet containing 1.4 mg/kg L-carnitine supplemented with 1 g L-carnitine (obtained from Lonza, Basel, Switzerland) per kilogram of diet. As such, the diet contains $1.0014 \text{ g LC / kg} = 1 \text{ g LC/kg} + 0.0014 \text{ g (1.4 mg) LC/kg}$.

We further gathered from the study the body weight of rats in the treatment group is 0.335 kg. As such, the 18 g feed/day with L-carnitine supplemented at 1.0014 g/kg of the diet is equal to:

18 g/day x 1.0014 g/kg L-carnitine x 1 kg/ 1,000 g x 1,000 mg/g ÷ 0.335 kg =
53.8 mg/kg bw/day

The 53.8 mg/kg bw/day is comparable to the “approximately 50 mg/kg bw/day” reported by FSANZ (2014).

- **FDA Question #8):** *The Itabashi et al. (1988b) study is discussed in the FSANZ (2014) document. FSANZ states that necropsy results revealed an increase in absolute and relative liver weight in the 3,000 mg/kg body weight group. This was not mentioned in the current GRAS notice. Please explain whether these effects are considered adverse, and if not, why not.*

Ausnutria Response:

Ausnutria did not mention this observation in the GRAS notification because both the study authors and FSANZ did not view the increase in absolute and relative liver weights as an adverse effect. In retrospect, we should have discussed the finding in the GRAS notification. Briefly, as noted by FDA, the *Itabashi et al. (1988b)* study found that the parent rats given 3,000 mg/kg L-carnitine chloride showed an increase in the liver weight and an increase in the liver/body weight. However, in the absence of histological correlates, the authors of the study considered the increased absolute and relative liver weights of the 3000 mg/kg bw/day parent rats to be non-adverse. Notably, FSANZ also drew the same conclusion in its own review. Ausnutria concurred with these assessments.

- **FDA Question #9):** *Kikumori et al. (1988b) conducted a 13-week study in dogs. Please briefly discuss this study.*

Ausnutria Response:

In the 13-week study, the toxicity of L-carnitine chloride with continuous oral administration of 50, 200, and 800 mg/kg LC-80 to 4 beagle dogs of each sex in the 50-mg/kg group and 6 of each sex in each of the other groups were investigated, along with recovery during a 5-week drug-free period. ^{6/} Vomiting and diarrhea occurred transiently after administration in the 800-mg/kg group, but no other toxicity symptoms were found, and no dogs died. The vomiting and diarrhea ceased during the recovery period. Some statistically significant differences such as platelet counts were found. However, these were not dose-dependent, and no L-carnitine chloride-related changes were confirmed. The authors concluded that, on the basis of the above findings, the L-

^{6/} Kikumori, M., et al. (1988). "Subacute toxicity study of l-carnitine chloride in beagles." *Iyakuhin Kenkyu* 19(2): 244-260.

carnitine chloride maximum no-effect dose in this study can be considered to be 200 mg/kg/day in both males and females.

- **FDA Question #10):** For the Kikumori et al. (1988c) (chronic) study mentioned on page 40, please state the duration of the study.

Ausnutria Response:

The duration of the chronic study is 53 weeks.

- **FDA Question #11):** On page 46, the notifier discusses short-term and subchronic toxicity studies of tartaric acid and its salts (section 6.2.4.2). The second paragraph of this section provides the reference of Inoue et al. (2015) for the study being discussed. At the end of the same paragraph, the reference for the same study is given as Down et al. (1977). Please confirm that the reference for this paragraph should be Inoue et al. (2015).

Ausnutria Response:

The reference should be Inoue et al. (2015). We apologize for the error.

- **FDA Question #12):** On page 46, the notifier states that “A NOAEL was identified at 60 mg/kg bw/day for males and 68 mg/kg bw/day for females, respectively.” in the Inoue et al. (2015) study. This Japanese article is also available in English at the journal’s website (https://www.jstage.jst.go.jp/article/tox/28/2/28_2014-0058/pdf/-char/en).
 - a. The English language article states, “According to the data of the present study, the no-observed-adverse-effect level (NOAEL) is judged to be 0.125% in both sexes (males, 0.075 g/kg body weight/day; females, 0.082 g/kg body weight/day).” Please explain the discrepancy between your NOAEL values and those given in the article by the study authors.
 - b. We note that the correct name of the test article in this study is potassium hydrogen tartrate. Please state whether you concur.

Ausnutria Response:

- a. The 0.075 g/kg bw/day and 0.082 g/kg bw/day for males and females respectively identified by the study authors refer to the level of DL-potassium hydrogen tartrate. The 60 mg/kg bw/day for males and 68 mg/kg bw/day for females used in GRN 993 refers to the level of DL-tartaric acid. Ausnutria originally obtained these values from EFSA review of tartaric acid-tartrates (EFSA Journal 2020;18(3):6030, Page 39). The GRAS notification should have attributed these values to the EFSA review.

When we calculate the NOAEL values based on DL-tartaric acid, the NOAELs are 60 mg/kg bw/day in males and 65.4 mg/kg bw/day in females, which are slightly different than the 60 mg/kg bw/day for males and 68 mg/kg bw/day reported in the EFSA review. EFSA did not provide their calculations; we therefore cannot explain how EFSA arrived at its NOAELs. Below, we provide our calculations for the NOAEL in males and females.

Below is the conversion calculation:

- MW of DL-potassium hydrogen tartrate = 188.177 g/mol
- MW of DL-tartaric acid = 150.087 g/mol

Males: $60 \text{ mg/kg bw/day} = 0.075 \text{ g/kg} \times 1000 \text{ mg/g} \times \div 188.177 \text{ g/mol} * 150.087 \text{ g/mol}$

Females: $65.4 \text{ mg/kg bw/day} = 0.082 \text{ g/kg bw/day} \times 1000 \text{ mg/g} \times \div 188.177 \text{ g/mol} * 150.087 \text{ g/mol}$

As such, the NOAEL values for males and females (when based on DL-tartaric acid) we calculated are 60 mg/kg bw/day and 65.4 mg/kg bw/day, respectively. We apologize for any confusion.

- b. Yes, we agree with the agency the correct name of the test article should be DL-potassium hydrogen tartrate (PHT).
- **FDA Question #13):** *For the Packman et al. (1963) study mentioned on page 46, the sodium salt of tartaric acid was administered at a dietary concentration of 7.7%. In its GRAS notice, the notifier states that this dietary concentration corresponds to approximately 550 mg/kg bw/day. Please explain how you arrived at this value.*

Ausnutria Response:

The value reported in the GRAS notification is the same as that reported by EFSA in its 2020 review (EFSA Journal 2020;18(3):6030, Page 39). The GRAS notification should have attributed these values to the EFSA review.

Below, we provide our calculations that identify the same concentration level of 550 mg/kg bw/day. In **Table 1** of the study (**Appendix D**), the authors provided the total food intake for the treatment group as 17.0 kg and the number of animals in each treatment group and the mean body weights at weeks 0, 4, 8, 16, and 22. The weekly food intake for each animal, when taking into consideration the number of animals in each group at various timepoints, can be calculated as:

17 kg ÷ (14 animals x 4 weeks (week 4- week 0) + 10 animals x 4 weeks (week 8- week 4) + 5 animals x 8 weeks (week 16- week 8) + 5 animals x 6 weeks (week 22- week 16)) = 0.1 kg

Because the sodium tartrate constitutes up to 7.7% of the total diet, the daily intake of sodium tartrate can be calculated as:

0.1 kg x 7.7% ÷ 7 days/week x 1000 g/kg = 1.1 g/day

We gather from **Table 1** that the body weight is roughly 2 kg per animal, as such, the dietary consumption of sodium tartrate can be calculated as:

1.1 g/day ÷ 2 kg bw x 1000 mg/g = 550 mg/kg bw/day

- **FDA Question #14):** For the Hunter et al. (1977) study:
 - a. Please state the NOAEL, if any.
 - b. On page 47, the notifier states, “Survival rate from 78 weeks was lower in the rats receiving 42,240, 60,160 or 76,800 ppm monosodium L-Tartaric acid than controls.” In the original publication by Hunter et al. (1977) the study authors state, “During the first 78 weeks of treatment, survival among treated rats was similar to that of the control group. However, during the final six months of the study the number of rats that died from the groups receiving mono- sodium L(+) tartrate at dietary levels of 42 240, 60 160 or 76 800 ppm was significantly less than in the control group.” (page 265). Fewer rats died at the top 3 doses; hence the survival rate was higher for these groups than that of the controls. Please state whether you concur.

Ausnutria Response:

- a) The authors of the study did not provide an NOAEL, however, based on the findings of the study, we believe the NOAEL for monosodium L-tartrate can be considered as 3,100 mg/kg bw/day for male and 4,100 mg/kg bw/day for female. These are equivalent to 2,460 mg/kg bw/day L tartaric acid for males and 3,200 mg/kg bw/day for females.
 - b) Yes, we agree. The rats receiving L tartaric acid had higher survival rates than the control group. The authors of the study suspected this was correlated with the lower food intake of these groups and the resultant reduced body weight gain. We apologize for the mis-statement and should have used the language in the GRAS notification from the study, “During the first 78 weeks of treatment, survival among treated rats was similar to that of the control group. However, during the final six months of the study the number of rats that died from the groups receiving mono- sodium L(+) tartrate at dietary levels of 42 240, 60 160 or 76 800 ppm was significantly less than in the control group.”
- **FDA Question #15):** For the Younes et al. (2020) study described on pages 47-48, please state what the developmental toxicity NOAELs, if any, are in the rat, hamster, and rabbit developmental toxicity studies of tartaric acid.

Ausnutria Response:

The NOAELs for developmental toxicity reported by *Younes et al. (2020)* for different animal species are summarized in the table, below.

Animal species	NOAEL for developmental toxicity studies of tartaric acid
Mice	274 mg/kg bw per day
Rat	181 mg/kg bw per day
Hamster	225 mg/kg bw per day
Rabbit	215 mg/kg bw per day for 13 days

* * *

We trust the agency will agree we have addressed each of the agency's questions. If any additional questions arise in the course of your review, please contact us, preferably by telephone or e-mail, so that we can provide a prompt response.

Sincerely,



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Appendix A. NHANES 2015-2016 Food Code Names (Infant Formula)

Infant formula, NFS
Infant formula, NS as to form (Similac Expert Care Alimentum)
Infant formula, ready-to-feed (Similac Expert Care Alimentum)
Infant formula, powder, made with water, NFS (Similac Expert Care Alimentum)
Infant formula, powder, made with tap water (Similac Expert Care Alimentum)
Infant formula, powder, made with plain bottled water (Similac Expert Care Alimentum)
Infant formula, powder, made with baby water (Similac Expert Care Alimentum)
Infant formula, NS as to form (Similac Advance)
Infant formula, ready-to-feed (Similac Advance)
Infant formula, liquid concentrate, made with water, NFS (Similac Advance)
Infant formula, powder, made with water, NFS (Similac Advance)
Infant formula, liquid concentrate, made with tap water (Similac Advance)
Infant formula, liquid concentrate, made with plain bottled water (Similac Advance)
Infant formula, liquid concentrate, made with baby water (Similac Advance)
Infant formula, powder, made with tap water (Similac Advance)
Infant formula, powder, made with plain bottled water (Similac Advance)
Infant formula, powder, made with baby water (Similac Advance)
Infant formula, NS as to form (Similac Advance Organic)
Infant formula, ready-to-feed (Similac Advance Organic)
Infant formula, powder, made with water, NFS (Similac Advance Organic)
Infant formula, powder, made with tap water (Similac Advance Organic)
Infant formula, powder, made with plain bottled water (Similac Advance Organic)
Infant formula, powder, made with baby water (Similac Advance Organic)
Infant formula, NS as to form (Similac Sensitive)
Infant formula, ready-to-feed (Similac Sensitive)
Infant formula, liquid concentrate, made with water, NFS (Similac Sensitive)
Infant formula, powder, made with water, NFS (Similac Sensitive)
Infant formula, liquid concentrate, made with tap water (Similac Sensitive)
Infant formula, liquid concentrate, made with plain bottled water (Similac Sensitive)
Infant formula, liquid concentrate, made with baby water (Similac Sensitive)
Infant formula, powder, made with tap water (Similac Sensitive)

Infant formula, powder, made with plain bottled water (Similac Sensitive)
Infant formula, powder, made with baby water (Similac Sensitive)
Infant formula, NS as to form (Similac for Spit-Up)
Infant formula, ready-to-feed (Similac for Spit-Up)
Infant formula, powder, made with water, NFS (Similac for Spit-Up)
Infant formula, NS as to form (Similac Expert Care NeoSure)
Infant formula, ready-to-feed (Similac Expert Care NeoSure)
Infant formula, powder, made with water, NFS (Similac Expert Care NeoSure)
Infant formula, powder, made with tap water (Similac Expert Care NeoSure)
Infant formula, powder, made with plain bottled water (Similac Expert Care NeoSure)
Infant formula, powder, made with baby water (Similac Expert Care NeoSure)
Infant formula, NS as to form (Similac Go and Grow)
Infant formula, powder, made with water, NFS (Similac Go and Grow)
Infant formula, NS as to form (Enfamil Newborn)
Infant formula, ready-to-feed (Enfamil Newborn)
Infant formula, powder, made with water, NFS (Enfamil Newborn)
Infant formula, powder, made with tap water (Enfamil Newborn)
Infant formula, powder, made with plain bottled water (Enfamil Newborn)
Infant formula, powder, made with baby water (Enfamil Newborn)
Infant formula, NS as to form (Enfamil Infant)
Infant formula, ready-to-feed (Enfamil Infant)
Infant formula, liquid concentrate, made with water, NFS (Enfamil Infant)
Infant formula, liquid concentrate, made with tap water (Enfamil Infant)
Infant formula, liquid concentrate, made with plain bottled water (Enfamil Infant)
Infant formula, liquid concentrate, made with baby water (Enfamil Infant)
Infant formula, powder, made with water, NFS (Enfamil Infant)
Infant formula, powder, made with tap water (Enfamil Infant)
Infant formula, powder, made with plain bottled water (Enfamil Infant)
Infant formula, powder, made with baby water (Enfamil Infant)
Infant formula, NS as to form (Enfamil A.R.)
Infant formula, ready-to-feed (Enfamil A.R.)
Infant formula, powder, made with water, NFS (Enfamil A.R.)
Infant formula, powder, made with tap water (Enfamil A.R.)

Infant formula, NS as to form (Enfamil EnfaCare)
Infant formula, ready-to-feed (Enfamil EnfaCare)
Infant formula, powder, made with water, NFS (Enfamil EnfaCare)
Infant formula, powder, made with plain bottled water (Enfamil A.R.)
Infant formula, powder, made with baby water (Enfamil A.R.)
Infant formula, NS as to form (Enfamil Gentlease)
Infant formula, ready-to-feed (Enfamil Gentlease)
Infant formula, powder, made with water, NFS (Enfamil Gentlease)
Infant formula, powder, made with tap water (Enfamil EnfaCare)
Infant formula, powder, made with plain bottled water (Enfamil EnfaCare)
Infant formula, powder, made with baby water (Enfamil EnfaCare)
Infant formula, powder, made with tap water (Enfamil Gentlease)
Infant formula, powder, made with plain bottled water (Enfamil Gentlease)
Infant formula, powder, made with baby water (Enfamil Gentlease)
Infant formula, NS as to form (Enfamil Enfagrow Toddler Transitions)
Infant formula, ready-to-feed (Enfamil Enfagrow Toddler Transitions)
Infant formula, powder, made with water, NFS (Enfamil Enfagrow Toddler Transitions)
Infant formula, powder, made with tap water (Enfamil Enfagrow Toddler Transitions)
Infant formula, powder, made with plain bottled water (Enfamil Enfagrow Toddler Transitions)
Infant formula, powder, made with baby water (Enfamil Enfagrow Toddler Transitions)
Infant formula, NS as to form (Enfamil Enfagrow Toddler Transitions Gentlease)
Infant formula, powder, made with water, NFS (Enfamil Enfagrow Toddler Transitions Gentlease)
Infant formula, powder, made with tap water (Enfamil Enfagrow Toddler Transitions Gentlease)
Infant formula, powder, made with plain bottled water (Enfamil Enfagrow Toddler Transitions Gentlease)
Infant formula, powder, made with baby water (Enfamil Enfagrow Toddler Transitions Gentlease)
Infant formula, NS as to form (PediaSure)
Infant formula, ready-to-feed (PediaSure)
Infant formula, with fiber, NS as to form (PediaSure Fiber)
Infant formula, with fiber, ready-to-feed (PediaSure Fiber)
Infant formula, NS as to form (Gerber Good Start Gentle)

Infant formula, ready-to-feed (Gerber Good Start Gentle)
Infant formula, liquid concentrate, made with water, NFS (Gerber Good Start Gentle)
Infant formula, powder, made with water, NFS (Gerber Good Start Gentle)
Infant formula, liquid concentrate, made with tap water (Gerber Good Start Gentle)
Infant formula, liquid concentrate, made with plain bottled water (Gerber Good Start Gentle)
Infant formula, liquid concentrate, made with baby water (Gerber Good Start Gentle)
Infant formula, powder, made with tap water (Gerber Good Start Gentle)
Infant formula, powder, made with plain bottled water (Gerber Good Start Gentle)
Infant formula, powder, made with baby water (Gerber Good Start Gentle)
Infant formula, NS as to form (Gerber Good Start Protect)
Infant formula, powder, made with water, NFS (Gerber Good Start Protect)
Infant formula, powder, made with tap water (Gerber Good Start Protect)
Infant formula, powder, made with plain bottled water (Gerber Good Start Protect)
Infant formula, powder, made with baby water (Gerber Good Start Protect)
Infant formula, NS as to form (Gerber Graduates Gentle)
Infant formula, NS as to form (Gerber Graduates Protect)
Infant formula, NS as to form (Store Brand)
Infant formula, liquid concentrate, made with water, NFS (Store Brand)
Infant formula, powder, made with water, NFS (Store Brand)
Infant formula, ready-to-feed (Store Brand)
Infant formula, liquid concentrate, made with tap water (Store Brand)
Infant formula, liquid concentrate, made with plain bottled water (Store Brand)
Infant formula, liquid concentrate, made with baby water (Store Brand)
Infant formula, powder, made with tap water (Store Brand)
Infant formula, powder, made with plain bottled water (Store Brand)
Infant formula, powder, made with baby water (Store Brand)
Infant formula, NS as to form (Enfamil ProSobee)
Infant formula, ready-to-feed (Enfamil ProSobee)
Infant formula, liquid concentrate, made with water, NFS (Enfamil ProSobee)
Infant formula, powder, made with water, NFS (Enfamil ProSobee)
Infant formula, liquid concentrate, made with tap water (Enfamil ProSobee)
Infant formula, liquid concentrate, made with plain bottled water (Enfamil ProSobee)
Infant formula, liquid concentrate, made with baby water (Enfamil ProSobee)

Infant formula, powder, made with tap water (Enfamil ProSobee)
Infant formula, powder, made with plain bottled water (Enfamil ProSobee)
Infant formula, powder, made with baby water (Enfamil ProSobee)
Infant formula, NS as to form (Enfamil Enfagrow Toddler Transitions Soy)
Infant formula, powder, made with water, NFS (Enfamil Enfagrow Toddler Transitions Soy)
Infant formula, NS as to form (Similac Isomil Soy)
Infant formula, ready-to-feed (Similac Isomil Soy)
Infant formula, liquid concentrate, made with water, NFS (Similac Isomil Soy)
Infant formula, powder, made with water, NFS (Similac Isomil Soy)
Infant formula, liquid concentrate, made with tap water (Similac Isomil Soy)
Infant formula, liquid concentrate, made with plain bottled water (Similac Isomil Soy)
Infant formula, liquid concentrate, made with baby water (Similac Isomil Soy)
Infant formula, powder, made with tap water (Similac Isomil Soy)
Infant formula, powder, made with plain bottled water (Similac Isomil Soy)
Infant formula, powder, made with baby water (Similac Isomil Soy)
Infant formula, NS as to form (Similac Expert Care for Diarrhea)
Infant formula, ready-to-feed (Similac Expert Care for Diarrhea)
Infant formula, NS as to form (Gerber Good Start Soy)
Infant formula, ready-to-feed (Gerber Good Start Soy)
Infant formula, liquid concentrate, made with water, NFS (Gerber Good Start Soy)
Infant formula, powder, made with water, NFS (Gerber Good Start Soy)
Infant formula, liquid concentrate, made with tap water (Gerber Good Start Soy)
Infant formula, liquid concentrate, made with plain bottled water (Gerber Good Start Soy)
Infant formula, liquid concentrate, made with baby water (Gerber Good Start Soy)
Infant formula, powder, made with tap water (Gerber Good Start Soy)
Infant formula, powder, made with plain bottled water (Gerber Good Start Soy)
Infant formula, powder, made with baby water (Gerber Good Start Soy)
Infant formula, NS as to form (Gerber Graduates Soy)
Infant formula, NS as to form (Store Brand Soy)
Infant formula, ready-to-feed (Store brand Soy)
Infant formula, liquid concentrate, made with water, NFS (Store Brand Soy)
Infant formula, powder, made with water, NFS (Store Brand Soy)
Infant formula, powder, made with tap water (Store Brand Soy)

Infant formula, powder, made with plain bottled water (Store Brand Soy)
Infant formula, powder, made with baby water (Store Brand Soy)
Infant formula, NS as to form (Enfamil Nutramigen)
Infant formula, ready-to-feed (Enfamil Nutramigen)
Infant formula, liquid concentrate, made with water, NFS (Enfamil Nutramigen)
Infant formula, powder, made with water, NFS (Enfamil Nutramigen)
Infant formula, NS as to form (PurAmino)
Infant formula, powder, made with water, NFS (PurAmino)
Infant formula, NS as to form (Enfamil Pregestimil)
Infant formula, ready-to-feed (Enfamil Pregestimil)
Infant formula, powder, made with water, NFS (Enfamil Pregestimil)
Infant formula, ready-to-feed, low iron (Enfamil Premature 20 Cal)
Infant formula, ready-to-feed, with iron (Enfamil Premature 20 Cal)

Appendix B. 30x10g samples from three different production lots (i.e., Batch 85027, Batch 93391, and Batch 96472 on *C. sakazakii*) when tested with ISO 22964:2017

Sample reference	Parameter	Unit	Value
L carnitine L tartrate batch 85027, procut 1000049	Cronobacter spp	/10 g	Negative
L carnitine L tartrate batch 85027, procut 1000049	Cronobacter spp	/10 g	Negative
L carnitine L tartrate batch 85027, procut 1000049	Cronobacter spp	/10 g	Negative
L carnitine L tartrate batch 85027, procut 1000049	Cronobacter spp	/10 g	Negative
L carnitine L tartrate batch 85027, procut 1000049	Cronobacter spp	/10 g	Negative
L carnitine L tartrate batch 85027, procut 1000049	Cronobacter spp	/10 g	Negative
L carnitine L tartrate batch 85027, procut 1000049	Cronobacter spp	/10 g	Negative
L carnitine L tartrate batch 85027, procut 1000049	Cronobacter spp	/10 g	Negative
L carnitine L tartrate batch 85027, procut 1000049	Cronobacter spp	/10 g	Negative
L carnitine L tartrate batch 85027, procut 1000049	Cronobacter spp	/10 g	Negative
L carnitine L tartrate batch 85027, procut 1000049	Cronobacter spp	/10 g	Negative
L carnitine L tartrate batch 85027, procut 1000049	Cronobacter spp	/10 g	Negative
L carnitine L tartrate batch 85027, procut 1000049	Cronobacter spp	/10 g	Negative
L carnitine L tartrate batch 85027, procut 1000049	Cronobacter spp	/10 g	Negative
L carnitine L tartrate batch 85027, procut 1000049	Cronobacter spp	/10 g	Negative
L carnitine L tartrate batch 85027, procut 1000049	Cronobacter spp	/10 g	Negative
L carnitine L tartrate batch 85027, procut 1000049	Cronobacter spp	/10 g	Negative
L carnitine L tartrate batch 85027, procut 1000049	Cronobacter spp	/10 g	Negative
L carnitine L tartrate batch 85027, procut 1000049	Cronobacter spp	/10 g	Negative
L carnitine L tartrate batch 85027, procut 1000049	Cronobacter spp	/10 g	Negative
L carnitine L tartrate batch 85027, procut 1000049	Cronobacter spp	/10 g	Negative
L carnitine L tartrate batch 85027, procut 1000049	Cronobacter spp	/10 g	Negative

Thirteen pages have been removed in accordance with copyright laws. The removed reference citations are:

Brandsch, C., and K. Eder. "Reproductive performance of rats supplemented with l-carnitine." *Journal of animal physiology and animal nutrition* 87.7-8 (2003): 301-308.

E. W. Packman, D. D. Abbott, and J. W. E. Harrison. "Comparative Subacute Toxicity for Rabbits of Citric, Fumaric, and Tartaric Acids." *Toxicology and Applied Pharmacology* 5, 163-167 (1963)