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Research Article

Genotoxicity and subchronic oral toxicity studies of L-carnitine and L-carnitine L-tartrate

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ABSTRACT

L-Carnitine is an endogenous substance that is biosynthesized from theamino acids lysine and methionine, and is also obtained from an omnivorous diet. The safety of L-carnitine and L-carnitine L-tartrate, as a dietary source of L-carnitine, was assessed in a subchronic toxicity study and in two genotoxicity assays. In a 90-day subchronic study, rats received diets containing 0, 2,500, 12,500, or 50,000 ppm L-carnitine L-tartrate for a period of 90 days, followed by a four-week recovery period. No treatment-related effects were noted on mortality, ophthalmology, hematology, gross pathology, or histopathology. Increases observed in food and water consumption, changes in absolute and relative seminal vesicle weights,and effects noted in urinalysis evaluations were deemed to be of no toxicological significance, as they were either considered to be a physiological response, were transient and disappeared at the end of the recovery period, or were not accompanied by any microscopic changes. L-carnitine displayed no mutagenic activity in various bacterial strains at concentrations up to 5,000 μg/plate both in the presence or absence of metabolic activation, nor did it induce chromosome aberrations in human lymphocytes. The results of these experiments support the safety of L-carnitine and L-carnitine L-tartrate as a dietary source of L-carnitine.

Keywords: L-carnitine; L-carnitine L-tartrate; Subchronic; Toxicity; Safety; Genotoxicity

Abbreviations: ALP: Alkaline Phosphatase; EFSA: European Food Safety Authority; FPNUs: Foods for Particular Nutritional Uses; GLP: Good Laboratory Practices; LDH: Lactate Dehydrogenase; NOAEL: No-Observed-Adverse-Effect Level; OECD: Organization for Economic Co-operation and Development; UK: United Kingdom

INTRODUCTION

L-Carnitine is a naturally-occurring substance found in all mammalian species. It is biosynthesized from the amino acids lysine and methionine, where lysine provides the carbon backbone of L-carnitine and methionine makes up the 4-*N*-methyl groups [1-3]. The predominant site of L-carnitine biosynthesis is the liver, although, it also is biosynthesized in the kidney and brain [4-6]. Dietary sources contribute to approximately 75% of the body's store of L-carnitine [7]. Background dietary intake of L-carnitine occurs as a result of its natural occurrence in the diet, mainly in products of animal origin, with lamb, beef, and pork, being the richest sources of dietary L-carnitine [8,2].

L-Carnitine has a number of physiological functions, the most prominent being the transport of long-chain fatty acids into mitochondria. By acting as a shuttle and an essential co-factor, L-carnitine facilitates the β-oxidation of fatty acids, and as such performs a crucial role in cellular metabolism of fat and energy production [9,10].

The safety of L-carnitine(in the form of L-carnitine chloride) has previously been evaluated in a number of subchronic and chronic oral toxicity studies in Sprague-Dawley rats and beagle dogs [11-14]. Overall, the chronic administration of L-carnitine chloride by gavage for one year was not associated with toxicologicallysignificant adverse effects in rats at doses of up to 737 mg/kg body weight/day [13] or in dogs at doses of up to 200 mg/kg body weight/day [12]. These doses of L-carnitine chloride are equivalent to 601 and 163 mg L-carnitine/kg body weight/day, respectively. In these studies, the study authors based the no-observed-adverse-effect level (NOAEL) determinations upon observed gastrointestinal symptoms. However, in both species, gastrointestinal effects observed at high doses were considered to be physiological responses, typically associated with large bolus doses, rather than evidence of systemic toxicity [13-14] and therefore, the NOAEL could be established at a higher dose.

In the following study, the potential toxicity of L-carnitinefrom dietary intakes of L-carnitine L-tartrate was studied at higher L-carnitine intakes than tested previously in a 90-day subchronic

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toxicity study. In addition, an Ames assay and a chromosome aberration test were conducted to assess the mutagenic potential of L-carnitine.

MATERIALS AND METHODS

The test substance for all the studies was supplied by Lonza AG. For the subchronic toxicity study, the test substance, L-carnitine L-tartrate (batch no. 00202, purity 99.9%), was supplied as a white crystalline solid.L-Carnitine L-tartrate is the salt of L-carnitineand tartaric acid, consisting of approximately 68% L-carnitine and 32% L-tartaric acid. Upon receipt at the testing facility (LPT Laboratory of Pharmacology and Toxicology KG, Germany), the test substance was inspected and the physico-chemical parameters such as color, consistency, physical state, melting point, and pH values were determined and compared with information provided by the supplier.

For the *in vitro*bacterial reverse mutation assay (Ames test), L-carnitine was provided an off-white powder (batch no. 0350-000-01) with a purity of 99.6%. For the *in vitro* chromosome aberration test, the test substance, L-carnitine (batch no.: 0350-000-01), had a purity of 99% and was kept in a cool and dry place prior to use.

Subchronic toxicity study

Animal treatment and test article administration

The study was conducted atLPT Laboratory of Pharmacology and Toxicology KG (Germany)in accordance with the Organization for Economic Co-operation and Development (OECD) Principles of Good Laboratory Practices (GLP). Male and female Crl:CD rats were obtained from Charles River Deutschland (Sulzfeld, Germany) at five to sixweeks of age, and quarantined and acclimatized for seven days. Animals were assessed for health conditions and allocated to groups based on body weights by computer randomization. Animals were housed individually in Makrolontype III cages, and provided food (ssniff®R/M-H V1530, ssniff Spezialdiäten Gmbh, Soest, Germany) and tap water *ad libitum*. The composition of the ssniff®R/M-H V1530 diet is provided in Table 1. During the study animals were housed in a facility designed to maintain appropriate environmental conditions (19-25°C, 40-70% relative humidity, 12-h light/dark cycle). To prepare the test diet, L-carnitine L-tartrate at various concentrations of 2,500, 12,500, and 50,000 ppm was mixed with the control pelleted diet using an impact mill. During the course of the study, the test diets were freshly prepared once a week. Homogeneity, concentration, and stability of the test diets were confirmed throughout the study period.

Groups of 10 male and 10 female rats were fed test diets containing 0 (control), 2,500 (low-dose), 12,500 (mid-dose) and 50,000 (high-dose) ppm L-carnitine L-tartrate. Additional groups of 20 animals (5/sex/group) received control diets or diets containing 50,000 ppm L-carnitine L-tartrate for 90 days followed by a four-week recovery period.

Clinical observations, body weight, food and water consumption

Animals were observed for general clinical signs at least once daily. All animals were inspected for signs of morbidity and mortality twice daily (once before and once after treatment). Detailed clinical observations were made on all animals once before the first exposure and once a week thereafter. Body weights were measured prior to treatment, on the first day of treatment, and once weekly

Table 1: The composition of the ssniff®R/M-H V1530 diet.

Ingredients	Average content in the diet
Energy (MJ/kg)	12.2
Crude protein (%)	19
Crude fat (%)	3.3
Crude fiber (%)	4.9
Ash (%)	6.7
Minerals (Average % content in the diet)	
Calcium	1
Phosphorous	0.7
Sodium	0.25
Magnesium	0.2
Potassium	0.9
Amino acids (Average % content in the diet)	
Lysine	1
Methionine	0.3
Cystine	0.3
Glycine (%)	0.9
Leucine (%)	1.3
Isoleucine (%)	0.7
Arginine (%)	1.2
Phenylalanine (%)	0.9
Tryptophan (%)	0.25
Histidine (%)	0.5
Tyrosine (%)	0.6
Asparaginic acid (%)	1.7
Glutamic acid (%)	3.8
Valine (%)	0.9
Threonine (%)	0.7
Trace elements (ppm)	
Manganese	90
Copper	12
Zinc	75
Iodine	2
Iron	220
Selenium	0.2
Cobalt	2
Vitamins (per kg/diet)	
Vitamin A (IU)	15,000
Vitamin D3 (IU)	1,000
Vitamin E (mg)	100
Vitamin B1 (mg)	10
Vitamin B2 (mg)	20
Vitamin B6 (mg)	12
Vitamin B12 (µg)	80
Biotin (µg)	400
Pantothenic acid (mg)	30
Choline chloride (mg)	1,600
Folic acid (mg)	4
Nicotinic acid (mg)	60
Vitamin K3 (mg)	5
Inositol (mg)	50

thereafter. Food consumption measurements were taken once at pre-treatment and once weekly during the treatment and recovery period. Water consumption was quantitatively determined during treatment weeks 6 and 12.

Ophthalmological examinations and urinalysis

Ophthalmological examinations were conducted on all surviving animals in the last week of the main study (week 13) and during the recovery period (week 17). For urinalysis, urine was collected from all animals in the main study on day 86 and from all animals in the recovery period on day 118 (all animals were fasted overnight), and evaluated for the following parameters: specific gravity (using an Atago Refractometer), color and turbidity (examined visually), volume, and pH (using a pH meter). Sodium, potassium, and chloride were analyzed using a flame photometer. The following parameters also wereexamined using quantitative indicators of analyte concentration (Combur 10® test, Roche Diagnostics GmbH, Mannheim, Germany): protein, glucose, ketone body, bilirubin, occult blood, urobilinogen, and nitrite. Following centrifugation of urine samples, macroscopic examinations were carried out by examining the deposits for the presence of the epithelial cells, leucocytes, erythrocytes, organisms, crystalluria, and further constituents such as sperm and casts.

Hematology and clinical biochemistry

Prior to necropsy, blood samples were collected from the retrobulbar venous plexus under light ether anesthesia from all animals fasted overnight in the main study on day 86 and all fasted animals in the recovery period on day 118. In ethylene diamine tetraacetic acid-treated blood, hematological parameters measured using Advia™ 120 hematology analyzer (Bayer Vital GmbH & Co. KG, Fernwald, Germany) included: erythrocytes count, white blood cell count, differential blood count (neutrophilic, eosinophilic and basophilic granulocytes), lobularity index, reticulocytes, hematocrit value, hemoglobin content, platelet count, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration. In citrate-treated blood, thromboplastin time and activated partial thromboplastin time were assessed according to the methods of Quick [15] and Bell and Alton (1954), respectively.

Blood collected for clinical biochemistry analysis was treated with heparin and the serum was examined for the following parameters using a Konelab 20i clinical biochemistry analyzer (Labsystems GmbH, Frankfurt, Germany): aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), γ-glutamyl transpeptidase, total bilirubin, total protein, albumin, globulin, albumin/globulin ratio, total cholesterol, triglyceride, glucose, blood urea nitrogen, creatinine, inorganic phosphate, calcium, sodium, potassium, and chloride.

Clinical pathology and histopathology

All rats in the main study were anesthetized by ether and following blood sample collection, were exsanguinated on days 91 and 92. Necropsy for all animals allocated to the recovery period was performed on day 119. A standard list of organs were weighed, and the relative weights calculated. Tissues fixed for histopathological evaluation included: trachea (including larynx), tongue, esophagus, stomach, small intestine (duodenum, jejunum, and ileum), large intestine (cecum, colon, and rectum), pancreas, aorta, urinary bladder, vagina, spinal cord, sciatic nerve (right and left), sternum, femur (right and left), submandibular lymph node (right and left), eyeball/optic nerve (right and left), skeletal muscle (thigh), mammary gland, uterus (including cervix and oviducts), ovaries, epididymis, brain (cerebrum, cerebellum, medulla), prostate, testicles, seminal vesicle, salivary gland, thymus, spleen, pituitary, liver, lungs, nasal cavity (turbinate), thyroid, adrenal, heart, Zymbal gland, and skin. All stored organs and tissues of each animal in the control and high-dose groups were embedded, sectioned, stained with hematoxylin-eosin, and examined microscopically.

Statistical analysis

For quantitative data including body weight, food and water consumption, hematology, clinical biochemistry, urinalysis, and relative organ weights means and standard deviations were calculated and statistical tests were performed based on Dunnett's multiple comparison test. All statistical analysis were performed at the $p<0.05$ level of significance. Clinical pathology data were analyzed using Fisher's exact test.

In vitro bacterial reverse mutation assay (Ames test)

This study was conducted at Safepharm Laboratories Limited, United Kingdom (UK), in compliance with UK GLP standards, and in accordance with the requirements of the OECD Guideline for Testing of Chemicals No. 471 (OECD, 1997). The bacterial reverse mutation assay, using the plate incorporation method, was conducted in histidine-requiring *Salmonella typhimurium*(*S. typhimurium*) strains TA98, TA100, TA1535, and TA1537, and tryptophan-requiring *Escherichia coli* (*E. coli*) WP2*uvrA*$^-$pKM101 strain. All strains were stored at -196°C in a Statebourne liquid nitrogen freezer. Prior to use, characterization checks were carried out on the master strains to determine the amino-acid requirement, presence of *rfa*, R factors, *uvrB* and *uvrA* mutations, and the spontaneous reversion rate. The bacterial strains were cultured in nutrient broth at 37°C for approximately 10 hours. The assay was performed with or without metabolic activation using the S9 fraction from the livers of phenobarbitone/β-naphthoflavone (80/100 mg/kg body weight/day)-treated rats.Before testing, 50 mg/mL of the test material dissolved in sterile distilled water was filtered through a 0.2 µm sterile filter. This solution was then used to prepare serial dilutions in water.

Dilutions of thetest article were prepared in sterile distilled water. The test substance solution (0.1 mL), 0.1 mL of fully-grown bacterial culture and 0.5 mL S9-mix or 0.2 M sodium phosphate (pH 7.5) were added to 2 mL molten top agar (containing 0.6% agar, 0.5% NaCl and 1 mM L-histidine. HCl/1mM biotin for the *S. typhimurium* strains or supplemented with 1 mM tryptophan for the *E. coli* strain). The ingredients were thoroughly mixed and equally distributed onto the surface of Vogel-Bonner Minimal agar plates (30 mL/plate). Following incubation at 37°C for approximately 48 hours, the frequency of revertant colonies was assessed using a Domino colony counter. This procedure was repeated in triplicate for each tester strain at five concentrations (50, 150, 500, 1,500, and 5,000 µg/plate). The dose levels were selected based on the results of a preliminary test that was carried out in two tester strains (TA100 and WP2*uvrA*$^-$ pKM101) at concentrations of 0, 0.15, 0.5, 1.5, 5, 15, 50, 150, 1,500, and 5,000 µg/plate to determine the toxicity of the test material. The test material was not toxic to the strains of bacteria at the concentrations tested (results not shown).

Negative (solvent) and positive controls were also used in parallel with the test material. Positive control mutagens in the absence of S9 included *N*-ethyl-*N'*-nitro-*N*-nitrosoguianidine for TA100, TA1535,

and WP2*uvrA*- pKM101 tester strains, 9-aminoacridinefor TA1537 tester strain, and 4-nitroquinoline-1-oxide for TA98. Positive controls used in the presence of S9 included 2-aminoanthracene for TA100, TA1535, TA1537, and WP2*uvrA*- pKM101 tester strains, and benzo[a]pyrene for TA98 tester strain. If a two-fold or greater increase in the mean number of revertants was observed compared to the number of revertants on negative control plates, the test substance was considered to be mutagenic.

In vitro chromosome aberration test in human lymphocytes

The study was conducted at Harlan Laboratories Ltd. (Switzerland) in accordance with the OECD Principles of GLP, and in compliance with the OECD Guidelines for Testing of Chemicals No. 473 (OECD, 1997). The potential of L-carnitine to induce structural chromosomal aberrations was assessed in human lymphocytes in two independent experiments. All experiments were conducted in duplicate.Blood samples were obtained from healthy donors (a 24-year-old male and a 31-year-old female) not receiving medication. Blood cultures were set up in bulk within 24 hours after collection. The culture medium consisted of Dulbecco's modified eagle medium/Ham's F12 medium (1:1) (DMEM:F12, Life Technologies GmbH, Eggenstein, Germany) containing 10% (v/v) fetal calf serum provided by PAA Laboratories GmbH (Cölbe, Germany) and supplemented with 0.1 mL solution of penicillin (10,000 U/mL) and streptomycin (10,000 µg/mL) (Seromed, Berlin, Germany). In addition, the culture medium was supplemented with 0.05 mL phyto hemagglutinin (final concentration 3 µg/mL, Seromed, Berlin, Germany), 0.05 mL heparin (25,000 USP'U/mL, Nattermann, Köln, Germany), and 0.1 mL HEPES (final concentration 10 mM, Serva, Heidelberg, Germany). Deionized water acted as a negativecontrol, and cyclophosphamide (Aldrich Chemie, Steinheim, Germany) and ethylmethane sulfonate (Acros Organics, Geel, Belgium) acted as positive controls in the absence and presence of metabolic activation, respectively.

The dose selection was based on a preliminary cytotoxicity test performed in duplicate with 10 concentrations of the test substance (ranging from 10.5 to 1,620 µg/mL), as well as a solvent and positive control. The test conditions were similar to those required for the main study. Exposure time was four hours (with and without S9 mix), and the preparation interval was 22 hours after start of the exposure. Cytotoxicity was characterized by the percentage of mitotic suppression in comparison to the controls by counting 1,000 cells per culture in duplicate. No cytotoxicity occurred at the concentrations tested (data not shown).

Two separate experiments were conducted, in which cells were seeded in 10 mL of culture medium in 75-cm^3 sterile cell culture flasks (Greiner, Frickenhausen, Germany), and incubated at 37°C in humidified air containing 5.5% CO_2. Cells were treated with the test substance at concentrations of 529, 925.7, and 1,620 µg/mL, and incubated for four hours. After the incubation period, the S9 and the test substance were washed out with saline G, and the culture medium was replaced with freshly added medium (for treatment without S9 mix) or serum-free medium (for treatment with S9 mix), and incubated with fresh medium for 18 hours. For the treatment with metabolic activation, 50 µL S9 mix/mL medium was used. In the second experiment, cells were continuously treated with the test substance (without S9 mix) for 22 hours. All cultures were incubated at 37°C in humidified air containing 5.5% CO_2.

To arrest the cells in metaphase, colcemid (Fluka, Neu-Ulm, Germany) at a final concentration of 0.2 µg/mL was added to cultures three hours before the end of the incubation period. Cells were then harvested by centrifugation, supernatants removed, and cells were re-suspended in 5 mL hypotonic potassium chloride (0.0375 M). Cells were allowed to stand at 37°C for 20-25 min, centrifuged to remove hypotonic solution, and fixed with a mixture of methanol and glacial acetic acid (3:1). Fixed cell suspensions were dropped on clean glass slides and stained with Giemsa (Merck, Darmstadt, Germany). Blind analysis was conducted for 100 well-spread metaphases per specimen. Only metaphases with 46 ± 1 centromer regions were included in the analysis. Structural aberrations recorded included chromatid breaks, chromatid exchanges, deletions and chromosomal disintegrations. The Fisher's exact test was performed to compare incidences of structural aberrations in the negative control to that of the other treatment groups. Statistical significance was designated at $p<0.05$. The incidences of cells with only chromosomal gaps were excluded from statistical analyses. The test article was judged to be positive for chromosome aberration-inducing potential, if the number of structural aberrations in the treated groups were statistically significantly increased compared to the negative control group in a dose-dependent manner.

RESULTS

Subchronic toxicity study

In the subchronic toxicity study, the mean intakes of L-carnitine L-tartrate for the low-, mid-, and high-dose were calculated to be 196, 1,018, and 4, 365 mg/kg body weight/day for male rats, and 218, 1,118, and 4,935 mg/kg body weight/day for female rats, respectively. No mortality was observed in animals of the control, low-dose or mid-dose groups. One male and one female in the high-dose group died prematurely on test days 86 and 118, respectively, during examination due to anesthesia and the blood withdrawal procedure. As such, the deaths were not considered treatment-related.

Animals in the low-dose and mid-dose groups did not show any clinical signs of systemic toxicity. Soft feces were noted in all animals of the high-dose groupsstarting from test day six onwards. The occurrence of soft stool lasted during the first eight days of the recovery period and had disappeared on day nine of the recovery period. A quantitative determination of the drinking water consumption in test weeks 6 and 12 revealed a statistically significant increase ($p \leq 0.05$) in the relative drinking water intake in high-dose groups ranging from 27-35%. This lasted during the first 8 days of the recovery period and disappeared on day nine (Figure 1a,b).

Compared to control animals, food consumption was significantly increased by 9-14% in high-dose males in test weeks 6 to 8, 10, 11, and 13. In the recovery period, the food consumption of high-dose male rats was significantly increased compared to controls by about 25 and 15% in test weeks 14 and 15, respectively. A significant increase in food consumption (up to 17%) also was observed in high-dose females during weeks six to eight compared to their respective controls. No significant changes were observed during the recovery period (Figure 2a,b). There were no statistically significant differences in the body weight or body weight gain in the treatment groups compared to control animals during the main study or recovery period (results not shown).

No treatment-related differences in hematology parameters were observed between the control and the treatment groups during the

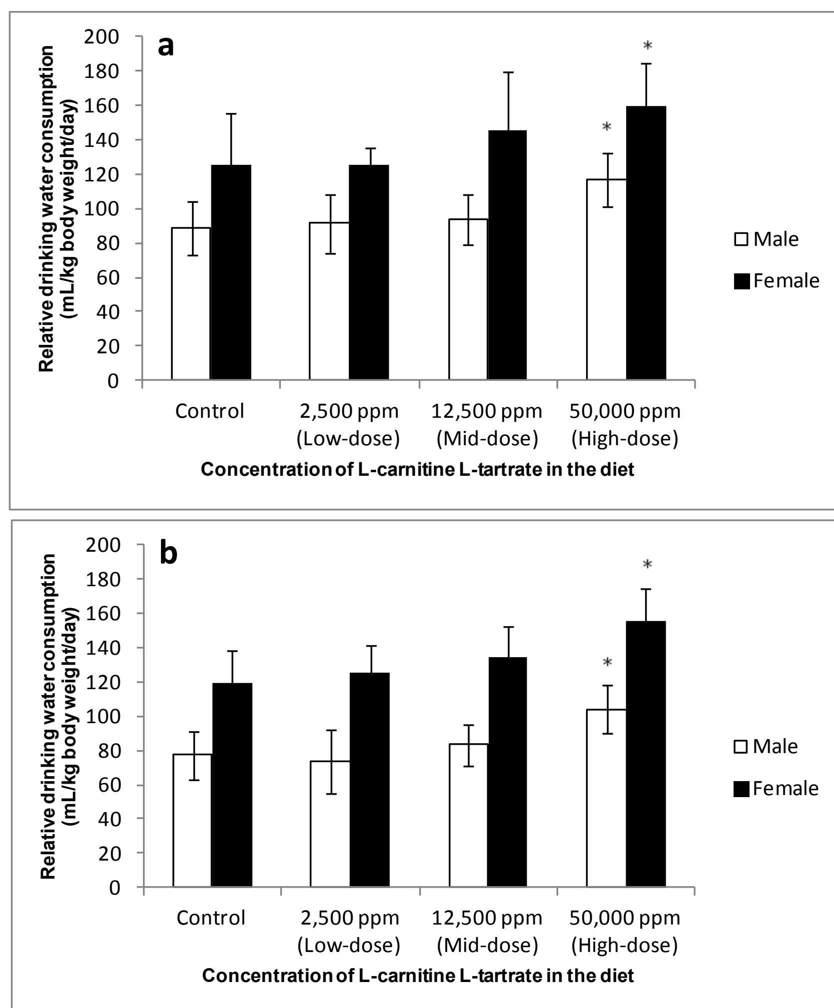

Figure 1: Relative drinking water consumption in test weeks 6 (a) and 12 (b) following oral exposure to L-carnitine L tartrate for 13 weeks.

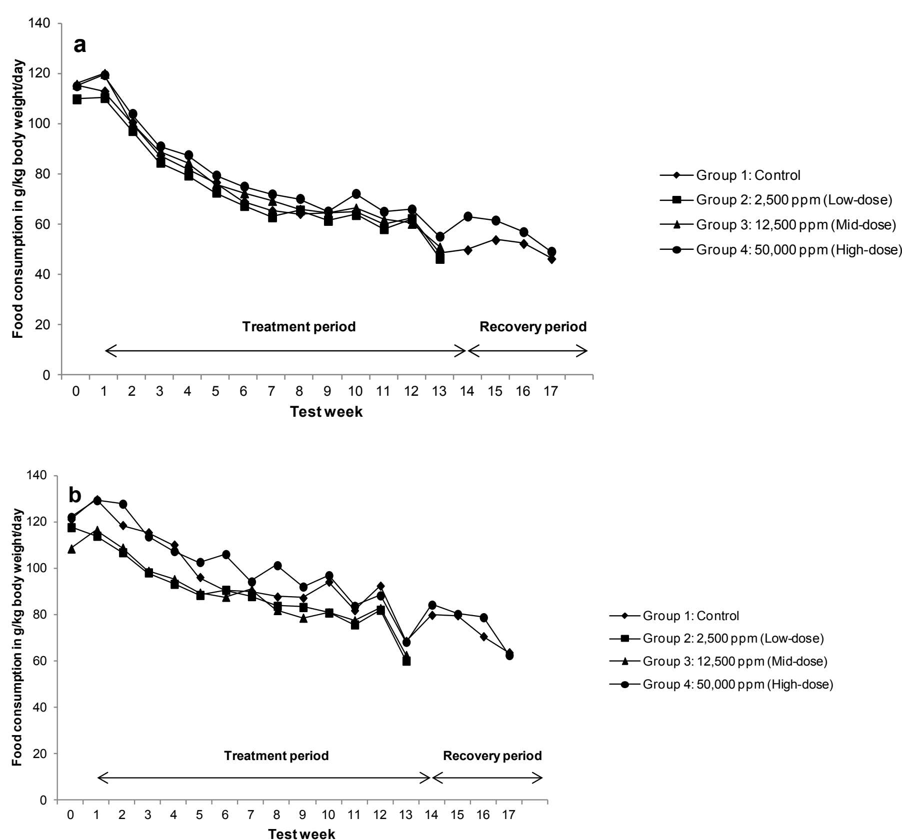

Figure 2: Food consumption following oral exposure to L-carnitine L-tartrate for 13 weeks in male (a) and female (b) rats.

study period or at the end of the recovery period. A significant decrease (45.7%) in the number of reticulocyteswas observed in high-dose females after the 4-week recovery period when compared with the control group (Table 2).

Notreatment-related changes were noted in the clinical biochemistry parameters during the treatment or recovery period. A significant decrease (14%) in ALP activity and a significant increase (80.1%) in LDH levels were observed in high-dose males during the study period. In addition, a significant increase (23.3%) in glucose levels was noted in high-dose males during the recovery period. During the study period, a significant decrease (7.5%) in albumin/globulin ratio was observed in high-dose females. Also, a significant decrease (19%) in bilirubin levels was noted in high-dose females during the recovery period (Table 3).

The urine pH values were slightly, but statistically significantly, decreased in high-dose groups in week 13. In high-dose females, there was a marginal, but statistically significant, increase (1.9%) in the specific gravity value compared to the corresponding control. This parameter was also increased in high-dose males, but the change was not statistically significant. All changes observed during the main study subsided during the recovery period, and no significant changes were observed (Table 4). Ophthalmological examination of the ocular structures of the rats revealed no treatment-related lesions in the eyes or the optic region at the end of the 13-week study period or during the recovery period (data not shown).

The relative and absolute seminal vesicle weights of the high-dose male rats were significantly decreased by 38.1 and 34.8%, respectively, at the end of 13 weeks of treatment. No differences in seminal vesicle weights were noted following the recovery period. A statistically significant reduction of the relative uterus weights (by 25.1, 24.2, and 31.4%) was observed in the animals of the

Table 2: Hematology parameters following oral exposure to L-carnitine L-tartrate for 13 weeks.

	Treatment groups					
	Main study				Recovery period	
Parameters	0 ppm	2,500 ppm	12,500 ppm	50,000 ppm	0 ppm	50,000 ppm
	Control	(Low-dose)	(Mid-dose)	(High-dose)	(Control)	(High-dose)
Males						
RBC (10^{12}/L)	9.38 ± 0.45	9.33 ± 0.41	9.28 ± 0.40	9.24 ± 0.40	9.02 ± 0.61	9.30 ± 0.19
WBC (10^9/L)	9.30 ± 1.67	8.21 ± 1.61	8.62 ± 1.32	7.60 ± 2.16	8.44 ± 1.23	8.70 ± 0.81
HCT (%)	47.3 ± 2.5	46.3 ± 1.5	46.4 ± 1.5	46.2 ± 1.3	44.0 ± 3.2	46.6 ± 2.3
HGB (mmol/L)	9.70 ± 0.43	9.57 ± 0.3	9.51 ± 0.26	9.49 ± 0.35	8.92 ± 0.59	9.56 ± 0.4
PCT (10^9/L)	1310.6 ± 171.1	1322 ± 112.8	1249.4 ± 137.3	1384.4 ± 189.8	1228 ± 162.2	1137.4 ± 245.7
LI (mmol/L)	2.571 ± 0.107	2.482 ± 0.091	2.444 ± 0.091	2.521 ± 0.195	2.602 ± 0.070	2.554 ± 0.088
Reticulocytes (%)	19.5 ± 2.7	19.1 ± 2.2	18.82.7	20.5 ± 5.0	19.6 ± 2.3	19.8 ± 3.3
Differential Blood Count (% of total leucocytes)						
Neutrophil s	17.21	16.92	19.34	17.72	22.96	22.42
Lymphocytes	76.26	76.16	74.46	77.03	69.74	71.84
Monocytes	3.07	3.27	3	2.83	4.34	3.1
Eosinophils	1.93	2.16	2.1	1.43	2.12	1.74
Large unstained cells	0.5	0.39	0.2	0.36	0.76	0.74
Basophils	0.5	0.39	0.2	0.36	0.1	0.16
Females						
RBC (10^{12}/L)	8.61 ± 0.31	8.73 ± 0.39	8.34 ± 0.94	8.35 ± 0.35	8.54 ± 0.45	8.78 ± 0.65
WBC (10^9/L)	5.47 ± 1.45	6.72 ± 1.87	6.11 ± 1.89	5.28 ± 1.45	4.34 ± 0.75	4.92 ± 0.75
HCT (%)	44.7 ± 1.8	45.6 ± 1.8	42.6 ± 3.9	44.2 ± 1.2	45.4 ± 1.5	46.6 ± 3.6
HGB (mmol/L)	9.31 ± 0.45	9.54 ± 0.35	8.94 ± 0.78	9.21 ± 0.30	9.38 ± 0.35	9.58 ± 0.84
PCT (10^9/L)	1298.5 ± 165.2	1265.2 ± 92.1	1355.7 ± 231.1	1307.9 ± 245	1254.4 ± 120.3	1309.4 ± 137.6
LI (mmol/L)	2.369 ± 0.130	2.339 ± 0.134	2.345 ± 0.1	3.328 ± 0.140	2.380 ± 0.027	2.368 ± 0.104
Reticulocytes (%)	22 ± 5.6	23.7 ± 5.6	23.7 ± 8.0	27 ± 12.5	30.2 ± 11.2	16.4 ± 6.2*
Differential Blood Count (% of total leucocytes)						
Neutrophils	16.12	17.23	25.14	17.58	22.32	20.74
Lymphocytes	77.81	76.57	69.26	76.45	71.98	74.12
Monocytes	2.99	2.86	2.71	2.61	2.72	2.48
Eosinophils	1.78	1.96	1.68	2	2.28	1.86
Large unstained cells	0.71	0.99	0.87	0.95	0.6	0.68
Basophils	0.58	0.41	0.35	0.38	0.1	0.08

Abbreviations: HCT=Hematocrit Value; HGB=Hemoglobin Content; LI=Lobularity Index; PCT=Platelet Count; RBC=Red Blood Cell count; WBC=White Blood Cell Count

Values are listed as mean ± SD, unless otherwise specified.

*Significantly different from control ($p \leq 0.05$) by Dunnett's multiple comparison test.

Table 3: Clinical biochemistry parameters following oral exposure to L-carnitine L-tartrate for 13 weeks.

	Treatment groups					
	Main study				Recovery period	
Parameters	0 ppm	2,500 ppm	12,500 ppm	50,000 ppm	0 ppm	50,000 ppm
	Control	(Low-dose)	(Mid-dose)	(High-dose)	(Control)	(High-dose)
Males						
Total bilirubin (µmol/L)	2.91 ± 0.3	2.81 ± 0.24	2.97 ± 0.38	2.87 ± 0.34	2.92 ± 0.18	3.16 ± 0.21
Total cholesterol (mmol/L)	1.848 ± 0.289	2.005 ± 0.358	2.01 ± 0.342	2.013 ± 0.464	1.840 ± 0.260	1.686 ± 0.244
Creatinine (µmol/L)	52.2 ± 3.2	52.9 ± 3.0	51.6 ± 2.8	54.4 ± 2.7	55.2 ± 4.0	57.2 ± 4.9
Glucose (mmol/L)	6.715 ± 1.036	6.875 ± 0.568	6.796 ± 0.315	6.295 ± 0.475	7.328 ± 1.050	9.036 ± 1.482*
Inorganic phosphate (mmol/L)	2.204 ± 0.0144	2.107 ± 0.128	2.142 ± 0.172	2.166 ± 0.104	2.124 ± 0.178	1.932 ± 0.209
Total protein (g/L)	63.4 ± 3.4	64.4 ± 1.8	64.9 ± 1.7	63.9 ± 3.8	67.0 ± 2.1	65.8 ± 3.2
Albumin (g/L)	31.8 ± 2.0	31.4 ± 0.9	31.7 ± 0.9	31.3 ± 2.0	31.9 ± 2.3	32.8 ± 1.4
Globulin (g/L)	31.6 ± 1.7	33.0 ± 2.0	33.2 ± 2.2	32.6 ± 2.4	35.1 ± 1.4	33.0 ± 2.6
Albumin/globulin ratio	1.004 ± 0.046	0.957 ± 0.075	0.962 ± 0.082	0.961 ± 0.073	0.910 ± 0.087	1.00 ± 0.079
α1-Globulin (g/L)	12.3 ± 1.0	12.9 ± 1.4	12.8 ± 1.0	13.4 ± 2.0	15.2 ± 1.9	13.1 ± 1.1
α2-Globulin (g/L)	4.5 ± 0.4	4.5 ± 0.3	4.9 ± 0.3	4.5 ± 0.3	4.9 ± 0.4	5.0 ± 0.6
β-Globulin (g/L)	12.4 ± 0.8	13.0 ± 0.7	13.0 ± 0.9	12.5 ± 1.1	12.8 ± 1.2	12.5 ± 1.4
γ-Globulin (g/L)	2.5 ± 0.8	2.6 ± 0.5	2.5 ± 0.5	2.3 ± 0.5	2.2 ± 0.7	2.3 ± 0.3
Triglycerides (mmol/L)	0.497 ± 0.232	0.435 ± 0.082	0.396 ± 0.050	0.578 ± 0.177	0.112 ± 0.639	0.814 ± 0.334
Urea (mmol/L)	7.077 ± 1.102	7.468 ± 0.995	7.553 ± 0.887	7.060 ± 0.998	7.280 ± 1.584	7.336 ± 1.071
Calcium (mmol/L)	2.623 ± 0.096	2.678 ± 0.085	2.669 ± 0.091	2.615 ± 0.086	2.734 ± 0.018	2.694 ± 0.050
Chloride (mmol/L)	106.4 ± 1.2	107.0 ± 0.7	107.4 ± 1.2	107.2 ± 1.7	106.4 ± 0.5	106.4 ± 0.5
Potassium (mmol/L)	3.245 ± 0.212	3.341 ± 0.201	3.391 ± 0.094	3.460 ± 0.359	3.168 ± 0.187	3.052 ± 0.188
Sodium (mmol/L)	139.4 ± 0.5	138.8 ± 0.6	138.8 ± .0.8	139.7 ± 1.3	145.6 ± 0.9	145.0 ± 0.7
Alanine aminotransferase (U/L)	37.4 ± 6.2	35.6 ± 3..6	47.9 ± 25.6	36.1 ± 7.2	37.4 ± 11.7	36.8 ± 102
Alkaline phosphatase (U/L)	75.5 ± 10.3	73.8 ± 10.1	72.4 ± 7.3	64.9 ± 7.0*	67.8 ± 7.5	71.0 ± 5.6
Aspartate aminotransferase (U/L)	68.2 ± 11.2	67.3 ± 5.0	86.3 ± 32.6	62.6 ± 10.0	55.6 ± 7.2	64.6 ± 11.0
Lactate dehydrogenase (U/L)	39.8 ± 21.1	38.4 ± 9.4	71.7 ± 68.7*	41.3 ± 22.3	45.2 ± 13	70.2 ± 24.2
γ-Glutamyltransferase (U/L)	7.8 ± 0.6	7.7 ± 0.9	7.6 ± 1.0	7.9 ± 0.7	9.2 ± 1.3	9.2 ± 1.3
Females						
Total bilirubin (µmol/L)	3.48 ± 0.53	3.46 ± 0.46	3.11 ± 0.37	3.12 ± 0.23	4.06 ± 0.57	3.28 ± 0.71*
Total cholesterol (mmol/L)	1.793 ± 0.307	1.599 ± 0.294	1.750 ± 0.259	1.909 ± 0.316	1.620 ± 0.313	1.838 ± 0.523
Creatinine (µmol/L)	56.9 ± 5.2	59.8 ± 2.0	58.6 ± 3.4	58.2 ± 6.2	58.4 ± 8.3	61.4 ± 5.0
Glucose (mmol/L)	5.928 ± 0.916	6.232 ± 0.888	6.036 ± 0.578	6.069 ± 0.718	6.474 ± 0.451	6.456 ± 0.445
Inorganic phosphate (mmol/L)	1.873 ± 0.313	1.986 ± 0.279	1.806 ± 0.316	1.787 ± 0.343	1.510 ± 0.286	1.558 ± 0.047
Total protein (g/L)	63.9 ± 2.2	64.7 ± 2.4	64.4 ± 4.1	63.9 ± 4.6	65.0 ± 2.6	67.4 ± 2.5
Albumin (g/L)	34.0 ± 1.5	34.8 ± 1.3	33.8 ± 3.2	32.7 ± 2.5	35.0 ± 3.2	35.8 ± 2.3
Globulin (g/L)	29.9 ± 1.2	29.9 ± 1.5	30.6 ± 2.3	31.2 ± 2.6	30.0 ± 2.7	31.6 ± 1.6
Albumin/globulin ratio	1.137 ± 0.050	1.168 ± 0.054	1.110 ± 0.125	1.052 ± 0.067*	1.182 ± 0.183	1.136 ± 0.101
α1-Globulin (g/L)	10.1 ± 0.8	9.9 ± 0.8	10.8 ± 1.3	10.5 ± 1.0	10.9 ± 1.9	11.8 ± 1.3
α2-Globulin (g/L)	4.5 ± 0.5	4.7 ± 0.3	4.7 ± 0.5	4.8 ± 0.5	4.4 ± 0.5	4.9 ± 0.5
β-Globulin (g/L)	12.2 ± 0.3	11.9 ± 0.6	11.8 ± 0.9	12.3 ± 1.0	11.3 ± 1.5	11.7 ± 0.5
γ-Globulin (g/L)	3.1 ± 0.6	3.3 ± 0.5	3.3 ± 1.0	3.6 ± 1.2	3.4 ± 1.0	3.2 ± 0.5
Triglycerides (mmol/L)	0.443 ± 0.09	0.402 ± 0.082	0.626 ± 0.681	0.436 ± 0.066	0.504 ± 0.115	0.480 ± 0.066
Urea (mmol/L)	7.344 ± 1.265	7.518 ± 1.872	7.226 ± 1.049	7.845 ± 1.669	8.196 ± 1.762	8.930 ± 3.089
Calcium (mmol/L)	2.719 ± 0.081	2.745 ± 0.053	2.674 ± 0.172	2.737 ± 0.073	2.632 ± 0.107	2.658 ± 0.044
Chloride (mmol/L)	107.2 ± 1.4	108.2 ± 1.5	107.4 ± 2.8	108.5 ± 1.0	107 ± 0.7	108.2 ± 1.3
Potassium (mmol/L)	3.068 ± 0.269	3.013 ± 0.185	2.931 ± 0.308	3.095 ± 0.170	3.096 ± 0.205	2.936 ± 0.167
Sodium (mmol/L)	138.8 ± 1.2	139.1 ± 1.2	138.7 ± 3.3	139.4 ± 0.7	145.4 ± 0.5	146.8 ± 1.5
Alanine aminotransferase (U/L)	35.2 ± 8.7	30.5 ± 7.6	30.8 ± 13.7	46.6 ± 35.4	33.0 ± 10.9	57.4 ± 50.2
Alkaline phosphatase (U/L)	47.9 ± 5.5	49.8 ± 7.2	47.8 ± 3.7	48.6 ± 5.9	49.2 ± 6.8	48.2 ± 7.8
Aspartate aminotransferase (U/L)	73.4 ± 17.3	77.8 ± 7.9	68.1 ± 10.2	87.4 ± 29.5	110.2 ± 89.2	62.0 ± 26.6
Lactate dehydrogenase (U/L)	37.7 ± 14.2	36.7 ± 9.6	32.7 ± 7.3	39.9 ± 9.0	110.2 ± 89.2	62.0 ± 26.6
γ-Glutamyltransferase (U/L)	7.7 ± 1.3	8.5 ± 0.8	8.5 ± 1.1	8.3 ± 2.0	10.4 ± 1.5	9.4 ± 2.3

Table 4: Urinalysis parameters following oral exposure to L-carnitine L-tartrate for 13 weeks .

	Treatment groups					
	Main study				Recovery period	
Parameters	0 ppm	2,500 ppm	12,500 ppm	50,000 ppm	0 ppm	50,000 ppm
	Control	(Low-dose)	(Mid-dose)	(High-dose)	(Control)	(High-dose)
Males						
Specific gravity (g/mL)	1.048 ± 0.013	1.049 ± 0.009	1.053 ± 0.013	1.058 ± 0.016	1.041 ± 0.006	1.048 ± 0.008
pH	7.39 ± 0.29	7.29 ± 0.25	7.54 ± 0.30	6.43 ± 0.09*	7.08 ± 0.27	6.92 ± 0.28
Urine volume (mL/kg bw/24 h)	22.89 ± 9.51	21.82 ± 5.19	19.74 ± 6.53	22.19 ± 12.13	17.61 ± 0.88	17.95 ± 4.05
Sodium (mmol/L)	80.65 ± 25.52	90.49 ± 21.61	111.56 ± 58.84	67.95 ± 41.21	36.98 ± 13.45	44.08 ± 10.84
Potassium (mmol/L)	271.88 ± 63.21	287.71 ± 55.98	342.51 ± 121.82	262.04 ± 94.46	204.06 ± 30.34	258.92 ± 45.23
Chloride mmol/L)	88.38 ± 21.32	91.35 ± 24.87	108.10 ± 44.89	102.94 ± 35.21	35.70 ± 16.34	39.12 ± 16.69
Females						
Specific gravity (g/mL)	1.045 ± 0.004	1.047 ± 0.009	1.050 ± 0.015	1.065 ± 0.018*	1.043 ± 0.004	1.045 ± 0.007
pH	6.72 ± 0.26	6.76 ± 0.48	6.46 ± 0.32	6.08 ± 0.11*	6.46 ± 0.60	6.54 ± 0.21
Urine volume (mL/kg bw/24 h)	26.60 ± 9.18	27.97 ± 11.13	27.39 ± 7.68	25.07 ± 12.27	23.04 ± 11.79	18.93 ± 11.27
Sodium (mmol/L)	79.80 ± 27.33	82.08 ± 21.92	71.23 ± 33.60	71.89 ± 32.45	73.34 ± 27.90	65.64 ± 28.30
Potassium (mmol/L)	274 ± 71.22	259.78 ± 59.47	277.75 ± 110.20	319.88 ± 119.45	205.56 ± 44.75	209.12 ± 41.56
Chloride (mmol/L)	94.81 ± 34.54	85.85 ± 15.34	76.70 ± 27.84	114.04 ± 29.97	54.24 ± 18.84	51.90 ± 17.66

bw = body weight
Values are listed as mean ± SD, unless otherwise specified.
*Significantly different from control ($p \leq 0.05$) by Dunnett's multiple comparison test.

low, mid-, and high-dose, respectively, after the 13-week treatment period. No significant changes in organ weights were observed during the recovery period (Table 5, absolute organ weight not shown).No test substance-related macroscopic or histopathological findings were observed at necropsy at the end the 13-week study period or following the recovery period.

In vitro bacterial reverse mutation assay (Ames test)

Using the direct plate incorporation method, L-carnitine did not cause a 2-fold or greater increase in the mean number of revertant colonies at concentrations up to 5,000 µg/plate in all strains tested, both in the presence or absence of metabolic activation compared to the negative control (Table 6). The experiment was repeated under the same conditions and comparable results were obtained (results not shown). Conversely, the concurrent positive controls induced more than a two-fold increase in the number of revertant colonies, while the negative controls were within the range of historical laboratory values, thus confirming the sensitivity of the test and the activity of the S9-mix (Table 6). L-Carnitine was not toxic to any strain tested as demonstrated by the absence of a decrease in the mean number of revertant colonies.

In vitro chromosome aberration test in human peripheral blood lymphocytes

No visible precipitation of the test substance in the culture medium was observed at the end of treatment. No relevant increase in the osmolarity or pH value was observed during both independent experiments. No biologically relevant increase in the number of cells carrying structural chromosome aberrations was observed. A dose-dependent increase in chromosomal aberration was observed in the absence of metabolic activation in the first experiment (0.5, 1.0, and 3.0% aberrant cells, excluding gaps) with a statistically significant increase at 1,620 µg/mL. Similarly, in the second experiment there was a slight dose-dependent increase in the proportion of cells with chromosome aberrations in the absence of metabolic activation (2.0, 3.0, and 3.5% aberrant cells, excluding gaps). These changes were not considered biologically relevant as the values were within the historical range (0.0-4.0% aberrant cells, excluding gaps). The positive control substances induced the expected significant increases in the incidence of structural chromosomal aberrations, thus confirming the sensitivity of the test (Table 7).

DISCUSSION

The safety of L-carnitine and its tartaric acid salt were evaluated in standard toxicological experiments designed to assess subchronic oral toxicity and potential genotoxicity. In a 90-daysubchronic study, no compound-related deaths or ophthalmological abnormalities were reported in rats fed diets containing L-carnitine L-tartrate at dietary intakes of up 50,000 ppm. There were no significant differences in body weight between the control and the treatment groups. Soft feces observed in all high-dose animals from day six onwardswere considered physiological response associated with large bolus doses, as effects disappeared at the end of the recovery period. The increase in water consumption noted in high-dose males and females was transient and disappeared at the end of the recovery period.Significant increases in food consumption observedin high-dose males and females were not accompanied by any changes in the body weight in either group and as such, were not considered a toxicological response. Any statistically significant changes observed in hematological or clinical biochemistry parameters were all minor in magnitude and were regarded as incidental findings, as they were not dose-related and occurred only in one sex, or were transient and also disappeared at the end of the recovery period. The effects noted in urinalysis evaluations were considered transient, as they also were not observed at the end of the recovery period. The significant decrease in absolute and relative seminal vesicle weights observed in high-dose males compared to their respective controlsfollowing 13 weeks of treatment were not observed after four weeks of recovery and were thus considered

Table 5: Relative organ weights following oral exposure to L-carnitine L-tartrate for 13 weeks.

Parameters	Treatment groups					
	Main study				Recovery period	
	0 ppm	2,500 ppm	12,500 ppm	50,000 ppm	0 ppm	50,000 ppm
	Control	(Low-dose)	(Mid-dose)	(High-dose)	(Control)	(High-dose)
Males (Relative organ weights in g/kg bw)						
Left adrenal	0.201 ± 0.057	0.201 ± 0.074	0.162 ± 0.047	0.151 ± 0.049	0.113 ± 0.010	0.131 ± 0.029
Right adrenal	0.149 ± 0.041	0.174 ± 0.053	0.147 ± 0.036	0.144 ± 0.015	0.128 ± 0.021	0.152 ± 0.034
Brain	4.83 ± 0.33	4.70 ± 0.32	4.81 ± 0.34	4.75 ± 0.42	4.26 ± 0.27	4.50 ± 0.31
Left gonad	4.73 ± 0.63	4.28 ± 0.93	4.55 ± 0.44	4.64 ± 0.46	4.04 ± 0.25	4.33 ± 0.49
Right gonad	4.65 ± 0.62	4.61 ± 0.36	4.57 ± 0.45	4.61 ± 0.49	4.02 ± 0.33	4.40 ± 0.50
Heart	3.13 ± 0.30	2.86 ± 0.14	2.98 ± 0.19	2.91 ± 0.28	2.67 ± 0.11	2.96 ± 0.25
Left kidney	4.03 ± 0.34	4.41 ± 0.22	4.232 ± 0.37	4.59 ± 0.43	4.31 ± 0.30	4.46 ± 0.27
Right kidney	4.24 ± 0.27	4.29 ± 0.22	4.32 ± 0.38	4.62 ± 0.54	4.41 ± 0.27	4.41 ± 0.48
Liver	40.4 ± 2.7	39.8 ± 2.4	39.5 ± 3.0	43.0 ± 3.8	41.4 ± 3.7	41.4 ± 4.2
Lungs	5.70 ± 0.73	5.62 ± 0.65	5.40 ± 0.23	5.22 ± 0.48	5.15 ± 0.35	4.80 ± 0.32
Pituitary	0.027 ± 0.007	0.026 ± 0.005	0.030 ± 0.008	0.027 ± 0.007	0.030 ± 0.003	0.039 ± 0.005
Spleen	2.56 ± 0.41	2.46 ± 0.34	2.60 ± 0.32	2.65 ± 0.45	2.18 ± 0.20	2.22 ± 0.06
Thymus	0.78 ± 0.18	0.75 ± 0.11	0.78 ± 0.12	0.73 ± 0.10	0.86 ± 0.18	0.68 ± 0.11
Thyroid	0.087 ± 0.019	0.070 ± 0.013	0.092 ± 0.022	0.077 ± 0.014	0.094 ± 0.015	0.104 ± 0.018
Seminal vesicle	5.145 ± 2.181	4.523 ± 1.449	4.488 ± 1.364	3.184 ± 1.029*	3.120 ± 0.368	3.0 ± 0.174
Prostate	2.533 ± 0.883	2.927 ± 1.155	2.822 ± 1.185	3.280 ± 0.795	3.308 ± 0.802	2.862 ± 0.695
Salivary glands	3.434 ± 0.388	3.516 ± 0.350	3.298 ± 0.403	3.768 ± 0.358	3.565 ± 0.095	3.605 ± 0.323
Females (Relative organ weights in g/kg bw)						
Left adrenal	0.269 ± 0.060	0.279 ± 0.066	0.289 ± 0.070	0.249 ± 0.042	0.241 ± 0.037	0.229 ± 0.023
Right adrenal	0.247 ± 0.048	0.273 ± 0.056	0.270 ± 0.067	0.234 ± 0.050	0.258 ± 0.044	0.277 ± 0.068
Brain	7.30 ± 0.44	7.12 ± 0.56	7.14 ± 0.58	7.68 ± 0.66	7.53 ± 0.54	7.18 ± 1.85
Left gonad	0.299 ± 0.084	0.284 ± 0.062	0.276 ± 0.073	0.292 ± 0.071	0.314 ± 0.086	0.346 ± 0.091
Right gonad	0.283 ± 0.068	0.312 ± 0.089	0.257 ± 0.051	0.288 ± 0.069	0.350 ± 0.095	0.264 ± 0.047
Heart	3.64 ± 0.35	3.48 ± 0.26	3.47 ± 0.27	3.40 ± 0.16	3.86 ± 0.74	3.74 ± 0.37
Left kidney	4.40 ± 0.41	4.08 ± 0.22	4.16 ± 0.26	4.46 ± 0.46	4.01 ± 0.28	4.04 ± 0.22
Right kidney	4.37 ± 0.39	4.09 ± 0.33	4.21 ± 0.28	4.42 ± 0.36	4.21 ± 0.26	3.16 ± 1.86
Liver	49.2 ± 13.6	44.4 ± 2.6	45.2 ± 8.0	47.2 ± .3	40.5 ± 3.9	39.9 ± 3.7
Lungs	7.30 ± 1.30	6.82 ± 0.60	7.18 ± 0.39	6.71 ± 0.35	7.51 ± 2.08	6.23 ± 0.38
Pituitary	0.051 ± 0.014	0.053 ± 0.011	0.060 ± 0.006	0.055 ± 0.011	0.069 ± 0.011	0.071 ± 0.008
Spleen	3.29 ± 0.42	3.26 ± 0.29	3.61 ± 0.42	3.48 ± 0.69	2.58 ± 0.58	2.84 ± 0.26
Thymus	1.23 ± 0.25	1.25 ± 0.25	1.09 ± 0.20	1.24 ± 0.58	1.07 ± 0.21	0.87 ± 0.11
Thyroid	0.128 ± 0.021	0.135 ± 0.033	0.123 ± 0.022	0.121 ± 0.033	0.167 ± 0.042	0.135 ± 0.014
Uterus	48.7 ± 12.8	36.5 ± 10.6*	36.9 ± 9.3*	33.4 ± 8.3*	39.5 ± 5.5	39.9 ± 9.8
Salivary glands	3.819 ± 0.442	3.623 ± 0.498	3.916 ± 0.451	3.873 ± 0.426	3.776 ± 0.252	3.696 ± 0.405

Values are listed as mean ± SD, unless otherwise specified.
*Significantly different from control (p≤0.05) by Dunnett's multiple comparison test.

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

In standard Ames assay, L-carnitine was shown to be non-mutagenic in either the presence or absence of metabolic activation. Specifically, L-carnitine failed to induce an increase in the incidence of reverse mutations above negative control values in all *S. typhimurium* and *E. coli* strains tested at concentrations up to 5,000 µg/plate. In addition, L-carnitine was not cytotoxic and did not display any clastogenic properties in human peripheral blood lymphocytes at concentrations up to1,620 µg/mL. The slight dose-dependent increase in chromosomal aberrations noted at 1,620 µg/mLwas within the historical laboratory range, and therefore, was not considered biologically relevant. These results are consistent with the findings of Hamaiet al. [16] who demonstrated that L-carnitine chloride was not mutagenic in the Ames test when incubated with various *S. typhimurium* and *E. coli* tester strains at concentrations up to 10,000 µg/plate in the presence or absence of metabolic activation. L-Carnitine chloride also tested negative

Table 6: Results of the bacterial reverse mutation test with L-carnitine.

Test substance concentration (µg/plate)	Mean number of colonies per plate[a]									
	Base-pair substitution type						Frame-shift type			
	TA100		TA1535		WP2*uvr*A-pKM101		TA98		TA1537	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
0	102 ± 10.1	98 ± 14.5	27 ± 7	15 ± 2.3	225 ± 4.9	213 ± 13	18 ± 1.7	23 ± 2	16 ± 6.4	13 ± 4.4
50	106 ± 7	93 ± 22.6	17 ± 6.7	13 ± 0.6	221 ± 13	207 ± 9.5	11 ± 0.6	22 ± 5.8	18 ± 1.5	13 ± 3.8
150	97 ± 6.6	89 ± 4.0	24 ± 3	12 ± 1.7	226 ± 13.2	206 ± 6	12 ± 2.6	21 ± 3.1	10 ± 4.4	13 ± 2.1
500	98 ± 5.7	84 ± 7.4	24 ± 7.2	12 ± 2.1	233 ± 13.2	208 ± 6.6	13 ± 2.6	23 ± 4.6	15 ± 4	13 ± 1
1,500	101 ± 4.9	99 ± 7.2	18 ± 2.9	14 ± 1.2	232 ± 8.1	210 ± 1.2	16 ± 3.2	25 ± 3.5	11 ± 3.5	12 ± 3.1
5,000	101 ± 3.5	95 ± 7.9	24 ± 1.2	12 ± 1	218 ± 9.6	220 ± 8.5	12 ± 1.5	19 ± 1.7	16 ± 2.5	14 ± 2.1
Positive controls	ENNG (3 µg/ plate)	2AA (1 µg/ plate)	ENNG (5µg/plate)	2AA (2 µg/plate)	ENNG (0.5 µg/plate)	2AA (10 µg/plate)	4NQO (0.2 µg/plate)	BP (5 µg/ plate)	9AA (80 µg/ plate)	2AA (2 µg/plate)
Mean number of colonies per plate	1,193 ± 44.7	1,293 ± 28.5	809 ± 195	130 ± 0.6	1,555 ± 42.8	1,883 ± 137.9	138 ± 2.1	255 ± 19.1	3,391 ± 23.5	518 ± 161.5

Abbreviations: 2AA: 2-aminoanthracene; 9AA: 9-aminoacridine; BP: benzo(a)pyrene; ENNG: *N*-ethyl-*N*´-nitro-*N*-nitrosoguanidine; 4NQO: 4-nitroquinoline-1-oxide
[a]Mean of triplicate measurements

Table 7: Results of the chromosome aberration assay with L-carnitine.

Concentration of L-carnitine (µg/mL)	Exposure (hours)	MI (% of control)	Structural aberrations (including gaps)[a] (%)	Structural aberrations (excluding gaps)[a] (%)	Structural aberrations (with exchanges) (%)
Without S9 metabolic activation					
Negative control (deionized water 10% v/v)	4	100	0	0	0
529	4	97.8	1	0.5	0
925	4	112.6	1.5	1	0
1,620	4	97.2	3	3.0*	0
Positive control[b]	4	77	12.5	12.5	7.5
Negative control	22	100	3	2.5	0
529	22	103.7	2	2	0.5
925	22	114.9	3	3	0
1,620	22	100.3	4	3.5	0
Positive control[b]	22	37.8	24.5	24.5*	3
With S9 metabolic activation					
Negative control	4	100	1.5	1.5	0
529	4	100	3.5	3	2
925	4	104.1	1	1	0
1,620	4	97.8	1	0	0.5
Positive control[c]	4	32.2	17.5	17.0*	0
With S9 metabolic activation					
Negative control	4	100	2.5	0.5	0
529	4	118.8	1	1	0
925	4	118.5	1.5	1.5	0
1,620	4	118.5	1.5	1	0
Positive control[d]	4	24.2	15	15.0*	1.5

Abbreviations: MI, mitotic index
*$p<0.05$
[a]Includes cells carrying exchanges
[b]Ethylmethanesulfonate (825 µg/mL)
[c]Cyclophosphamide (22.5 µg/mL)
[d]Cyclophosphamide (15 µg/mL)

(up to 5,000 µg/disk) in the Rec assay using *Bacillus subtilis* or in a chromosomal aberration assay conducted in Chinese hamster V79 cells (up to 10 mg/mL), in the presence and absence of metabolic activation [16].

In addition, several clinical studies following L-carnitine and L-carnitine L-tartrate consumption were shown to be well-tolerated with no adverse effects reported following daily oral ingestion of doses ranging from 2-3 g L-carnitine/day for up to three months

or a dose of 2 g/day for up to six months [17-39]. The only effects observed included mild gastrointestinal side effects; at higher intakes (>4 g/day) ingestion of L-carnitine in some individuals was associated with increased frequency of gastrointestinal symptoms, which is consistent with the gastrointestinal clinical signs observed at high doses in animal studies.

In 2003, the European Food Safety Authority (EFSA) evaluated the safety of L-carnitine L-tartrate as a source of L-carnitine in Foods for Particular Nutritional Uses (FPNUs). EFSA concluded that L-carnitine L-tartrate presents no safety concerns when used as a source of L-carnitine for use in FPNUs, provided that the acceptable daily intake for tartaric acid from all sources in the diet [0 to 30 mg/kg body weight/day for L-tartaric acid [40-41] exclusive of tartaric acid occurring naturally in the diet, is not regularly exceeded [42]. Specifically, EFSA noted that the safety of up to 3 g/day of L-carnitine L-tartrate (equivalent to 2 g/day of L-carnitine) has been established in adults.

In summary, the findings of an *in vitro* bacterial mutagenic assay and an *in vitro* chromosome aberration test indicate that L-carnitine does not have mutagenic or clastogenic properties. The effects noted in the 13-weekdietary subchronic study conducted with L-carnitine L-tartrate were not considered to be of toxicological concern, and therefore, the NOAEL was established to be the highest concentration tested. Taken together, these datasupport the safety of L-carnitine, as well as L-carnitine L-tartrate, as a dietary source of L-carnitine.

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The authors are employees of Lonz.

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