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Twenty-Eight-Day Repeated-Dose Oral Toxicity Study of Water-Miscible Coenzyme Q10 Preparation (Q10EP40) in Rats

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Q10EP40, a new water-miscible and emulsified floury preparation for dietary supplements, consists of coenzyme Q10 (CoQ10), fatty acid ester of glycerin, casein, dextrin and sodium carbonate. The safety of CoQ10 bulk substance itself has been evaluated by several subacute and chronic toxicity studies in animals. However, the safety of CoQ10 preparation produced with food ingredients has not yet been fully confirmed. The present study was conducted to evaluate the toxicity of Q10EP40 in Sprague-Dawley rats with gavage administration at concentrations of 500, 1000 or 2000 mg/kg for 28 days. No deaths were observed in any group, and there were no adverse effects on general condition, behavior, body weight or food consumption, results of urinalysis, hematology, blood chemistry, ophthalmological examination, gross pathological examination or histopathological examination, or organ weights. On the basis of these findings, the no-observed-adverse effect level (NOAEL) for Q10EP40 in Sprague-Dawley rats is considered to be 2000 mg/kg/day.

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Key words —— oral toxicity study, coenzyme Q10, rat, water-miscible, emulsified preparation

INTRODUCTION

Coenzyme Q10 (CoQ10) functions in mitochondria to accept electrons from NADH *via* flavin-containing centers and then passes them *via* cytochrome c reductase down the electron transport chain. The ability of CoQ10 to function in extramitochondrial reactions as an electron donor has led researchers to postulate a role for ubiquinol, the reduced form of CoQ10, as an endogenous antioxidant. Although it has long been established that ubiquinol protects membranes from oxidation,[1] the possibility that supplementation with CoQ10 might increase resistance to oxidative stress has been the subject of a growing body of research.[2–6] This growing interest is spurring active efforts to determine uses of CoQ10 as a dietary supplement. However, the physicochemical properties of CoQ10, including relatively large molecular weight and its poor water solubility, causes the variable bioavailability as well as difficulty or limitation in preparation of dietary supplements.

We have developed a new water-miscible preparation containing 40% (w/w) CoQ10, termed Q10EP40, using common food ingredients and formulation techniques. It is an emulsified floury preparation constituted from CoQ10, polyglycerol ester of fatty acid, casein, dextrin and sodium carbonate, and can therefore be used for preparation of dietary supplements such as beverages, jelly and chewable tablets, or as a food ingredient. In a bioavailability study with rats, the area under concentration time curve ($AUC_{0-24\,hr}$) value of plasma CoQ10 concentration after oral administration of Q10EP40 suspension in water at 30 mg/kg was nearly twice that obtained with administration of CoQ10 bulk solution in soy bean oil (unpublished data), indicating improved bioavailability of Q10EP40.

Although the safety of CoQ10 bulk substance itself has been evaluated in several toxicity studies, including 52-week chronic toxicity study in rats,[7–9] no data have been published concerning the toxicity of water-miscible preparations such as Q10EP40 with high bioavailability. It is desirable to evaluate the toxicity of Q10EP40, since its improved bioavailability or interactions among its components may affect expression of toxicity. Therefore, as a part of safety assessment of it, we performed a

28-day, repeated-dose oral toxicity study of Q10EP40 in Sprague-Dawley rats.

MATERIALS AND METHODS

Test Materials —— The components of Q10EP40 and its placebo are shown in Table 1. The CoQ10 bulk substance (coenzyme Q10; all in trans configuration) used in this study was manufactured by Kaneka Corp. (Osaka, Japan). Dextrin was purchased from Sanwa Starch Co., Ltd. (Nara, Japan), acid casein (30/60 mesh type) from Meggle Japan Co., Ltd. (Tokyo, Japan) and sodium carbonate from Tosoh Corp. (Tokyo, Japan). Fatty acid ester of monoglycerin/hexaglycerin (Sunsoft No. 621B/ Sunsoft No. Q18F) was manufactured and provided by Taiyo Kagaku Co., Ltd. (Mie, Japan). Q10EP40 was prepared as follows. Acid casein, sodium carbonate, monoglycerin fatty acid of ester and hexaglycerin fatty acid of ester were added to tenfold water warmed to approximately 65°C in a vessel. The batch was agitated for 1 hr using a homomixer, and CoQ10 and dextrin were added under mixing and run vigorously until homogenized, and then the batch was spray-dried. Placebo was performed in the same manner as above without CoQ10. Q10EP40 and placebo were stored protected from light at 12 to 23°C until preparation of dosing solutions.

Animals and Treatment —— Seventy-eight male and 78 female Sprague Dawley rats [Crj : CD(SD)IGS] were purchased from Charles River Japan (Kanagawa, Japan) at four weeks of age. After 2-week acclimatization, the rats were allocated to 5 groups of 10 rats per sex for toxicity study, and 4 satellite groups of 3 rats per sex for toxicokinetic analysis (CoQ10 concentration in plasma). Rats were housed individually in bracket-type metallic wire-mesh cages in animal rooms which were set to maintain a temperature at 23 ± 3°C, the relative humidity of 50 ± 20% and artificial lighting for 12 hr a day (07:00 to 19:00). They were received basal diet CRF-1 (Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water *ad libitum*. All animal procedures were conducted at Bozo Research Center Inc. (Shizuoka, Japan) in accordance with the guidelines for animal experimentation of the Japanese Association for Laboratory Animal Science.

Table 1. Formulations of Q10EP40 and Placebo

	Weight (g)	
	Active	Placebo
CoQ10	400	—
Dextrin	195	195
Acid casein	189	189
Fatty acid ester of glycerin	13	13
Sodium carbonate	203	203
Total	1000	600

Test chemical, Q10EP40 or placebo, was suspended in water (vehicle). Q10EP40 was administered at 500, 1000 or 2000 mg/kg/day and placebo at 1200 mg/kg/day (equivalent to 2000 mg/day in Q10EP40) once daily by oral gavage for 28 days (10 ml/kg body weight). To rats of the control group, water (vehicle) for injection was administered.

Observations and Measurements —— Unless otherwise noted, measurements were performed using ten rats. Clinical observations, which included recording any changes in general condition or behavior, were recorded twice daily during the administration period. Body weights and food consumption were measured on day 1 of administration and thereafter twice a week. Four-hour and 20-hr urine samples were collected on day 25. In urinalysis using 4-hr urine samples, pH, protein, ketone bodies, glucose, occult blood, bilirubin and urobilinogen were measured using AUTION Sticks-7EA and AM-4290 (ARKRAY Inc., Kyoto, Japan), and color and sediment were assessed by macroscopic or microscopic examination. Osmotic pressure, sodium, potassium and chloride were measured in 20-hr urine samples using OM-6030 (ARKRAY Inc.) and PVA-alphaII (A&T Corp., Kanagawa, Japan). Urinary volume for 24 hr was assessed for total volume of 4 and 20-hr samples. On ophthalmological examination, the anterior portion, transparent body and fundus oculi were assessed for 6 rats per group on day 25. After 28-day administration period, necropsy was performed for all rats except those of satellite groups. Rats were weighed and killed by exsanguinations under ether anesthesia after overnight fating. Blood samples were collected from the abdominal aorta for hematology and blood chemistry.

The following hematological parameters were measured using a Coulter T890 (Beckman Coulter Inc., U.S.A.) and ACL 100 (Instrumentation Laboratory Inc., U.S.A.) on samples collected using EDTA-2K as an anticoagulant: red blood cell (RBC), white blood cell (WBC), hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular

hemoglobin concentration (MCHC), reticulocytes and platelets. Prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen were measured using on ACL 100 coagulometer (Instrumentation Laboratory Inc.) on samples collected in tubes containing sodium citrate as an anticoagulant. Differential leukocytes were assessed by microscopic examination. The following blood chemistry measurements were performed using a Clinical Laboratory System TBA-120FR (Toshiba Corp., Japan) or Automatic Electrophoresis CLINISCAN SA-V (K.K. Herena Kenkyujyo, Saitama, Japan) on sera or plasma obtained by centrifugation: aspartate aminotransferase (AST) (glutamic-oxaloacetic transaminase, GOT), alanine aminotransferase (ALT) (glutamic pyruvate transaminase, GPT), lactate dehydrogenase (LDH), gamma-glutamiltranspeptidase (γ-GTP), alkaline phosphatase (AlP), total cholesterol, triglyceride (TG), phospholipid (PL), total bilirubin, glucose, blood urea nitrogen (BUN), creatinine, sodium (Na), potassium (K), chloride (Cl), calcium (Ca), inorganic phosphate (P), total protein (TP), albumin, albumin/globulin ratio (A/G), and globulin fraction.

The following organs were weighed (paired organs together) after dissection: brain, pituitary, thy-

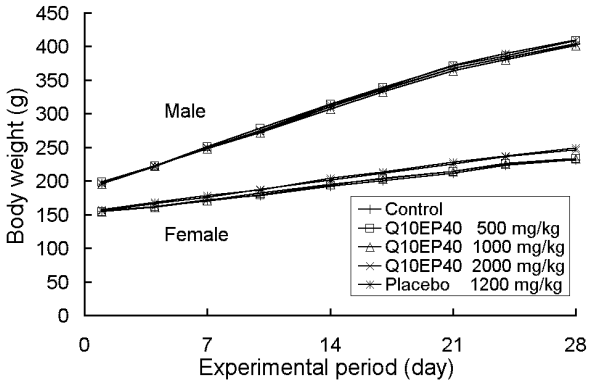

Fig. 1. Changes of Mean Body Weight of Rats during the 28-day Experimental Period

Each point is presented as a mean value of body weight for 10 rats in each group.

Table 2-1. Results of Urinalysis for Rats Treated with Q10EP40

Sex	Material	Dose (mg/kg)	Number	pH							Protein[a]			Ketone Body[b]	
				6	6.5	7	7.5	8	8.5	9	−	±	+	−	±
Male	Control	0	10	0	0	0	3	4	3	0	5	4	1	10	0
	Q10EP40	500	10	0	0	0	0	8	2	0	3	6	1	9	1
	Q10EP40	1000	10	0	0	0	1	5	4	0	2	7	1	10	0
	Q10EP40	2000	10	0	0	1	1	4	4	0	0	9	1	9	1
	placebo	1200	10	0	0	0	1	6	3	0	2	7	1	10	0
Female	Control	0	10	0	0	1	0	5	4	0	10	0	0	10	0
	Q10EP40	500	10	1	0	2	2	1	4	0	10	0	0	9	1
	Q10EP40	1000	10	0	1	0	4	1	3	1	10	0	0	9	1
	Q10EP40	2000	10	1	0	0	2	3	4	0	10	0	0	10	0
	placebo	1200	10	0	1	2	2	3	2	0	10	0	0	10	0

Sex	Material	Glucose[c]	Occult blood[d]				Bilirubin[e]	Urobilinogen[f]	Color[g]		
		−	−	±	+	++	−	±	LY	Y	DY
Male	Control	10	8	1	1	0	10	10	0	10	0
	Q10EP40	10	8	1	1	0	10	10	0	10	0
	Q10EP40	10	8	1	1	0	10	10	0	10	0
	Q10EP40	10	8	2	0	0	10	10	0	10	0
	placebo	10	8	1	1	0	10	10	0	10	0
Female	Control	10	10	0	0	0	10	10	0	10	0
	Q10EP40	10	8	1	0	1	10	10	0	10	0
	Q10EP40	10	10	0	0	0	10	10	0	10	0
	Q10EP40	10	10	0	0	0	10	10	0	10	0
	placebo	10	9	0	0	1	10	10	0	10	0

a) −, < 10 mg/dl; ±, 10–25 mg/dl; +, 26–85 mg/dl. *b*) −, < 5 mg/dl; ±, 5–7.5 mg/dl. *c*) −, < 30 mg/dl. *d*) −, 0.03 mg/dl; ±, 0.03–0.05 mg/dl; +, 0.06–0.15 mg/dl; ++, 0.16–0.75 mg/dl. *e*) −, < 0.5 mg/dl. *f*) ±, < 2.0 mg/dl. *g*) LY, light yellow; Y, yellow; DY, deep yellow.

Table 2-2. Results of Urinalysis for Rats Treated with Q10EP40

Sex	Material	Dose (mg/kg)	Number	RBC		WBC		SEC		
				−	±	−	±	−	±	+
Male	Control	0	10	10	0	9	1	0	9	1
	Q10EP40	500	10	10	0	10	0	0	10	0
	Q10EP40	1000	10	10	0	10	0	0	10	0
	Q10EP40	2000	10	9	1	10	0	0	10	0
	placebo	1200	10	9	1	10	0	0	9	9
Female	Control	0	10	10	0	10	0	0	10	0
	Q10EP40	500	10	10	0	10	0	0	9	1
	Q10EP40	1000	10	10	0	9	1	0	10	0
	Q10EP40	2000	10	10	0	10	0	0	10	0
	placebo	1200	10	9	1	10	0	0	10	0

Sex	Material	SREC		Cast	Crystallization			
					PS			CO
		−	±	−	−	±	+	−
Male	Control	10	0	10	6	4	0	10
	Q10EP40	10	0	10	7	3	0	10
	Q10EP40	10	0	10	7	2	1	10
	Q10EP40	10	0	10	7	3	0	10
	placebo	10	0	10	7	3	0	10
Female	Control	9	1	10	7	2	1	10
	Q10EP40	10	0	10	7	3	0	10
	Q10EP40	10	0	10	6	3	1	10
	Q10EP40	10	0	10	5	4	1	10
	placebo	10	0	10	6	2	2	10

SEC, Squamous Epithelial Cell. SREC, Small Round Epithelial Cell. PS, Phosphate salt. CO, Calcium Oxalate. −, Negative; ±, Slight; +, Mild.

roid (including parathyroid), adrenals, thymus, spleen, heart, lung (including bronchi), salivary glands (submandibular and sublingual gland), liver, kidneys, testes, seminal vehicle, prostate, ovaries and uterus. The following tissues from all rats except those of satellite groups were fixed in phosphate-buffered 10% formalin (however, the testis and epididymides were fixed in Bouin's solution, and eyeballs, Harderian glands and optic nerves in phosphate-buffered 3% glutaraldehyde/2.5% formalin mixture and then preserved in phosphate-buffered 10% formalin): adrenals, thoracic aorta, cecum, cerebellum, cerebrum, colon, duodenum, epididymis, esophagus, eyes, femur (including bone marrow), harderian gland, heart, ileum, jejunum, kidneys, liver, lungs (including bronchus), mammary gland (inguinal region), mesenteric lymph node, nasal cavity, rectum, optic nerve, ovary, oviduct, pancreas, parorchis, pituitary, prostate, rectum, sciatic nerve, seminal vesicle, femoral skeletal muscle, skin (inguinal region), spinal cord, spleen, sternum (including bone marrow), stomach, submandibular gland/sublingual gland, submandibular lymph node, thymus, thyroid (including parathyroid), tongue, trachea, urinary bladder, uterus, vagina and zymbal gland. These tissues were processed to paraffin sections, stained with hematoxylin and eosin, and examined histopathologically.

CoQ10 Concentration in Plasma of Satellite Group —— Blood samples were collected once from 3 rats of each satellite group on day 1 (1, 4 and 8 hr postdose) and on day 23 (predose and 1, 4 and 8 hr postdose). Rats were not fasted before blood collection. Blood (approximately 0.5 ml) was collected from a jugular vein of conscious rats into tubes containing sodium heparin. Plasma samples obtained by centrifugation were stored in a freezer (–23 to –18°C). These samples were analyzed for CoQ10 by an HPLC method modified and validated for support of this study, described in previous paper.[9] Toxicokinetic analysis of the data from days 1 and 23 included determinations of maximum concentration (C_{max}), time to reach the maximum concentration (T_{max}) and $AUC_{0-8\ hr}$.

Table 2-3. Water Intake and Results of Urinalysis for Rats Treated with Q10EP40

Sex	Material	Dose (mg/kg)	Number	Water intake (ml/24 hr)	Urine volume (ml/24 hr)
Male	Control	0	10	46 ± 7[a)]	13.4 ± 2.7
	Q10EP40	500	10	44 ± 8	14.4 ± 3.4
	Q10EP40	1000	10	45 ± 9	13.8 ± 3.4
	Q10EP40	2000	10	49 ± 9	14.5 ± 5.0
	placebo	1200	10	51 ± 10	13.5 ± 4.0
Female	Control	0	10	36 ± 9	8.1 ± 3.4
	Q10EP40	500	10	32 ± 5	8.9 ± 3.6
	Q10EP40	1000	10	37 ± 12	11.2 ± 5.7
	Q10EP40	2000	10	36 ± 6	10.6 ± 2.9
	placebo	1200	10	37 ± 5	9.7 ± 4.1
Sex	Material	Osmolality (mOsm/kg)	Na (mmol/24 hr)	K (mmol/24 hr)	Cl (mmol/24 hr)
Male	Control	1959 ± 384	2.33 ± 0.64	4.05 ± 0.96	2.87 ± 0.80
	Q10EP40	1831 ± 283	2.25 ± 0.39	4.04 ± 0.72	2.79 ± 0.50
	Q10EP40	1880 ± 374	2.19 ± 0.32	3.86 ± 0.56	2.64 ± 0.36
	Q10EP40	1803 ± 399	2.25 ± 0.44	3.73 ± 0.99	2.66 ± 0.71
	placebo	1848 ± 430	2.18 ± 0.20	3.63 ± 0.36	2.50 ± 0.18
Female	Control	2041 ± 438	1.44 ± 0.46	2.39 ± 0.93	1.70 ± 0.56
	Q10EP40	1911 ± 378	1.46 ± 0.41	2.46 ± 0.68	1.74 ± 0.48
	Q10EP40	1742 ± 549	1.50 ± 0.51	2.50 ± 0.86	1.80 ± 0.64
	Q10EP40	1830 ± 217	1.70 ± 0.52	2.88 ± 0.71	1.97 ± 0.59
	placebo	1976 ± 333	1.75 ± 0.67	2.89 ± 1.09	2.00 ± 0.72

a) Mean ± S.D.

Statistical Analysis —— Unless otherwise noted, data for body weight, food consumption, urinalysis (quantitative data), hematology, blood chemistry and organ weight were analyzed statistically for homogeneity of variance using Bartlett's test. If no significant heterogeneity was detected, Dunnett's test was used to compare differences of mean values between the control and each treatment group (significance level: two-tailed test, 1 or 5%). If significant heterogeneity was detected, the nonparametric Dunnett-type method was used to compare differences of mean rank between the control and each treatment group (significance level: two-tailed test, 1 or 5%).

RESULTS AND DISCUSSION

CoQ10 is an important mitochondrial redox component, and its reduced form (ubiquinol) is the only endogenously lipid-soluble antioxidant that animal cells can synthesize *de novo*. Its plasma and tissue concentrations decrease with aging and in a number of diseases. The antioxidant activity of ubiquinol is independent of effects of vitamin E, which inhibits the propagation of lipid peroxidation. Ubiquinol can efficiently sustain the effect of vitamin E by regenerating this vitamin from the tocopheroxyl radical.[10)] Supplementation of humans with CoQ10 increases the concentrations of ubiquinol in plasma and in all of its lipoproteins to increase the resistance of low-density lipoprotein to radical oxidation.[3)] CoQ10 has increasingly been used as an antioxidant for the treatment of a variety of diseases. Moreover, CoQ10 has gained popularity as a dietary supplement due to its beneficial effects.

Several toxicity studies have supported the safety of CoQ10 bulk substance itself.[7–9)] However, no data have been published concerning the toxicity of formulation products of CoQ10. Q10EP40 is constituted from CoQ10, dextrin, casein, sodium carbonate and polyglycerol ester of fatty acid. The Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA) have evaluated dextrin, casein, sodium carbonate and polyglycerol ester of fatty acid concerning acceptable daily intake (ADI) for man. Ac-

Table 3. Hematological Values in Rats Treated with Q10EP40

	Males				
Dose (mg/kg):	0 (Control)	500	1000	2000	placebo
RBC ($\times 10^4/\mu$l)	787 ± 37[a)]	780 ± 36	786 ± 34	774 ± 31	768 ± 36
Hb (g/dl)	15.8 ± 0.6	15.8 ± 0.5	15.8 ± 0.6	15.7 ± 0.7	15.5 ± 0.9
Ht (%)	47 ± 2	47 ± 1	47 ± 2	47 ± 2	46 ± 3
MCV (fl)	59.5 ± 1.3	60.0 ± 1.6	59.7 ± 1.2	60.1 ± 1.6	59.7 ± 1.1
MCH (pg)	20.1 ± 0.5	20.2 ± 0.5	20.2 ± 0.5	20.3 ± 0.7	20.1 ± 0.5
MCHC (%)	33.7 ± 0.4	33.7 ± 0.4	33.8 ± 0.4	33.7 ± 0.4	33.7 ± 0.3
Reticulocyte (%)	2.1 ± 0.3	1.9 ± 0.5	2.1 ± 0.5	2.0 ± 0.3	2.5 ± 0.7
Platelet ($\times 10^4/\mu$l)	114.4 ± 7.3	109.7 ± 10.0	104.0 ± 7.2*	111.0 ± 8.4	107.0 ± 9.3
PT (s)	13.5 ± 0.8	14.0 ± 0.7	14.6 ± 1.2	14.6 ± 1.5	13.2 ± 0.6
APTT (s)	19.8 ± 1.0	20.1 ± 1.4	20.2 ± 1.1	20.0 ± 2.2	18.6 ± 1.3
Fibrinogen (mg/dl)	348 ± 42	342 ± 42	343 ± 41	335 ± 26	321 ± 29
WBC ($\times 10^2/\mu$l)	101 ± 35	97 ± 33	100 ± 19	98 ± 29	112 ± 19
Differential leukocytes (%)					
Lymph.	89.7 ± 3.6	91.7 ± 2.4	89.4 ± 4.3	89.0 ± 5.5	91.9 ± 4.0
Stab.	0.1 ± 0.2	0.0 ± 0.0	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2
Seg.	9.5 ± 3.8	7.9 ± 2.4	9.9 ± 4.3	10.4 ± 5.2	8.9 ± 3.7
Eosino.	0.8 ± 0.8	0.4 ± 0.5	0.7 ± 0.5	0.5 ± 0.5	0.9 ± 0.7
Baso.	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Mono.	0.1 ± 0.2	0.1 ± 0.2	0.0 ± 0.0	0.1 ± 0.2	0.0 ± 0.0
Others	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Erythroblasts (/200 leukocyte)	0	0	0	0	0
	Females				
Dose (mg/kg):	0 (Control)	500	1000	2000	placebo
RBC ($\times 10^4/\mu$l)	764 ± 21	768 ± 40	780 ± 40	773 ± 28	776 ± 32
Hb (g/dl)	15.3 ± 0.5	15.5 ± 0.7	15.7 ± 0.5	15.5 ± 0.6	15.5 ± 0.6
Ht (%)	45 ± 1	46 ± 2	46 ± 1	45 ± 2	45 ± 2
MCV (fl)	58.5 ± 1.0	59.2 ± 1.8	58.5 ± 1.3	58.6 ± 1.3	58.9 ± 0.9
MCH (pg)	20.0 ± 0.4	20.2 ± 0.7	20.1 ± 0.6	20.1 ± 0.6	20.2 ± 0.5
MCHC (%)	34.2 ± 0.4	34.2 ± 0.4	34.3 ± 0.4	34.3 ± 0.4	34.3 ± 0.8
Reticulocyte (%)	1.8 ± 0.5	1.6 ± 0.4	1.4 ± 0.4	1.5 ± 0.3	1.5 ± 0.4
Platelet ($\times 10^4/\mu$l)	111.3 ± 15.6	109.2 ± 14.4	109.1 ± 9.1	109.7 ± 9.7	112.3 ± 7.8
PT (s)	12.7 ± 0.4	12.7 ± 0.4	12.8 ± 0.5	12.8 ± 0.5	12.9 ± 0.5
APTT (s)	14.3 ± 0.8	14.5 ± 0.4	15.0 ± 0.4	14.6 ± 0.9	14.5 ± 0.8
Fibrinogen (mg/dl)	238 ± 25	262 ± 27	250 ± 21	247 ± 23	257 ± 23
WBC ($\times 10^2/\mu$l)	61 ± 10	67 ± 18	58 ± 19	60 ± 12	67 ± 16
Differential leukocytes (%)					
Lymph.	90.2 ± 3.3	87.8 ± 3.9	87.4 ± 5.7	89.1 ± 4.1	89.3 ± 5.5
Stab.	0.1 ± 0.2	0.0 ± 0.0	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
Seg.	8.9 ± 3.7	11.5 ± 4.2	11.9 ± 5.5	10.4 ± 4.0	10.1 ± 5.2
Eosino.	0.9 ± 0.7	0.8 ± 0.5	0.6 ± 0.6	0.5 ± 0.5	0.6 ± 0.4
Baso.	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Mono.	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2
Others	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Erythroblasts (/200 leukocyte)	0	0	0	0	0

a) Mean ± S.D. Significantly different from control at $*p < 0.05$.

cording to the JECFA's evaluation, establishment of ADI for dextrin and casein was not deemed necessary and its ADI was expressed as "not specified," and ADI for casein was stated as "not limited." Moreover, toxicological evaluation of sodium carbonate by JECFA concluded that there appear to be no toxi-

Table 4. Blood Chemical Values in Rats Treated with Q10EP40

	Males				
Dose (mg/kg):	0 (Control)	500	1000	2000	placebo
AST (IU/l)	65 ± 6[a)]	64 ± 4	68 ± 4	68 ± 6	65 ± 5
ALT (IU/l)	31 ± 4	31 ± 3	31 ± 5	32 ± 3	30 ± 4
LDH (IU/l)	45 ± 9	47 ± 13	54 ± 14	51 ± 9	45 ± 5
γ-GTP (IU/l)	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
AlP (IU/l)	836 ± 251	723 ± 154	673 ± 157	727 ± 156	696 ± 112
Total cholesterol (mg/dl)	67 ± 10	57 ± 12	55 ± 8*	62 ± 14	63 ± 9
TG (mg/dl)	42 ± 14	56 ± 35	38 ± 20	39 ± 12	42 ± 19
PL (mg/dl)	109 ± 9	102 ± 16	96 ± 10	103 ± 17	103 ± 11
Total bilirubin (mg/dl)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Glucose (mg/dl)	147 ± 15	144 ± 11	151 ± 18	147 ± 19	146 ± 10
BUN (mg/dl)	14 ± 1	14 ± 1	14 ± 2	14 ± 1	13 ± 2
Creatinine ($\times$10 μg/dl)	26 ± 4	27 ± 4	27 ± 4	27 ± 4	29 ± 4
Na (mmol/l)	141 ± 1	141 ± 1	142 ± 1	141 ± 1	141 ± 1
K (mmol/l)	4.4 ± 0.2	4.4 ± 0.3	4.5 ± 0.3	4.6 ± 0.3	4.3 ± 0.3
Cl (mmol/l)	107 ± 1	107 ± 2	108 ± 1	108 ± 1	108 ± 1
Ca (mg/dl)	10.1 ± 0.2	10.0 ± 0.2	10.0 ± 0.3	10.0 ± 0.3	10.0 ± 0.3
P (mg/dl)	8.3 ± 0.8	8.7 ± 0.8	8.5 ± 0.9	8.6 ± 0.8	8.6 ± 0.8
TP (g/dl)	6.1 ± 0.2	6.0 ± 0.3	5.9 ± 0.3	5.9 ± 0.2	6.0 ± 0.3
Albumin (g/dl)	2.7 ± 0.1	2.7 ± 0.1	2.6 ± 0.1	2.7 ± 0.2	2.7 ± 0.1
A/G	0.77 ± 0.05	0.81 ± 0.08	0.80 ± 0.04	0.85 ± 0.09	0.80 ± 0.06
Albumin (%)	43.6 ± 1.4	44.8 ± 2.5	44.5 ± 1.2	45.7 ± 2.8	44.5 ± 1.7
Globulin α1 (%)	24.8 ± 1.7	23.7 ± 1.8	23.3 ± 1.9	22.7 ± 1.8*	23.8 ± 1.3
α2 (%)	9.5 ± 0.9	9.6 ± 0.7	9.1 ± 0.6	9.2 ± 0.7	9.0 ± 0.8
β (%)	17.0 ± 1.1	16.8 ± 1.1	17.5 ± 1.1	17.5 ± 1.4	17.4 ± 1.0
γ (%)	5.0 ± 0.8	5.1 ± 1.0	5.6 ± 1.2	4.9 ± 0.7	5.2 ± 0.6
	Females				
Dose (mg/kg):	0 (Control)	500	1000	2000	placebo
AST (IU/l)	64 ± 5	59 ± 6	62 ± 13	62 ± 8	66 ± 8
ALT (IU/l)	26 ± 3	26 ± 5	30 ± 14	28 ± 6	29 ± 5
LDH (IU/l)	42 ± 11	41 ± 8	41 ± 12	40 ± 6	46 ± 9
γ-GTP (IU/l)	2 ± 1	1 ± 0	1 ± 1	2 ± 1	1 ± 0
AlP (IU/l)	430 ± 94	418 ± 92	429 ± 81	437 ± 83	448 ± 172
Total cholesterol (mg/dl)	66 ± 6	66 ± 12	69 ± 8	64 ± 11	75 ± 16
TG (mg/dl)	5 ± 1	7 ± 3	9 ± 4	8 ± 5	8 ± 3
PL (mg/dl)	115 ± 10	114 ± 17	120 ± 13	115 ± 17	124 ± 22
Total bilirubin (mg/dl)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Glucose (mg/dl)	124 ± 17	119 ± 8	126 ± 12	122 ± 17	126 ± 11
BUN (mg/dl)	16 ± 3	17 ± 2	16 ± 3	16 ± 2	18 ± 4
Creatinine ($\times$10 μg/dl)	28 ± 4	28 ± 2	27 ± 4	27 ± 4	32 ± 8
Na (mmol/l)	140 ± 3	140 ± 2	140 ± 1	140 ± 1	140 ± 1
K (mmol/l)	4.6 ± 0.3	4.7 ± 0.3	4.7 ± 0.3	4.6 ± 0.3	4.6 ± 0.3
Cl (mmol/l)	111 ± 3	110 ± 2	109 ± 1	110 ± 2	110 ± 2
Ca (mg/dl)	9.5 ± 0.3	9.6 ± 0.4	9.7 ± 0.2	9.7 ± 0.3	9.5 ± 0.2
P (mg/dl)	7.4 ± 0.6	7.3 ± 0.7	7.4 ± 0.7	7.3 ± 0.7	7.2 ± 0.6
TP (g/dl)	6.0 ± 0.3	5.9 ± 0.3	6.0 ± 0.2	6.1 ± 0.4	6.0 ± 0.3
Albumin (g/dl)	2.9 ± 0.2	2.8 ± 0.2	2.9 ± 0.1	3.0 ± 0.2	2.9 ± 0.1
A/G	0.95 ± 0.06	0.94 ± 0.08	0.93 ± 0.06	0.96 ± 0.07	0.93 ± 0.09
Albumin (%)	48.6 ± 1.6	48.4 ± 2.6	48.0 ± 1.7	48.8 ± 1.9	48.2 ± 2.3
Globulin α1 (%)	19.8 ± 2.0	19.7 ± 2.6	20.4 ± 1.8	19.9 ± 2.0	20.4 ± 1.9
α2 (%)	8.7 ± 0.8	9.0 ± 1.0	8.8 ± 0.6	8.5 ± 0.6	8.7 ± 1.1
β (%)	16.2 ± 0.8	16.4 ± 1.0	16.2 ± 1.3	16.7 ± 1.1	16.0 ± 0.9
γ (%)	6.7 ± 1.0	6.4 ± 1.2	6.5 ± 1.1	6.1 ± 1.6	6.7 ± 1.6

a) Mean ± S.D. Significantly different from control at *$p < 0.05$.

Table 5. Organ Weights (g/100 g Body Weight) in Rats Treated with Q10EP40

	Males				
Dose (mg/kg):	0 (Control)	500	1000	2000	placebo
Body weight	376 ± 27[a)]	376 ± 25	370 ± 15	373 ± 14	378 ± 14
Brain	2.02 ± 0.07	1.99 ± 0.05	1.96 ± 0.07	1.96 ± 0.1	1.96 ± 0.05
Pituitary	12.9 ± 1.6	12.6 ± 1.4	12.7 ± 1.5	12.1 ± 2.0	12.4 ± 1.2
Thyroids	18.8 ± 2.9	18.4 ± 4.4	18.6 ± 3.9	18.1 ± 3.5	18.1 ± 3.5
Salivary glands	607 ± 60	634 ± 58	629 ± 72	583 ± 56	613 ± 56
Thymus	553 ± 123	553 ± 68	575 ± 89	477 ± 88	576 ± 86
Heart	1.22 ± 0.14	1.21 ± 0.14	1.15 ± 0.09	1.22 ± 0.09	1.23 ± 0.06
Lung	1.33 ± 0.08	1.35 ± 0.13	1.28 ± 0.09	1.31 ± 0.11	1.35 ± 0.09
Liver	11.06 ± 0.93	11.19 ± 1.37	10.77 ± 0.68	10.99 ± 0.63	11.12 ± 0.65
Spleen	0.67 ± 0.10	0.74 ± 0.12	0.71 ± 0.06	0.73 ± 0.12	0.75 ± 0.09
Kidneys	2.85 ± 0.29	2.82 ± 0.28	2.70 ± 0.20	2.75 ± 0.24	2.71 ± 0.23
Adrenals	58 ± 8	67 ± 11	58 ± 6	59 ± 8	59 ± 7
Testes	3.07 ± 0.36	3.10 ± 0.30	3.02 ± 0.24	3.14 ± 0.29	3.08 ± 0.23
Seminal vesicle	0.95 ± 0.15	0.92 ± 0.14	0.81 ± 0.08	0.92 ± 0.19	0.92 ± 0.16
Prostate	0.89 ± 0.12	0.89 ± 0.09	0.86 ± 0.08	0.84 ± 0.14	0.86 ± 0.07
Ovaries	—	—	—	—	—
Uterus	—	—	—	—	—
	Females				
Dose (mg/kg):	0 (Control)	500	1000	2000	placebo
Body weight	212 ± 15	216 ± 19	214 ± 26	227 ± 18	228 ± 14
Brain	1.87 ± 0.11	1.83 ± 0.05	1.85 ± 0.06	1.85 ± 0.06	1.89 ± 0.06
Pituitary	13.6 ± 1.3	13.5 ± 2.0	14.5 ± 2.0	14.5 ± 2.0	14.1 ± 2.1
Thyroids	13.9 ± 2.4	15.2 ± 3.9	13.6 ± 2.2	15.4 ± 3.5	15.4 ± 3.2
Salivary glands	419 ± 39	411 ± 44	420 ± 54	429 ± 31	419 ± 30
Thymus	508 ± 94	466 ± 122	492 ± 142	523 ± 67	496 ± 80
Heart	0.74 ± 0.06	0.77 ± 0.11	0.77 ± 0.11	0.82 ± 0.06	0.84 ± 0.08
Lung	1.01 ± 0.10	1.01 ± 0.10	1.01 ± 0.07	1.01 ± 0.06	1.04 ± 0.08
Liver	6.17 ± 0.60	6.29 ± 0.79	6.43 ± 0.85	6.73 ± 0.58	6.83 ± 0.47
Spleen	0.44 ± 0.04	0.46 ± 0.06	0.45 ± 0.06	0.46 ± 0.05	0.47 ± 0.07
Kidneys	1.68 ± 0.13	1.74 ± 0.20	1.77 ± 0.22	1.82 ± 0.17	1.74 ± 0.13
Adrenals	66 ± 10	67 ± 9	67 ± 9	63 ± 5	64 ± 5
Testes	—	—	—	—	—
Seminal vesicle	—	—	—	—	—
Prostate	—	—	—	—	—
Ovaries	78.5 ± 8.7	90.7 ± 15.2	84.4 ± 16.6	91.3 ± 12.6	80.7 ± 15.3
Uterus	531 ± 161	446 ± 115	514 ± 172	438 ± 120	522 ± 149

a) Mean ± S.D.

cological grounds to limit its use. On the other hand, ADI of 25 mg/kg/day is estimated for polyglycerol ester of fatty acid by JECFA. Piecing together above toxicological information on the constituents of Q10EP40, it appears that Q10EP40 is safe. However, its improved bioavailability or interactions among its components may affect expression of toxicity. As a part of safety assessment, Q10EP40 was submitted to a 28-day repeated-dose oral toxicity study in rats.

During the experimental period of 28-day repeated oral administration of Q10EP40 at 500, 1000 and 2000 mg/kg/day, neither death nor remarkable change in general condition was observed in the treated groups (included placebo). Changes in body weights during the experiment are shown in Fig. 1. There was no change of body weight gain in any group during the experiment between the control and treated groups for either sex, and there were no remarkable differences in food consumption between the control and treated groups for either sex.

The results of urinalysis are shown in Tables 2-

Table 6. Gross Pathological Findings in Rats Treated with Q10EP40

		Dose (mg/kg):	0	500	1000	2000	placebo
		Number:	10	10	10	10	10
Males		Grade					
Adrenal	Focus, white (bilateral)	±	0	1[a)]	0	0	0
Epididymis	Small (unilateral)	±	1	0	0	0	0
Kidney	Concave (unilateral)	±	0	1	0	0	0
	Dilatation, renal pelvis (unilateral)	±	1	1	0	0	1
Spleen	Elevation	±	0	1	0	0	0
Testis	Small (unilateral)	±	1	0	0	0	0
Females		Grade					
Eye	Dark red, eyeball (unilateral)	±	0	0	0	0	1
Kidney	Dilatation, renal pelvis (unilateral)	±	0	0	0	0	1
Liver	Hepatodiaphragmatic nodule	±	0	0	1	0	0
Lung (Bronchus)	Focus, yellow	±	0	0	1	0	0
Stomach	Focus, dark red, glandular stomach	±	1	0	0	1	0
Thyroid (Parathyroid)	Small (unilateral)	±	0	0	1	0	0

a) Number of rats exhibiting pathological findings. ± , slight.

1–2-3. Although amounts of urinary protein were aboundingly detected in males as compared with females, there were no significant differences between the control and treated groups for males by statistical analysis using non-parametric type Dunnett's multiple comparison test (Joint ranking). The results of hematology and blood chemistry are shown in Tables 3 and 4. On hematological examination, a significant decrease in number of platelets was observed in males of the 1000 mg/kg group, but not of the 2000 mg/kg group. On blood chemical examination, a significant decrease in total cholesterol was observed in males of the 1000 mg/kg group. Since this change was not dose–dependent, it appeared to be unrelated to the test materials. The globulin α1 ratio was significantly lower in males of the 2000 mg/kg group, compared with the control group. Since there were no changes in other associated parameters (*i.e.* albumin and other globulin ratios), the change was considered incidental.

Organ weights are shown in Table 5. There were no significant differences in any organ weight between the control and treated groups for either sex. On ophthalmological examination, unilateral hemorrhage of the iris and lack of mydriasis were observed in a female rat of 2000 mg/kg group. These changes were considered to be unrelated to exposure to the test material, since these changes are occasionally observed in the strain of rats. The gross pathological and histopathological findings are summarized in Tables 6 and 7. On gross pathological examination, slight changes were observed in some organs. The changes were not dose–dependent, or there were no significant alterations between the control and treated groups. On histopathological examination, several findings in kidney were observed in males, but not in females. The cause for differences between males and females in kidney is not explicit, however, the findings in males were considered to be within physiological variation since the findings were observed in control group. The incidence of infiltration of lamina propria at cecum in females was higher than that in males. However, these findings were assessed not to be of toxicity, because the changes were slight, and this finding is occasionally observed as a spontaneous change in rats. Other slight findings were also observed in some tissues. However, since these findings were low incidence or observed in the control group, they were considered spontaneous lesions.

The results of toxicokinetic analysis were as follows. The concentration of CoQ10 in plasma increased quickly in both sexes, following the first administration on day 1. C_{max} on day 1 at 500, 1000 and 2000 mg/kg of Q10EP40 was 1.08, 1.74 and 1.77 μg/ml in males, and 1.18, 1.28 and 1.37 μg/ml in females, respectively. $AUC_{0-8\ hr}$ on day 1 at 500, 1000 and 2000 mg/kg of Q10EP40 was 6.68, 9.81 and 10.82 μg hr/ml in males, and 6.95, 8.22 and 8.88 μg hr/ml in females, respectively. T_{max} on day 1 was 4 hr after administration in both males and females. Following the administration on day 23, the concentration of CoQ10 in plasma increased quickly in both sexes. C_{max} on day 23 at 500, 1000 and

Table 7. Histopathological Findings in Rats Treated with Q10EP40

			Males			Females		
		Dose (mg/kg):	0	2000	placebo	0	2000	placebo
Findings	Grade	Number:	10	10	10	10	10	10
Epididymis								
Decreased, sperm	mild		1[a)]	0	0	—	—	—
Eye								
Hemorrhage, vitreous body	slight		0	1	1	0	0	0
Hemorrhage, anterior chamber	slight		0	0	0	0	0	1
Retinal rosette	slight		2	0	0	1	1	1
	mild		2	0	0	1	0	1
Kidney								
Dilation, pelvic	mild		1	0	1	0	0	1
Basophilia, tubular	slight		1	2	2	0	0	0
Eosinophilic body, tubular cell	slight		2	0	1	0	0	0
Urinary cast	slight		0	0	2	0	0	0
Cell infiltration, interstitial	slight		0	0	2	0	0	0
Large intestine, cecum								
infiltration, lamina propria	slight		1	0	1	1	4	3
Large intestine, colon								
Cell infiltration, lamina propria	slight		1	0	0	0	0	0
Liver								
Hepatodiaphragmatic nodule	present		0	0	0	0	0	0
Microgranuloma	slight		0	1	3	1	0	1
Lung (bronchus)								
Mineralization, arterial wall	slight		0	1	1	0	0	0
Cell infiltration	slight		1	0	1	0	0	0
	mild		1	0	0	0	0	0
Accumulation, foamy cell	mild		0	0	0	0	0	0
Metaplasia, osseous	present		1	2	0	0	1	0
Pancreas								
Atrophy, acinar, focal	slight		1	0	2	0	0	1
Pituitary								
Aberrant craniopharyngeal tissue	present		0	0	0	0	1	0
Prostate								
Cell infliltration	slight		2	3	3	—	—	—
Spleen								
Hematopoiesis, extramedullary	slight		2	2	1	0	0	0
Hyperplasia, follicular, focal	mild		0	0	0	0	0	0
Stomach								
Erosion, glandular stomach	slight		0	0	0	1	2	0
Testis								
Atrophy, seminiferous tubular	slight		1	0	0	—	—	—
	severe		1	0	0	—	—	—

a) Number of rats exhibiting pathological findings.

2000 mg/kg of Q10EP40 was 1.73, 2.30 and 2.76 μg/ml in males, and 1.41, 1.68 and 1.68 μg/ml in females, respectively. $AUC_{0-8\,hr}$ on day 23 at 500, 1000 and 2000 mg/kg of Q10EP40 was 10.75, 14.79 and 16.66 μg hr/ml in males, and 8.62, 8.57 and 10.96 μg hr/ml in females, respectively. T_{max} on day 23 was 4 hr in males and 1 hr in females after administration. The results of toxicokinetic analysis revealed absorption of CoQ10 in rats given Q10EP40.

In conclusion, there were no toxic changes related to the administration of Q10EP40 or its pla-

cebo in rats given 2000 mg/kg/day, and the no-observed-adverse effect level (NOAEL) of Q10EP40 in Sprague-Dawley rats was estimated to be 2000 mg/kg/day. To the best of our knowledge, this is the first report concerning a subacute oral toxicity study of a CoQ10 preparation in rats, and it supports the safety of use of Q10EP40 in preparation of dietary supplements.

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