52-Week Oral Gavage Chronic Toxicity Study with Ubiquinone in Rats with a 4-Week Recovery†

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Potential ubiquinone (CoQ10; a natural fermentation product) toxicity was assessed in rats administered CoQ_{10} by oral gavage for 1 year at 100, 300, 600, and 1200 mg/(kg day). No adverse changes in mortality, clinical signs, body weight, food consumption, or clinical pathology results occurred. CoQ_{10} had elimination half-lives ranging from 10.7 to 15.2 h. At 1200 mg/(kg day), a high incidence of orange, granular, lumenal exudate in nasal turbinates occurred; microscopically, findings similar to those in the turbinates were occasionally observed in small granulomas within lung alveoli. A dose-related increased incidence of vacuolated macrophages (mesenteric lymph nodes) and vacuolated hepatic periportal cells was noted. Neither were associated with tissue damage or organ dysfunction, so they were not considered to be adverse. The nasal turbinate and lung findings were probably secondary to incidental exposure to crystallized test material. Overall, CoQ_{10} was well tolerated by male and female rats at dose levels up to 1200 mg/(kg day).

Keywords: *Ubiquinone; coenzyme Q_{10}; toxicity; rats; pharmacokinetics*

INTRODUCTION

Coenzyme Q (CoQ_{10}), or ubiquinone, 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. Also, the ability of CoQ_{10} to function in extramitochondrial reactions as an electron donor has led researchers to postulate a role for ubiquinol, the reduced form of ubiquinone, as an endogenous antioxidant. Although it has been long established that ubiquinol protects membranes from oxidation (Mellors and Tappel, 1966), the possibility that supplementation with CoQ_{10} might serve to increase resistance to oxidative stress has been the subject of a growing body of literature (Stocker et al., 1991; Mohr et al., 1992; Weber et al., 1994; Yamashita and Yamamoto, 1997). It is beyond the scope of the present manuscript to exhaustively cite the research for and against this hypothesis; interested readers are referred to review collections (Beyer, 1991; Littarru et al., 1996, 1997). Among possible medical uses of CoQ_{10}, therapy of cardiovascular diseases has received the most attention (Folkers et al., 1990; Mortenson, 1993).

Spurred by this growing interest in the potential use of CoQ_{10} as a dietary supplement, this research was undertaken to more rigorously test the chronic toxicity (i.e., 52 weeks of daily oral gavage treatment) of CoQ_{10} in rats in an experimental design following principles for the safety assessment of food additives (FDA, 1982). Dose levels were selected on the basis of the literature (Chiba et al., 1972) which concluded that ubiquinone, delivered in suspensions prepared in 10% gum arabic, produced no signs of toxicity at dose levels of up to 1000 mg/(kg day) for 5 weeks and 600 mg/(kg day) for 26 weeks in Wistar rats. In this 52-week study using CD rats, ubiquinone was administered as a solution in corn oil, and the high-dose level of ubiquinone, 1200 mg/(kg day), was twice that used in the 26-week study. Additionally, the scope of the present research included a kinetic assessment of ubiquinone exposure as well as a study of its potential to bioaccumulate. These have not been studied in the context of chronic exposure in rats.

MATERIALS AND METHODS

Materials. Ubiquinone (coenzyme Q_{10}; all in trans configuration), CoQ_{10}, lot Q5002, used in this study was a natural fermentation product manufactured and provided by Kaneka Corp. (Osaka, Japan). Analytical determinations showed this lot to contain only 0.23% coenzyme Q_9 and 0.02% water. The value for analysis of CoQ_{10} was 101.4%. Commercial corn oil [Mazola (Best Foods Division, Englewood Cliffs, NJ)], lots APR1397A and A17A5, with peroxide values of less than 1 were used as the carrier for CoQ_{10}.

Animals. Male and female Crl:CD(SD)BR VAF/Plus rats were obtained from the Portage, MI, facility of Charles River Laboratories, Inc., on September 5, 1995. The animals were approximately 46 days old at initiation of treatment. The males weighed from 198 to 266 g and the females weighed from 133 to 201 g at initiation of treatment. Environmental controls for the animal room were set to maintain 19−25 °C, a relative humidity of 50% ± 20%, and a 12-h light/12-h dark cycle. Certified Rodent Diet #5002 meal (PMI Feeds, Inc.) was provided ad libitum, except when animals were fasted. Water was provided ad libitum. The animals (247 males and 247 females) were assigned computer-generated random numbers and allocated to groups according to the relative rank of the random numbers. The experimental design is shown in Table 1.

Dose Preparation, Analysis, and Administration. Dose preparations were mixed approximately every 2 weeks. Each

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Table 1. Experimental Design

group	dose level (mg/kg)[a]	dose concn (mg/mL)	no. of animals male	no. of animals female
Toxicity Animals				
1 (control)	0	0	30[b]	30[b]
2 (low)	100	33.3	20	20
3 (mid)	300	100	20	20
4 (mid-high)	600	200	30[b]	30[b]
5 (high)	1200	400	30[b]	30[b]
Toxicokinetic Animals				
6 (mid)	300	100	39[c]	39[c]
7 (mid-high)	600	200	39[c]	39[c]
8 (high)	1200	400	39[c]	39[c]

[a] The dose volume was 3 mL/kg. The control group received the carrier only. [b] Ten animals/sex (selected at random before the 52 week scheduled sacrifice) were treated for at least 52 weeks; then treatment was discontinued, and the animals were observed for reversibility, persistence, or delayed occurrence of toxic effects for at least 4 weeks posttreatment. [c] Three animals/(sex group) were bled before dosing on day 1 and sacrificed. Three animals/(sex group) were bled at each of nine intervals after dosing on day 1 (total of 27 animals/(sex group)) and sacrificed. Nine animals/(sex group) continued on study for blood collection at weeks 4, 13, 26, and 52.

concentration was prepared independently. The test material was transferred to a foil-wrapped, labeled container with a portion of the carrier that had been heated to approximately 50 °C and mixed using a magnetic stir plate and stir bar until the test material dissolved. The dose preparations were dispensed into labeled, amber glass containers with stir bars for daily dose administration and stored in a refrigerator set to maintain 2 ± 8 °C. Each day prior to dosing, containers for the control and 100-, 300-, and 600-mg/(kg day) dose preparations were warmed in a water bath at approximately 40 °C to dissolve any precipitated test material. Similarly, containers for the 1200-mg/kg dose preparation were warmed in a water bath at approximately 50 °C.

For the concentration ranges of the dose preparations, the mixtures appeared to be solutions. Stability under the conditions of use was verified by HPLC analysis of stored dose preparations using a validated method. Using the same method, periodic analyses of the dose preparations were conducted during the study to verify proper concentrations. The HPLC method employed was similar to that of the procedure used for the plasma level determinations (vide infra) except that a precolumn was not employed, and the range for the assay was validated in a different matrix (corn oil). The coefficients of variation were 1.6% and 2.6% at the extremes of the validated range (33−500 mg/mL), respectively.

The dose preparations were administered once daily by oral gavage during treatment. The dose preparations were given at a dose volume of 3 mL/kg. Individual doses were calculated on the basis of the most recently recorded body weights. Animals were dosed at approximately the same time each day (approximately 4−6 h into light cycle). During dose administration, dose preparations were maintained in a water bath set to maintain approximately 40 °C.

Antemortem Data Collection. The animals were observed twice daily (a.m. and p.m.) for mortality and morbidity. Signs of poor health or abnormal behavior were recorded as they were observed. At least once each week, each toxicity animal was removed from its cage and examined. Any unusual or abnormal findings were recorded. Body weights and food consumption were recorded weekly during treatment and recovery. Ophthalmic examinations were done before initiation of treatment for all animals and at approximately 13, 26, 39, and 52 weeks of treatment for toxicity animals. The pupils were dilated with 0.5% Mydriacyl, and the anterior portion of the eye, optic media, and ocular fundus were examined using an indirect ophthalmoscope.

Blood and urine samples were collected from 10 toxicity animals/(sex group) (the first 10 surviving animals/(sex group)) during weeks 5, 14, 27, 53, and 57. Animals were fasted overnight, and urine was collected (approximately 16 h) before blood sampling; water was provided ad libitum. Blood was collected directly from a jugular vein from conscious animals. Animals were bled in random order. A standard battery of hematology (EDTA anticoagulant) and clotting function tests (sodium citrate anticoagulant), serum chemistry, and urine chemistry and urinalysis determinations were evaluated (see Table 2).

Table 2. Clinical Pathology Parameters

Hematology	
red blood cell count	
hemoglobin	
hematocrit	
mean corpuscular volume	
mean corpuscular hemoglobin	
mean corpuscular hemoglobin concn	
platelet count	
prothrombin time (weeks 27, 53, and 57 only)	
activated partial thromboplastin time (weeks 27, 53, and 57 only)	
white blood cell count	
differential blood cell count	
nucleated red blood cell count	
corrected white blood cell count	
segmented neutrophil count	
band neutrophil count	
lymphocyte count	
monocyte count	
eosinophil count	
basophil count	
blood cell morphology	
Serum Chemistry	
glucose	aspartate aminotransferase
urea nitrogen	alanine aminotransferase
creatinine	γ-glutamyl transferase
tot. protein	alkaline phosphatase
albumin	calcium
globulin	inorg phosphorus
albumin/globulin ratio	sodium
total bilirubin	potassium
cholesterol	chloride
triglycerides	serum protein electrophoresis
phospholipids	
Urinalysis	
vol (approximately 16 h)	bilirubin
specific gravity	blood
pH	urobilinogen
protein	microscopic examination of sediment
glucose	appearance
ketones	
Urine Chemistry	
sodium	fractional clearance of
potassium	sodium
chloride	potassium
	chloride

Most hematology variables were determined using a Coulter S-Plus IV whole blood automated analyzer. Differential leukocyte count and blood cell morphology slides were prepared using a Geometric Data Hemastainer and read manually. Prothrombin time was measured using a Coulter/IL ACL 3000 coagulation analyzer. Clinical chemistry and urine chemistry variables were determined using an Hitachi 704 random access chemistry analyzer, except that urinary fractional clearance of electrolytes (Duncan and Prasse, 1986) were calculated. Serum globulin was the difference between the values for total protein and albumin. Urinalysis was evaluated manually using Ames Multistix and light microscopy.

Toxicokinetic Analyses. Blood samples were collected once from three toxicokinetic animals/(sex group interval) on days 1 (predose and approximately 1, 2, 4, 6, 8, and 12 h postdose), 2 (24 h postdose), and 3 (36 and 48 h postdose). Six animals/(sex group) were used for subsequent collections during weeks 4, 13, 26, and 52; blood was collected from one

set of three toxicokinetic animals/(sex group interval) 24 h postdose and from a second set of three toxicokinetic animals/(sex group interval) at the approximate time-to-peak concentration (t_{max}, determined to be 4 h postdose from day 1 data). Animals were not fasted before blood collection. Blood [approximately 1.0 mL (days 1, 2, and 3) and 1.5 mL (weeks 4, 13, 26, and 52)] was collected under yellow fluorescent lighting from a jugular vein of conscious animals into tubes containing sodium heparin (samples were protected from light as soon as possible after collection). Samples were held at room temperature until centrifugation and processing under yellow fluorescent lighting. Plasma was harvested at room temperature and was protected from light by placing it in covered containers. Plasma was divided into aliquots, transferred into extract tubes under yellow fluorescent lighting, and stored in a freezer set to maintain -20 ± 10 °C. Before analysis, samples were thawed, mixed well, and extracted without further centrifugation.

Plasma samples were analyzed for ubiquinone by an HPLC method (Reahal and Wrigglesworth, 1992; Scalori et al., 1990) modified and validated for support of this study. Ubiquinone and its internal standard (ISTD), CoQ_7, were extracted from heparin-treated rat plasma by liquid−liquid extraction. The plasma proteins were precipitated with ethanol followed by deionized water, and the precipitated samples were extracted three times by vigorous shaking with hexane. The hexane extracts were combined and evaporated to dryness. The residue was reconstituted in ethanol and injected for analysis by HPLC using a precolumn (Brownlee Newguard RP-18; 15 mm × 3.2 mm, 7 μm particle size): column (YMC ODS-A 150 mm × 4.6 mm, 5 μm particle size) combination and ultraviolet absorbance detection (275 ηm). Peak height ratios of ubiquinone/ISTD were calculated. Flow rates for the methanol/hexane (90:10) mobile phase were 1.0 mL/min, run times were approximately 20 min, column temperature was 35 °C, and ubiquinone and ISTD retention times were approximately 18.4 and 6.8 min, respectively. The calibration curves were obtained by weighted (1/concentration) least-squares linear regression analysis using CALC Version 2.0, a program that calculates the line of best fit of the data that have been collected and processed using the Hewlett-Packard HP1000 (model A990) computer with 3350A Laboratory Automation System. The equations of the calibration curves were then used to calculate the concentration of ubiquinone in the animal samples and quality control (QC) samples from their peak height ratios. The lower limit of quantitation was 0.1 μg/mL for ubiquinone in rat plasma, calibration curves were linear between 0.1 and 10 μg/mL, and relative standard deviations at these extremes were 4.5% and 1.0%, respectively.

Noncompartmental toxicokinetic analysis (Gibaldi and Perrier, 1982) of the data from days 1, 2, and 3 included (if appropriate) determinations of C_{max}, T_{max}, $AUC_{0-\infty}$, terminal half-life ($t_{1/2}$), and terminal elimination rate constant (K_e). Plasma levels and accumulation factors were reported for data from weeks 4, 13, 26, and 52.

Anatomical Pathology. A necropsy was done on each toxicokinetic and toxicity animal that died on test or was sacrificed at an unscheduled interval. During weeks 53 (terminal sacrifice) and 57 (recovery sacrifice), toxicity animals were fasted overnight, anesthetized with sodium pentobarbital, weighed, exsanguinated by severing axillary blood vessels, and necropsied. Animals were necropsied in random order. The necropsy included a macroscopic examination of the external surface of the body; all orifices; the cranial cavity; the brain and spinal cord; the nasal cavity and paranasal sinuses; and the thoracic, abdominal, and pelvic cavities and viscera. For all toxicity animals at the scheduled and unscheduled sacrifices, adrenals, brain, heart, kidneys, liver, lungs, ovaries with oviducts, pituitary, prostate, salivary glands (submaxillary), seminal vesicles, spleen, testes, thymus, thyroids with parathyroid, and uterus were weighed; paired organs were weighed separately. Organ-to-body weight percentages and organ-to-brain weight ratios were calculated. Macroscopic lesions were collected from each toxicokinetic animal that died on test or was sacrificed at an unscheduled interval; the lesions were preserved in 10% phosphate-buffered formalin. The tissues listed in Table 3 or representative samples were collected from each toxicity animal and preserved in 10% phosphate-buffered formalin.

Table 3. Tissues Collected for Histopathology

adrenals
aorta
brain (at least three different levels)
cecum
cervix
colon
duodenum
epididymides
esophagus
external auditory sebaceous gland (Zymbal's gland)
eyes
femur with bone marrow (articular surface of the distal end)
Harderian gland
heart
ileum
jejunum
kidneys
lesions
liver
lungs with mainstem bronchi
lymph nodes (submaxillary and mesenteric)
mammary gland (males and females)
nasal turbinates
ovaries with oviducts
pancreas
pituitary
prostate
rectum
salivary glands (submaxillary)
sciatic nerve
seminal vesicles
skeletal muscle (thigh)
skin
spinal cord (cervical, midthoracic, and lumbar)
sternum with bone marrow
stomach
testes (preserved in Bouin's solution for scheduled sacrifice animals only)
thymus
thyroids with parathyroid
tongue
trachea
urinary bladder
uterus
vagina

Tissues (as appropriate) were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically from each control animal and each toxicity animal given 1200 mg/(kg day) sacrificed at the terminal sacrifice and from each toxicity animal that died on test or was sacrificed at an unscheduled interval. Macroscopic lesions, lungs, liver, mesenteric lymph node, spleen, kidneys, and nasal turbinates were also examined microscopically from each toxicity animal given 100, 300, or 600 mg/(kg day) sacrificed at the terminal sacrifice and from each animal at the recovery sacrifice.

Statistical Analyses. Only data collected on or after the first day of treatment were analyzed statistically. One-way analysis of variance [ANOVA (Winer, 1971)] was used to analyze body weights; body weight gains; food consumption; clinical chemistry and hematology values (except blood cell morphology); urine pH, volume, and specific gravity; organ weights; organ-to-body weight percentages; and organ-to-brain weight ratios. Levene's test (Levene, 1960) was done to test for variance homogeneity. In the case of heterogeneity of variance at $p \leq 0.05$, transformations were used to stabilize the variance. ANOVA was done on the homogeneous or transformed data. If the ANOVA was significant, Dunnett's multiple comparison *t*-test (Dunnett, 1964) was used for

Table 4. Incidence of Death of Toxicity Animals[a]

	group									
	males					females				
week	1	2	3	4	5	1	2	3	4	5
8		1								
10	1									
13	1									
28									1	
33								1		
35					1					
38				1						
48				1						
50									1	
52				1						
56					1					

[a] Dose levels for groups 1−5 were 0, 100, 300, 600, and 1200 mg/(kg day), respectively.

pairwise comparisons between treated and control groups. For each sex, groups 2−5 were compared with group 1 (control). Group comparisons were evaluated at the 5.0%, two-tailed probability level.

RESULTS

Dose Analyses. After 20 days under refrigerated conditions and then 4 h at 40 °C, results of stability analyses indicated that the average concentration of duplicate samples were 98.2% and 91.0% of the initial theoretical concentrations of 33.3 and 400 mg/mL, respectively. Therefore, the dose preparations were stable for 20 days under refrigerated conditions at concentrations ranging from 33.3 to 400 mg/mL. Periodic routine dose preparation analyses of all levels ranged from 95.4% to 116% of the theoretical concentrations indicating target concentrations were achieved.

Antemortem Observations and Survival. All high-dose toxicity animals had orange feces during treatment. The orange color of the feces was probably due to the presence of the orange colored CoQ_{10} in the feces and was not considered a toxic response. During treatment, red nasal discharge was observed in one control female, one mid-high-dose female, three high-dose males, and four high-dose females. Labored, irregular, or audible respiration was noted throughout the groups, with a higher incidence in mid-high-dose males and females and high-dose males. Other incidental findings included palpable tissue masses, sores, scabs, alopecia, discolored haircoat, and excessive salivation.

Eleven toxicity animals (eight males and three females; Table 4) died or were sacrificed at unscheduled intervals during the study. Two control males were sacrificed; one during week 10 and one during week 13. At necropsy, both animals had a bilaterally or unilaterally enlarged renal pelvis that contained semifluid material or calculi. Microscopically, correlating inflammatory changes in the urinary system directly contributed to the poor health of these animals. Similar macroscopic and microscopic lesions in the urinary system were also observed in one male given 100 mg/(kg day) that was sacrificed during week 8 and in one female given 600 mg/(kg day) that died during week 28. Malignant lymphoma caused the death (during week 35) of one male given 1200 mg/(kg day) and the morbidity of one female given 300 mg/(kg day) sacrificed during week 33. The cause of death for the remaining five animals that died or were sacrificed at unscheduled intervals could not be determined by microscopic evaluation of the tissues. These included three males given 600 mg/(kg day) one each that died during weeks 38, 48, and 52; one male given 1200 mg/(kg day) that died during week 56; and one female given 600 mg/(kg day) that was sacrificed during week 50.

Body Weights and Food Consumption. Body weight data are depicted in Figure 1. There were no significant differences in body weights for males. In general, mean body weights for the females given 300 mg/(kg day) or higher were slightly higher (less than 10% difference) than the control group females, but no statistical differences in mean body weight between CoQ_{10}-treated females and the control females occurred beyond week 17. The slight differences were not considered to be a toxic response.

Statistically significant increases in food consumption were noted at weeks 2 through 5 and 7 for the females

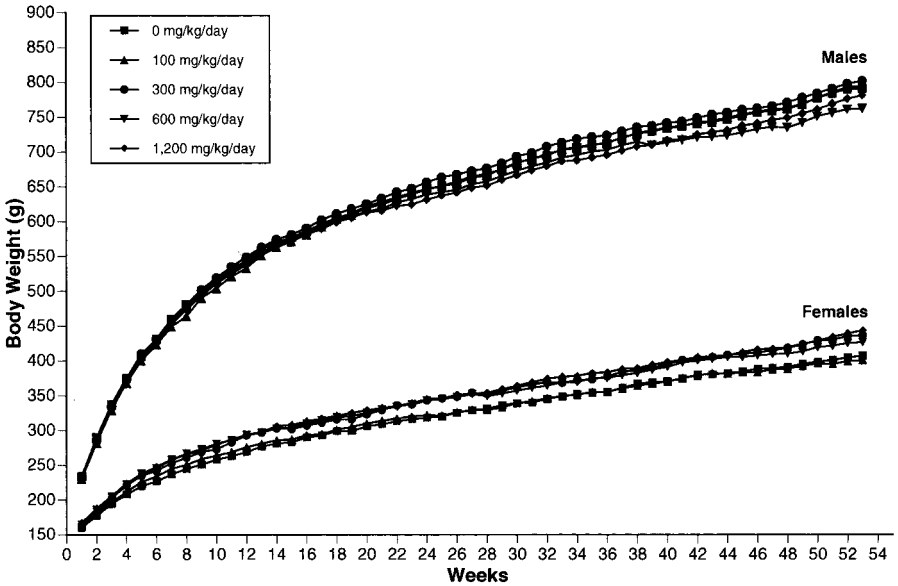

Figure 1. Mean (n = 20 or 30/(sex group) at week 1) body weights for males and females administered 0, 100, 300, 600, or 1200 mg/(kg day) of CoQ_{10}. Significant differences were detected for females at 300 mg/(kg day) (week 12), at 600 mg/(kg day) (weeks 4−12, and 14), and at 1200 mg/(kg day) (weeks 4−17). The range of coefficients of variation across all groups and intervals were 5.8−14.7 and 6.7−19.8 for males and females, respectively.

Table 5. Clinical Chemistry or Hematological Parameters with Changes in Female Rats Potentially Related to CoQ_{10} Administration

	week no.				
group	5	14	27	53	57
ALT: Alanine Aminotransferase Activity (IU/L)					
1	30	30	80	41	38
2	35	44	74	58	ND[a]
3	30	39	53	58	ND
4	31	34	45	53	58[b]
5	31	33	45	69[b]	79[b]
MCV: Mean Corpuscular Volume (fL)					
1	57	54	56	56	56
2	56	53	54	55	ND
3	57	53	55	55	ND
4	57	53	54	54	54[b]
5	56	53	54	53[b]	53[b]
MCH: Mean Corpuscular Hemoglobin (pg)					
1	21.3	20.3	20.7	20.7	21.4
2	21.0	19.8	20.1	20.8	ND
3	21.0	19.8	20.2	20.6	ND
4	21.1	19.8	20.1	19.9[b]	20.3
5	20.9	19.7	20.0	19.3[b]	20.2

[a] ND = not determined. [b] Significantly different from the mean for group 1 ($p < 0.05$).

administered 600 mg/(kg day) and during weeks 1 through 5, 7, 9, 11, 12, 45, and 46 for females administered 1200 mg/(kg day). The slightly higher food consumption may explain, in part, the slightly higher body weights for these groups.

Ophthalmology. Animals selected for the study had no lesions at the prestudy examination. No test material-related ophthalmic observations were noted at the week 13, 26, 39, and 52 examinations. Examination of the recovery animals was not done because there were no test material-related ophthalmic observations noted during treatment.

Clinical Pathology. Hematology, clinical chemistry, urinalysis, and urine chemistry were determined from samples collected during weeks 5, 13, 26, and 53 during treatment and during week 57 following treatment. Administration of CoQ_{10} had no clear effects on clinical pathology results. Of uncertain relationship to administration of CoQ_{10} were mildly lower mean corpuscular volume for females given 1200 mg/(kg day), mildly lower mean corpuscular hemoglobin for females given 600 or 1200 mg/(kg day), and mildly higher alanine aminotransferase for females given 1200 mg/(kg day). These small differences were apparent only at weeks 53 and 57 (Table 5), were absent for males, and were not considered adverse. There were no correlative findings for the differences in mean corpuscular volume and mean corpuscular hemoglobin; red blood cell count, hemoglobin concentration, or hematocrit were unaffected. The small difference for alanine aminotransferase may have been associated with pathological alterations in the liver that were observed microscopically (i.e., minimal to slight lymphohistiocytic inflammation associated with areas of periportal cell vacuolation) but was not indicative of significant hepatocellular degeneration or necrosis.

Anatomical Pathology. There were no organ weight changes detected statistically that were considered to represent a test material-related response. The incidence of selected macroscopic and microscopic observations related to CoQ_{10} administration is shown in Table 6.

Table 6. Incidence of Anatomical Pathology Findings Associated with Administration of Ubiquinone for 52 Weeks (Week 53 Necropsy)

		mg of ubiquinone/(kg day)				
organ/description of finding	sex	0	100	300	600	1200
no. examined	M	18	19	20	17	19
	F	20	20	19	18	20
lung bronchi						
inflammation, granulomatous	M	0	0	3	2	3
	F	0	1	0	0	2
infiltrate, macrophage (foamy)	M	2	6	12	14	8
	F	2	5	8	8	4
nasal turbinates						
granular material (macroscopic)	M	0	0	0	0	17
	F	0	0	0	0	19
lumenal exudate (microscopic)	M	0	0	0	0	9
	F	0	0	0	0	18
mesenteric lymph node						
infiltrate, macrophage (foamy)	M	0	12	19	15	17
	F	0	19	18	18	20
liver						
inflammation, lymphohistiocytic	M	3	1	2	1	4
	F	1	4	5	7	10
vacuolation, periportal	M	0	0	1	3	5
	F	0	4	9	10	15

Administration of CoQ_{10} was associated with an increase in the incidence of large, finely vacuolated (foamy) macrophages in the mesenteric lymph nodes (Figure 2, upper left) and an increase in the incidence of vacuolated cells in the periportal areas of the liver (Figure 2, upper right). The incidence and severity of the latter finding tended to increase with dose, and both findings were more prominent in females. Treated females also had an increased incidence of minimal to slight periportal lymphohistiocytic inflammation.

Macroscopically, males and females given 1200 mg/(kg day) had a high incidence of orange granular material in the nasal turbinates; in addition, some treated animals had light-colored foci or areas in lungs. Microscopically, the material in the nasal turbinates appeared as a lumenal exudate that was homogeneous, slightly eosinophilic, and contained crystalline-shaped spaces or patterns (Figure 2, lower left). The crystalline-shaped spaces suggested the presence of material that dissolved during tissue processing. Spaces similar to those observed microscopically in the turbinates were occasionally observed in small granulomas within the alveoli of the lung (Figure 2, lower right). The lungs of treated animals also exhibited an increase in vacuolated (foamy) alveolar macrophages.

Following a 4-week recovery, some treated animals continued to have orange granular material in the nasal turbinates and light-colored foci or areas in the lungs. The incidence, severity, and appearance of the foamy macrophage infiltrate in mesenteric lymph nodes, hepatic periportal cell vacuolation, alveolar macrophage infiltrate, and lumenal exudate in nasal turbinates were similar to those observed at week 53 (results not shown). All remaining macroscopic and microscopic observations in animals sacrificed at weeks 53 and 57 were considered incidental and unrelated to the test material.

Toxicokinetic Analyses. Table 7 and Figures 3 and 4 have results of the toxicokinetic evaluations. The mean CoQ_{10} plasma levels in males (Figure 3) and females (Figure 4) were comparable at the different dose levels on day 1, but the variability between the individual animals was high. Similarly, there was no consistent sex effect across the different dose levels on

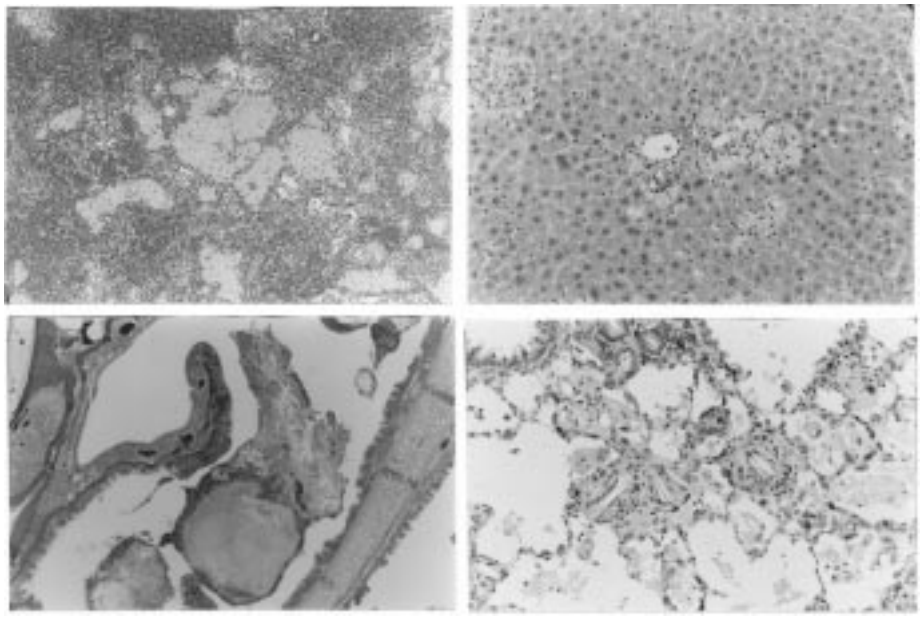

Figure 2. Representative photomicrographs from H&E stained 6 μm sections of selected organs from rats treated for 52 weeks with 1200 mg/(kg day) of CoQ_{10}. Note the appearance of large, finely vacuolated foamy macrophages in mesentreric lymph nodes (upper left) and vacuolated cells in the periportal areas of the liver (upper right). The material in the nasal turbinates (lower left) appeared as a lumenal exudate that was homogeneous, slightly eosinophilic, and contained crystalline-shaped spaces or patterns. The crystalline-shaped spaces suggested the presence of material that dissolved during tissue processing. Spaces similar to those observed microscopically in the turbinates were occasionally observed in small granulomas within the alveoli of the lung (lower right). The lungs of treated animals also exhibited an increase in vacuolated (foamy) alveolar macrophages.

Table 7. Ubiquinone Toxicokinetic Parameters[a] in Rats (Day 1)

dose level (mg/(kg day))	sex	C_{max} (μg/mL)	t_{max} (h)	AUC_{0-t} (μg·h/mL)	$AUC_{0-\infty}$ (μg·h/mL)	K_e (h^{-1})	$t_{1/2}$ (h)
300	M	1.63	4.0	28.0	32.0	0.0455	15.2
	F	1.54	2.0	25.9	NA	NA	NA
	M, F	1.43	2.0	27.0	30.7	0.0554	12.5
600	M	2.24	12.0	51.9	NA	NA	NA
	F	1.21	6.0	30.3	33.3	0.0649	10.7
	M, F	1.33	6.0	41.1	46.4	0.0502	13.8
1200	M	1.64	4.0	38.6	NA	NA	NA
	F	1.40	4.0	34.0	NA	NA	NA
	M, F	1.52	4.0	36.3	NA	NA	NA

[a] Each value represents data determined from up to 3 values per collection time point. [b] NA = not applicable.

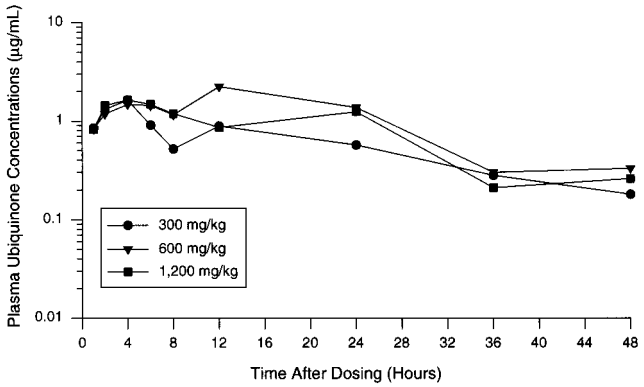

Figure 3. Mean CoQ_{10} plasma levels in males ($n = 3$ at each time point) after the first administration of 300, 600, or 1200 mg/kg of CoQ_{10}. The coefficients of variation ranged from 8.8 to 92.1%, with an overall average of 36.2%. All pretreatment CoQ_{10} values were below the limit of quantitation of 0.1 μg/mL, and no means were plotted for time 0.

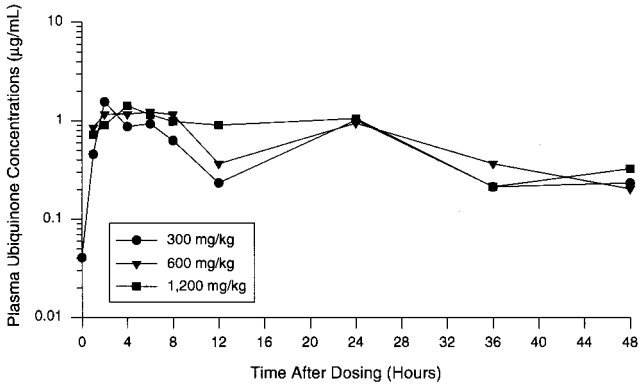

Figure 4. Mean CoQ_{10} plasma levels in females ($n = 3$ at each time point) after the first administration of 300, 600, or 1200 mg/kg of CoQ_{10}. The coefficients of variation ranged from 9.1 to 75.8%, with an overall average of 38.6%. All pretreatment CoQ_{10} values were below the limit of quantitation of 0.1 μg/mL for 600 or 1200 mg/kg groups, and no means were plotted for time 0. A female in the 300 mg/kg group had a pretreatment value (time 0) of 0.11 μg/mL; this was included in calculating the plotted mean value (assumes values of 0 for the other two rats).

the other collection days. Following the first dose on day 1, plasma CoQ_{10} concentrations rose slowly, with an overall mean t_{max} ranging between 4.0 and 12.0 h in males and 2.0 and 6.0 h in females. The increase in both C_{max} and AUC_{0-t} in males and females were less than proportional to the increase in the dose level. This may be caused by phagocytic activity of reticuloendothelial

Table 8. Accumulation Ratios[a] for Ubiquinone in Rat Plasma

dose level (mg/kg)	sex	week no. 4	13	26	52
300	M	1.06	3.44	2.30	2.60
	F	0.883	2.85	3.43	3.85
	all rats	1.08	3.5	3.16	3.54
600	M	0.757	2.06	1.46	1.66
	F	1.77	3.56	2.89	4.93
	all rats	1.45	3.36	2.55	3.65
1200	M	1.27	1.65	1.46	1.87
	F	0.98	3.29	2.10	4.23
	all rats	1.12	2.40	1.76	2.96

[a] Accumulation ratio = $(C_{max})_{ss}/(C_{max})_{D1}$. $(C_{max})_{ss}$ is the mean maximum plasma concentration (n = 3/sex) of CoQ_{10} measured during weeks 4, 13, 26, and 52, and $(C_{max})_{D1}$ is the maximum concentration calculated from the day 1 kinetic evaluation.

cells in lymphoid tissue and liver as a clearance mechanism for excess CoQ_{10} in lymph or plasma. This is supported by histopathology results indicating that there were increased numbers of enlarged cells of these types in mesenteric lymph nodes and in liver of CoQ_{10}-treated animals. Correspondingly, the liver has been shown to be the target organ for accumulation of CoQ_{10} in pharmacokinetic studies using oral and intravenous administration (Reahal and Wrigglesworth, 1992; Scalori et al., 1990). CoQ_{10} was slowly eliminated in rats, with a $t_{1/2}$ of 15.2 h in males at the 300-mg/(kg day) dose and 10.7 h in females at the 600-mg/(kg day) dose. These dose levels were selected based on shorter duration toxicity studies (13 weeks), which showed no toxic effects for CoQ_{10} at dose levels of as high as 600 mg/kg, rather than by a pharmacokinetic assessment of CoQ_{10} that may have led one to choose a lower dose range for testing (below the point of exposure saturation). There was marked accumulation of CoQ_{10} in rats at the different dose levels following daily gavage dosing for 52 weeks that was apparent from week 13 until the end of the study (Table 8).

DISCUSSION

Systemic microscopic changes associated with administration of CoQ_{10} were limited to an increased incidence of large, finely vacuolated (foamy) macrophages in mesenteric lymph nodes and an increase in the incidence of vacuolated cells in the periportal areas of the liver. These findings were not associated with damage to surrounding cells and appeared not to adversely affect the function of the liver or lymph nodes in which the cells were noted. Only one of the 10 animals administered 1200 mg/(kg day) was noted with vacuolated macrophages in mesenteric lymph node in a 4-week rat toxicity study completed in the same laboratory with aged CoQ_{10} (Williams et al., unpublished findings, 1997). In a 26-week duration toxicity study with CoQ_{10} suspended in 10% gum arabic and administered to Wistar rats at dose levels as high as 600 mg/(kg day), no such findings were reported (Chiba et al., 1972). This suggests treatment duration (28 days or 6 months versus 1 year), vehicle composition (suspension in 10% gum arabic versus solution in warm corn oil) or strain of rat (Wistar versus CD) could be factors important to the finding of vacuolated cells at the light microscopic level. Speculatively, the mononuclear phagocytic cells were probably enlarged due to phagocytic activity in removing absorbed CoQ_{10} from lymph or circulating blood. This activity may also contribute to the less than proportional increases in C_{max} and AUC_{0-t} noted with increasing dose. Saturated absorption of CoQ_{10} from the gastrointestinal tract is another probable causative factor in the less than dose proportional increases in circulating CoQ_{10}. That orange material was found in the feces of rats given 1200 mg/(kg day) indicates CoQ_{10} is incompletely absorbed. Overall, these findings indicate that the circulating plasma levels of CoQ_{10} are not effectively increased by dose levels of CoQ_{10} above 300 mg/kg.

CoQ_{10} has a demonstrated propensity to accumulate in liver, spleen, and plasma with repeated dosing over 8 days (Zhang et al., 1995) with the greatest accumulation noted in liver. Liver exhibited an approximate 4-fold accumulation compared with that seen 8 days after a single dose. This research also showed that the hepatic CoQ_{10} accumulation after a single dose was related to dose level over the range of 3−60 μmol/kg, but further increases were not apparent at a dose of 120 μmol/kg (approximately 100 mg/kg). The type of cell involved in the uptake of the CoQ_{10} was not established in this research, but it was shown that the liver concentrations of CoQ_{10} had returned to pretreatment levels within 10 days after treatment was stopped indicating that the elimination was fairly rapid. This is in contrast to the presence of the vacuolated mononuclear phagocytic cells observed microscopically in liver and lymph nodes, which was still apparent following a 4-week recovery in the present study. However, the dose levels and duration of treatment were much higher in the present study which could be factors in the persistence of the microscopic finding. It would be of interest in future research to evaluate the specificity of hepatic accumulation of CoQ_{10} within liver, lymph nodes, and spleen to determine if there is selective uptake of CoQ_{10} by reticuloendothelial cells and to provide more correlation between the microscopic and biochemical studies.

Local macroscopic findings associated with the administration of CoQ_{10} consisted of light colored areas in lungs and orange granular material in the nasal turbinates. Microscopic changes consisted of small granulomas in lungs (some contained crystalline-shaped spaces characteristic of crystalline material dissolved during tissue processing) and lumenal exudate in nasal turbinates with crystalline-shaped spaces similar to observations seen in lungs. These findings suggest that crystalline CoQ_{10} was inspired into the nasal turbinates and lungs. These findings were likely associated with the irregular or audible respiration observed during treatment.

Crystalline CoQ_{10} could gain access into nasal turbinates and lungs through several means. First, the dose solutions contain dissolved CoQ_{10} close to saturation levels in corn oil at approximately 40 °C. Small amounts of crystalline CoQ_{10} could have precipitated on the outside of the dosing needle, the surface of which is cooler than the solution in the needle itself. This could be rubbed off in the buccal cavity during the dosing process. Second, any drop of dose solution clinging to the dosing needle after or during withdrawal from the lower esophagus could be deposited in the back of the throat as the needle was withdrawn. Third, during grooming, crystalline CoQ_{10} present in the fur could be inhaled. Regardless of the actual mechanism of exposure, it was concluded that the findings in nasal turbinates and lungs were not systemically mediated toxic responses. Rather, they were likely secondary to

incidental exposure to crystallized test material and unlikely to be mimicked under conditions of typical exposure or usage in humans.

Administration of CoQ_{10}, when dissolved in a corn oil carrier, to rats by oral gavage at dose levels up to 1200 mg/(kg day) for 52 weeks was associated with an increased incidence of large, finely vacuolated (foamy) macrophages in mesenteric lymph nodes and an increase in the incidence of vacuolated cells in the periportal areas of the liver. Although these findings represent systemic responses to CoQ_{10}, they were thought to have resulted from phagocytosis of absorbed test material. The lesions do not appear to be associated with tissue damage or organ dysfunction and, thus, are not considered to be adverse changes. Additionally, it was concluded that the findings in nasal turbinates and lungs were not systemically mediated toxic responses. Rather, they were likely secondary to incidental exposure to crystallized test material and were unlikely to be mimicked under conditions of typical exposure or usage in humans.

Overall, even the high-dose of CoQ_{10}, 1200 mg/(kg day) for 52 weeks, was well tolerated by male and female Crl:CD(SD)BR VAF/Plus rats. The 100−1200 mg/(kg day) dose range used in this rat is 60−720 times that of a 100-mg daily supplement in a 60-kg human. This statement only implies achievement of an applied dosage exaggeration over a typical supplement level in humans, and it is realized that metabolic and physiologic differences between human and rat exist that would influence tissue utilization or responses to CoQ_{10}.

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