

CANTOX

HEALTH SCIENCES INTERNATIONAL

Revised New Dietary Ingredient Notification for KANEKA QH™

Submitted by: CANTOX HEALTH SCIENCES
INTERNATIONAL
1011 U.S. Highway 22 West, Suite 200
Bridgewater, New Jersey
08807

On behalf of: Kaneka Corporation
Functional Foods Development Division
3-2-4, Nakanoshima, Kita-Ku
Osaka 530-8288, Japan

April 28, 2005

CANTOX Offices:

Bridgewater, NJ, USA
908.429.9202

Mississauga, ON, Canada
905.542.2900

Reading, Berkshire, UK
+44 (0)118 935 7162

CONFIDENTIAL

SECTION 1

The name and complete address of the manufacturer or distributor of the dietary supplement that contains a new dietary ingredient, or of the new dietary ingredient.

The manufacturer of the new dietary ingredient is:

Kaneka Corporation
Functional Foods Development Division
3-2-4, Nakanoshima, Kita-Ku
Osaka 530-8288, Japan

Direct correspondence to:

David H. Bechtel, Ph.D., DABT
Senior Scientific Consultant
CANTOX U.S. Inc.
1011 U.S. Highway 22, Suite 200
Bridgewater, NJ 08807
Phone: 908-429-9202
Fax: 908-429-9260

SECTION 2

The name of the new dietary ingredient.

KANEKA QH™ brand of ubiquinol.

SECTION 3

Description of the dietary supplement or dietary supplements that contain the dietary ingredient including (i) the level of the dietary ingredient in the dietary supplement, and (ii) the conditions of use recommended or suggested in the labeling of the dietary supplement, or if no conditions of use are recommended or suggested in the labeling of the dietary supplement, the ordinary conditions of use of the supplement.

The dietary supplement containing KANEKA QH™ will be in softgel capsule form. The KANEKA QH™ softgel capsules will be clearly labeled and promoted as a dietary supplement. A description of the number of softgel capsules per serving size will appear on the label, and each serving of the dietary supplement will contain 50 mg of KANEKA QH™. Consumption of up to 6 servings per day will be suggested or recommended in the label directions, resulting in a maximum daily consumption of up to 300 mg KANEKA QH™ (equivalent to 6 mg/kg/day for a 50 kg body weight person).

SECTION 4

The history of use or other evidence of safety establishing that the dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe, including any citation to published articles or other evidence that is the basis on which the distributor or manufacturer has concluded that the dietary supplement will reasonably be expected to be safe.

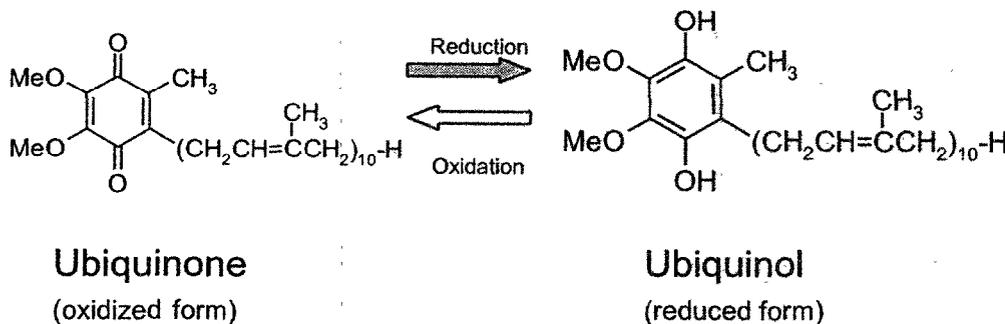
4.1 CHEMICAL COMPOSITION

4.1.1 Chemical Identity of Ubiquinol

Ubiquinol is the two-electron reduction product of coenzyme Q₁₀ (CoQ₁₀), a naturally-occurring, lipid-soluble nutrient (Frei *et al.*, 1990; Schoepp, 1997; Pepping, 1999). The term CoQ refers to a class of homologous benzoquinones that have been identified in all plants and animals, as well as in a majority of microorganisms (Budavari *et al.*, 1996; Nohl *et al.*, 1998). Benzoquinone homologs consist of a redox active quinoid moiety, and a hydrophobic side chain comprised of 6 to 10 isoprenoid units, depending on the species (Ibrahim *et al.*, 2000; Matthews *et al.*, 1998; Lenaz, 2001). In humans and most mammals, including dogs, the predominant form of coenzyme Q is coenzyme Q₁₀ (CoQ₁₀), which consists of 10 isoprenoid units in the side chain (Ramasarma, 1985). In rats and mice, the primary form is coenzyme Q₉, which contains 9 isoprenoid units. However, low levels of coenzyme Q₁₀ have also been reported in rats and mice (Battino *et al.*, 1992). Coenzyme Q₁₀ and its reduced form are also referred to as ubiquinone (or ubiquinone-10) and ubiquinol (or ubiquinol-10), respectively.

The conversion between ubiquinone, the oxidized form, and ubiquinol the reduced form, is shown in Figure 1 below.

Figure 1 Structures of coenzymes Q

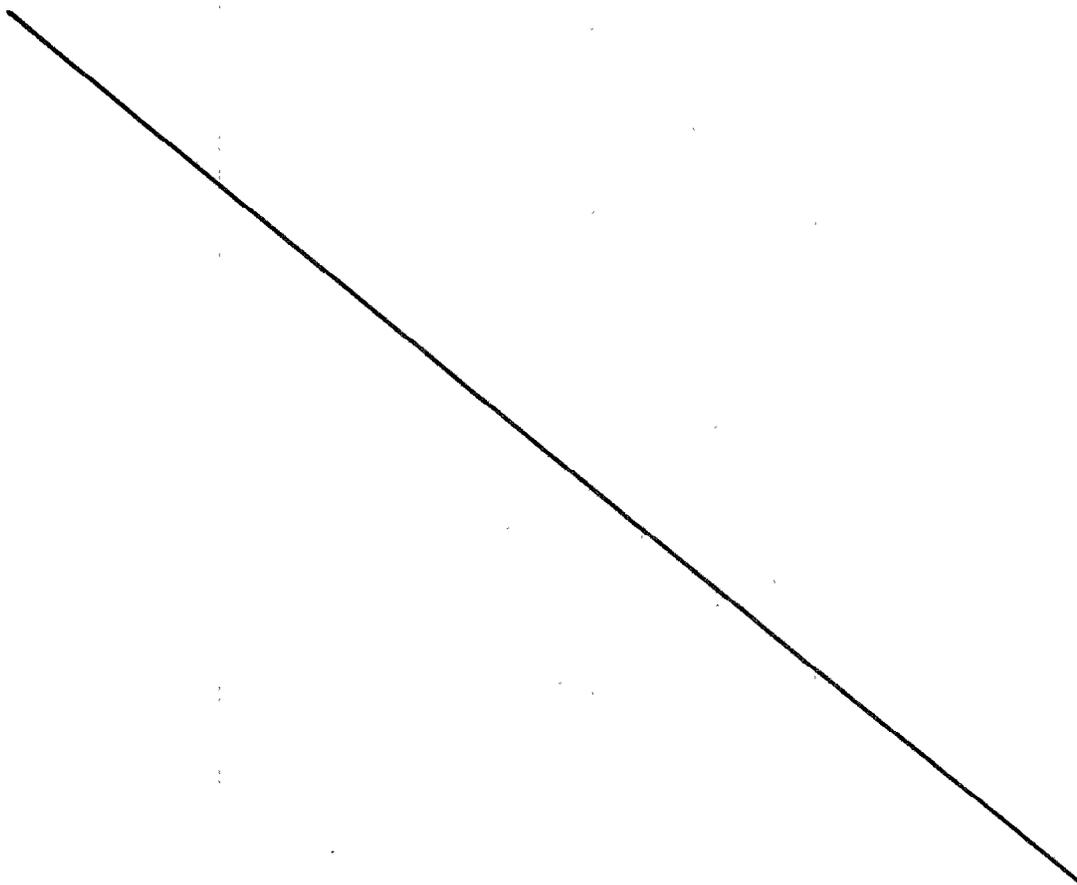


4.1.2 Physical and chemical properties of Ubiquinol

Stability: Ubiquinol is easily oxidized in air, forming ubiquinone.

- Molecular formula : $C_{59}H_{92}O_4$
- Chemical name: 2-[(2E,6E,10E,14E,18E,22E,26E,30E,34E)-3,7,11,15,19,23,27,31,35,39-Decamethyl-2,6,10,14,18,22,26,30,34,38-tetracontadecaenyl]-5,6-dimethoxy-3-methyl-1,4-benzenediol
- CAS Registry No.: 992-78-9

4.2 MANUFACTURE OF KANEKA QH™



4.3 BIOCHEMICAL CONSIDERATIONS AND BIOAVAILABILITY

4.3.1 Biochemical Considerations

4.3.1.1 Occurrence

4.3.1.1.1 Occurrence in Humans

Ubiquinol is the two-electron reduction product of coenzyme Q₁₀ (CoQ₁₀), a naturally-occurring, lipid-soluble nutrient (Frei *et al.*, 1990; Schoepp, 1997; Pepping, 1999). The term CoQ refers to a class of homologous benzoquinones that have been identified in all plants and animals, as well as in a majority of microorganisms (Budavari *et al.*, 1996; Nohl *et al.*, 1998). Benzoquinone homologs consist of a redox active quinoid moiety, and a hydrophobic side chain comprised of 6 to 10 isoprenoid units, depending on the species (Ibrahim *et al.*, 2000; Matthews *et al.*, 1998; Lenaz, 2001). In humans and most mammals, including dogs, the predominant form of coenzyme Q is coenzyme Q₁₀ (CoQ₁₀), which consists of 10 isoprenoid units in the side chain (Ramasarma, 1985). In rats and mice the primary form is coenzyme Q₉, which contains 9 isoprenoid units, however, low levels of coenzyme Q₁₀ have also been reported (Battino *et al.*, 1992). Coenzyme Q₁₀ and its reduced form are also referred to as ubiquinone (ubiquinone-10) and ubiquinol (ubiquinol-10), respectively.

Coenzyme Q₁₀ is located in the hydrophobic interior of nearly every cellular membrane, and to varying degrees in all tissues (Lass and Sohal, 1999; Nohl *et al.*, 1998). Since its discovery in 1957 by Crane and colleagues (Bertelli and Ronca, 1990), coenzyme Q₁₀ has been extensively

studied for its key role in mitochondrial energy production, where it acts as both an electron carrier and proton translocator during cellular respiration and adenosine triphosphate (ATP) production (Hughes *et al.*, 2002; Nohl *et al.*, 2001; Nohl *et al.*, 1998). Apart from its involvement in mitochondrial energy coupling, coenzyme Q₁₀ has also been shown to function in its reduced form (*i.e.*, ubiquinol) as an antioxidant in both mitochondria and lipid membranes (Forsmark-Andree *et al.*, 1997; Noack *et al.*, 1994). In addition, ubiquinol, like coenzyme Q₁₀, has been shown to be an integral part of virtually all living cells (Frei *et al.*, 1990).

Although coenzyme Q₁₀ becomes oxidized as a result of its antioxidant function, a substantial amount is maintained in its reduced state in the plasma membrane and endomembranes (Takahashi *et al.*, 1993), as well as in lipoproteins (Stocker and Frei, 1991). In the plasma membrane, reduction of coenzyme Q₁₀ is achieved through the involvement of several CoQ-reductases (*e.g.*, DT-diaphorase and NADPH-CoQ reductase) that may be either integral membrane proteins or cytosolic enzymes (Arroyo *et al.*, 2000). Stocker and Suarna (1993) also reported that natural ubiquinones are readily reduced after dietary uptake. While it is generally accepted that oxidized coenzyme Q₁₀ is the final product of its biosynthetic pathway, some authors (Stocker and Suarna, 1993; Schultz *et al.*, 1996) have proposed that the *de novo* synthesis of the hydroquinone also contributes, at least in part, to the high levels of ubiquinol observed *in vivo*. In fact, ubiquinol is the most common form of coenzyme Q₁₀ *in vivo* (Frei *et al.*, 1990), and represents more than 80% of the total ubiquinol-10 + coenzyme Q₁₀ pool in human plasma, intestine and liver (Edlund, 1988; Okamoto *et al.*, 1989; Åberg *et al.*, 1992). In the plasma of healthy adults, ubiquinol-10 accounts for approximately 95% of the total concentration, while ubiquinone-10 accounts for only 5% (Yamashita and Yamamoto, 1997); in human urine, ubiquinol-10 accounts for approximately 59% of the total ubiquinone-10 concentration (Okamoto *et al.*, 1989). Åberg *et al.* (1992) reported that high levels of reduction (70 to 100%) were also observed in human tissues including, the liver, pancreas, and intestine. Only in the brain and lung was most of the ubiquinone (approximately 80%) in the oxidized state. In contrast, the degree of ubiquinone reduction in all rat tissues was less than in corresponding human tissues.

Several authors have examined plasma concentrations of ubiquinol in human volunteers. For example, Kontush *et al.* (1997) reported that concentrations of ubiquinol-10 in plasma of young and aged controls were 0.66 and 0.77 μM , respectively, (0.57 $\mu\text{g/mL}$ and 0.67 $\mu\text{g/mL}$, respectively) while the percentage of total ubiquinol-10 + ubiquinone-10 was 85.7 and 83.1% in young and aged controls, respectively. Similarly, Miles *et al.* (2003) reported the plasma concentration of ubiquinol-10 in healthy adults was 1.07 $\mu\text{mol/L}$ (0.93 $\mu\text{g/mL}$), and Kaikkonen *et al.* (1999) reported values ranging from 0.5 to 2.0 $\mu\text{mol/L}$ (0.43 to 1.73 $\mu\text{g/mL}$). Since ubiquinol-10 can be rapidly recycled from its oxidized form by various electron transfer systems (Crane *et al.*, 1993), Stocker and Suarna (1993) suggested that the reduction of ubiquinone-10 to ubiquinol-10 by the liver plays an important role in maintaining its level in plasma.

Dietary supplementation with coenzyme Q₁₀ (100 to 300 mg/day) has been shown to increase concentrations of ubiquinol-10 in plasma and all of its lipoproteins (Mohr *et al.*, 1992). For example, in low-density lipoproteins (LDL), maximal supplementation is achieved following 4 to 5 days continuous coenzyme Q₁₀ supplementation, and at this time a 4 to 5-fold increase is also observed in LDL's ubiquinol-10 concentration [from 0.5 to 0.8, to 2.0 to 3.0 ubiquinol-10 molecules per LDL] (Thomas *et al.*, 1996; Mohr *et al.*, 1992). It is noteworthy that coenzyme Q₁₀ supplementation does not alter the ratio of ubiquinol-10 to ubiquinone-10 in LDL or plasma; the ratio remains constant with as much as 95% of the total coenzyme Q present as ubiquinol-10 (Mohr *et al.*, 1992). As such, Thomas *et al.* (1999) suggested that this finding was an indication that sufficient reducing potential is available to keep circulating coenzyme Q in the reduced form. Similarly, Takahashi *et al.* (1993) reported that a high ratio of ubiquinol to total ubiquinone (approximately 85%) was maintained even when serum concentrations of total ubiquinone were enhanced through oral supplementation with the oxidized form of ubiquinone. The authors suggested that this finding was an indication that the oxidized form of ubiquinone, when taken orally, is reduced to ubiquinol at the expense of reducing equivalents including, ND(P)H.

Several authors (Kontush *et al.*, 1997; Yamamoto and Yamashita, 1997, 1999; Lagendijk *et al.*, 1997; Wittenstein *et al.*, 2002) examined plasma ubiquinol and ubiquinone concentrations in patients with various pathological conditions (*e.g.*, hyperlipidemia, hepatitis, cirrhosis, hepatoma, coronary artery disease [CAD], diabetes mellitus), and reported that the ratio of ubiquinol to ubiquinone was decreased in these patients compared to healthy subjects. For example, Lagendijk *et al.* (1997) presented the following comparison of coenzyme Q₁₀ parameters between patients with CAD (n=40; mean age = 52.6) and controls (n=40; mean age = 52.6):

Parameter	CAD Patients (Mean)	Controls (Mean)
Ubiquinol (nmol/L)	1137.0	1145.1
Ubiquinone (nmol/L)	46.4	40.3
Ubiquinol/ubiquinone ratio	26.5	30.2

4.3.1.1.2 Occurrence in Animals

Aside from those investigations conducted in human volunteers, several authors have examined the concentration of reduced coenzymes Q, as well as the ratio of reduced forms to oxidized forms, in rodents. In mice, tissues involved in detoxification, such as the liver and kidney were shown to have high levels of reduced forms, which may, as Podda *et al.* (1996) and Tang *et al.* (2004) suggested, protect them from radicals escaping the P450 enzyme system. For example, according to Podda *et al.* (1996), concentrations of ubiquinol-9 and ubiquinol-10 in the liver and kidney were respectively, 42 and 81 nmol/g tissue, and 1.7 and 11 nmol/g tissue, compared to ubiquinol-9 and ubiquinol-10 concentrations of 1.6 and 0.6 nmol/g tissue in the brain, and 19 and 2.8 nmol/g tissue in the heart.

Tang *et al.* (2004) reported that mouse heart [mean (n=10): 897.5 nmol/g protein] and liver [mean (n=10): 281.3 nmol/g protein] contained appreciable concentrations of ubiquinol-9 compared to skeletal muscle [mean (n=10): 154 nmol/g protein] and brain [mean (n=10): 92.2 nmol/g protein]. For ubiquinol-10, mouse heart [mean (n=10): 83.8 nmol/g protein] reportedly contained a relatively appreciable amount, compared to the liver [mean (n=10): 4.6 nmol/g protein], skeletal muscle [mean (n=10): 6.5 nmol/g protein] and brain [mean (n=10): 26.7 nmol/g protein]. In addition, the authors reported that percentages of ubiquinol-9 in total CoQ₉ were 85.5% in the liver, 60% in the heart, 58.7% in the muscle, and 31.2% in the brain; percentages of ubiquinol-10 in total CoQ₁₀ were 88.6% in the liver, 60.9% in the heart, 57.9% in the muscle, and 35.3% in the brain. Tang *et al.* (2004) noted that according to Podda *et al.* (1996) the percentages of ubiquinol-9 in brain, heart and liver were approximately 14%, 7%, and 48%, respectively, while the percentages of ubiquinol-10 were 15% in brain and 12% in heart (due to the sensitivity of the ultraviolet (UV) detector, Podda *et al.* (1996) did not measure ubiquinone-10 in liver). Recognizing the percentages of reduced forms that they observed in mouse heart and liver were greater than those observed by Podda *et al.* (1996), Tang *et al.* (2004) suggested that such differences were the result of variations in sampling, extraction and analytical methods.

As mentioned previously, differences in the ratio of the reduced form to oxidized form have been reported in patients with various pathological conditions compared to healthy subjects. Similarly, variations in the ratio of the reduced form to oxidized form have also been shown to exist among different species. For example, in general, the reduced fraction (ubiquinol) has been reported to be higher in human than rat (Table 1).

Table 1 Coenzyme Q Concentration, Type and Extent of Reduction in Human and Rat Tissues

Tissue	Rat			Human		
	CoQ ₉	CoQ ₁₀	% Reduced	CoQ ₉	CoQ ₁₀	% Reduced
Heart	202	17	22	3	114	47
Kidney	124	22	42	3	67	73
Liver	131	21	87	2	55	95
Muscle	43	3	40	1	40	60
Brain	37	19	27	1	13	23
Pancreas	37	3	62	2	33	100
Spleen	23	9	18	1	25	87
Lung	17	2	12	1	8	24
Thyroid	44	7	45	1	25	68
Testis	32	5	49	1	11	78
Intestine	51	19	67	1	12	93
Colon	48	8	52	1	11	83
Ventricle	56	5	52	---	12	59

The values are presented in µg/g tissue. Data is taken from Åberg *et al.* (1992) and Runquist *et al.* (1995).

Takahashi *et al.* (1993) examined the concentrations of oxidized and reduced forms of ubiquinone homologues in rat tissues and subcellular fractions to clarify their distribution and physiological role. Concentrations (n=4 to 8) in tissues are shown in Table 2; the values are means, µg/g wet tissues or mL plasma.

Table 2 Concentrations of Oxidized and Reduced Forms of Ubiquinone in Rat Tissues and Subcellular Fractions

Tissue	Ubiquinone (UQ) Homologues Detected	t-UQ ^a Content	UQ _{red} Content	UQ _{red} (% of t-UQ) ^b
Plasma	UQ-9	0.48	0.39	82.1
	UQ-10	0.12	0.09	75.9
Liver	UQ-9	105.1	78	74.5
	UQ-10	18.8	12.7	67.4
Heart	UQ-9	188.4	17.4	9.3
	UQ-10	21.6	1.88	8.8
Kidney	UQ-9	122.6	19.9	16.2
	UQ-10	31.8	4.89	15.3
Spleen	UQ-9	75.9	22.4	29.8
	UQ-10	39.6	9.77	24.7

^a The sum of oxidized form of UQ (UQ_{ox}) and reduced form of UQ (ubiquinol) (UQ_{red}) of each of the homologues.

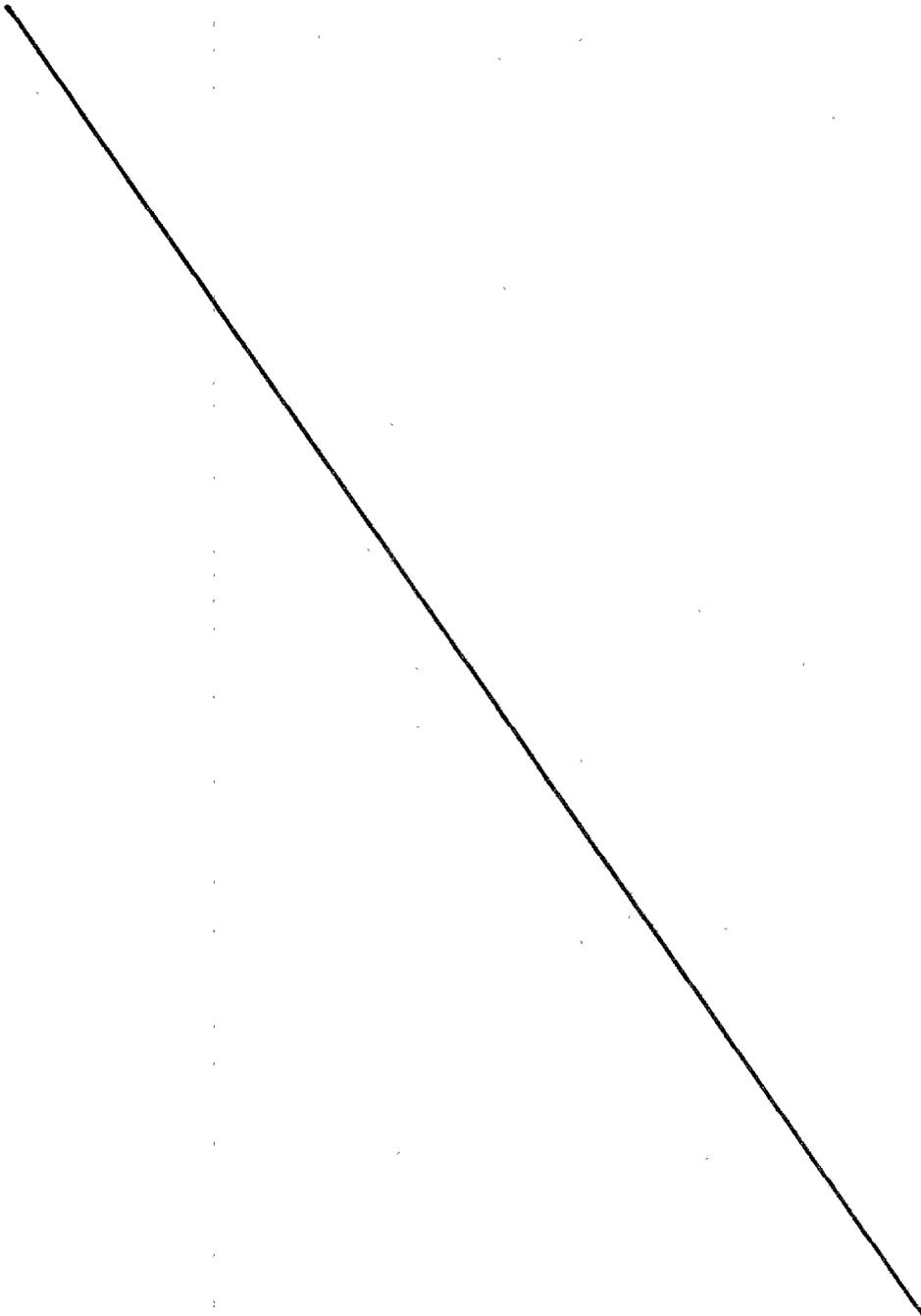
^b $\{[UQ_{red}]/t-UQ\} \times 100$

Similarly, UQ-9 and UQ-10 were detected in all blood cells isolated (*i.e.*, erythrocytes, ghost cells, endosomes, leukocytes, and platelets). Leukocytes and platelets, which have mitochondria, possessed higher concentrations of t-UQ-9 (total CoQ₉) and t-UQ-10 (total CoQ₁₀) than did erythrocytes, which do not have mitochondria. The UQ_{red} forms were below 10% of the t-UQ in erythrocytes and leukocytes, and they were not detected in platelets. With respect to subcellular distribution of UQ_{ox} and UQ_{red} homologues in rat liver and kidney, all fractions tested (*i.e.*, nuclei, mitochondria, crude lysosomes, crude microsomes, cytosol, plasma membranes) contained significant amounts of UQ-9 and UQ-10. The levels of the UQ_{red} forms reached 60-

70% of those of the t-UQ homologs in a majority of subcellular fractions of the liver, and accounted for approximately 25% of those in kidney.

Based on these findings, Takahashi *et al.* (1993) concluded that all rat tissues and subcellular fractions isolated from the liver and kidney contain significant amount of ubiquinone homologues. In addition, the authors noted that 70 to 80% of the total amounts of each UQ_{ox} and UQ_{red} homologue in the liver and plasma, as well as 20 to 30% of those in other tissues, exist as the reduced hydroquinone form.

4.3.1.1.3 Occurrence in Foods



4.3.1.2 Physiological Functions

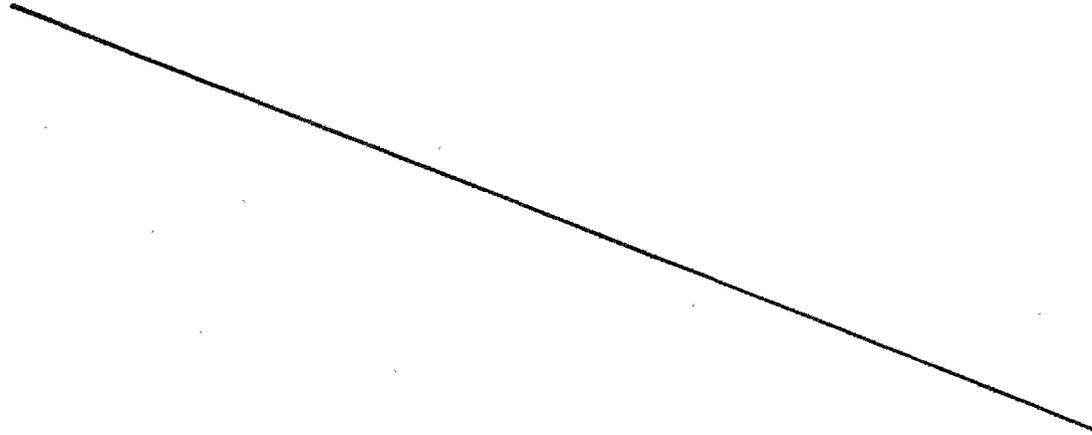
Ubiquinol-10, the reduced and most common form of coenzyme Q₁₀ *in vivo*, has been shown to be a potent lipophilic antioxidant for protection of lipids in a number of biological and model systems (Frei *et al.*, 1990; Ernster and Forsmark-Andree, 1993). Furthermore, according to Ernster and Dallner (1995), reduced coenzymes Q is the only known lipid-soluble antioxidant that animals can synthesize *de novo*, and for which there exist mechanisms that can regenerate it from its oxidized product formed as a result of its antioxidant activity.

4.3.2 Bioavailability

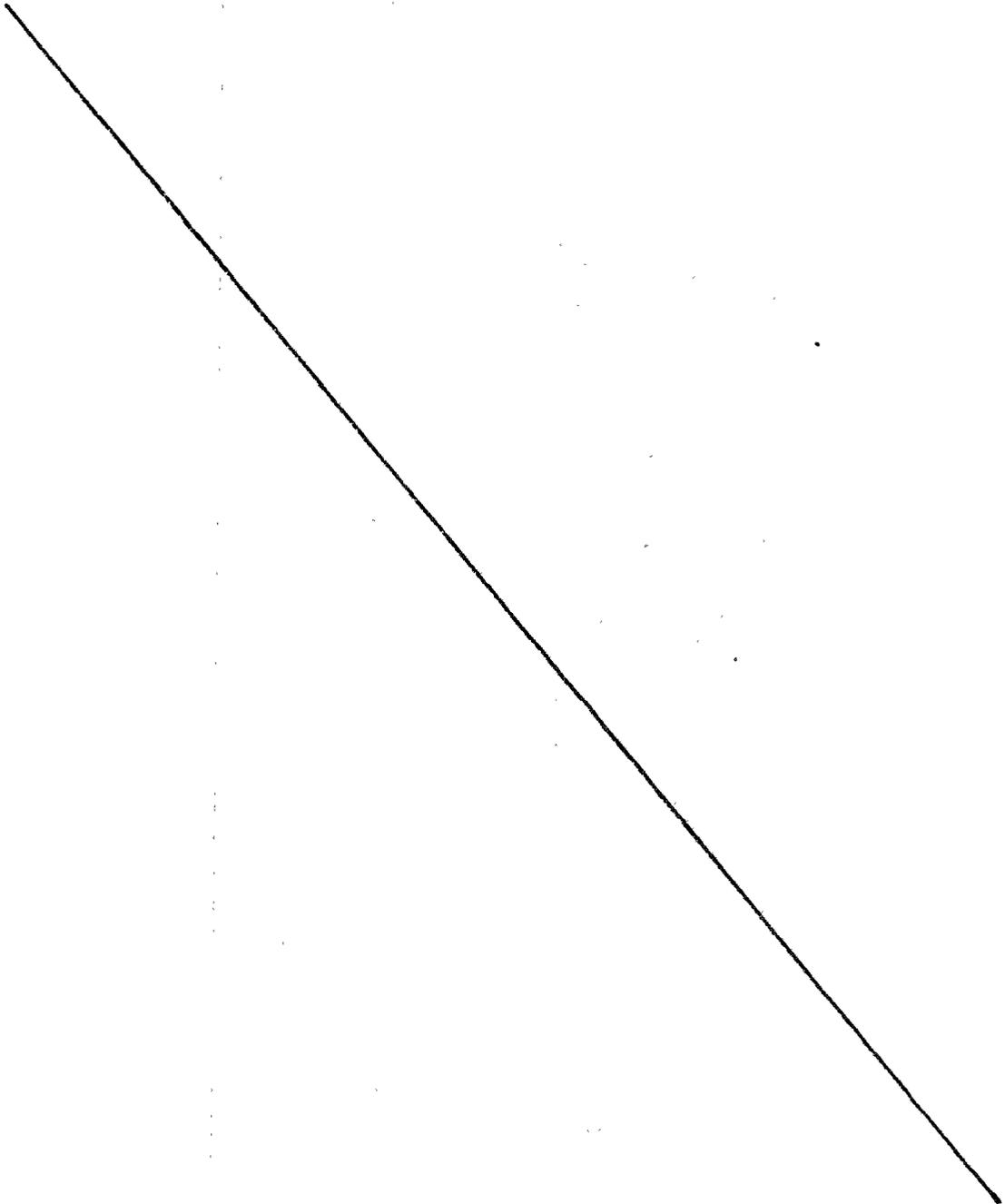
4.3.2.1 Bioavailability of Ubiquinol Compared to Ubiquinone



Figure 3 Comparison of Ubiquinol and Ubiquinone Bioavailability



4.3.2.2 Toxicokinetic Studies Conducted by Kaneka



PAGES 18 THROUGH 21

REDACTED IN ITS
ENTIRETY
CONTAINS
TRADE SECRET
CONFIDENTIAL
COMMERICAL
INFORMATION

4.3.2.3 Published Bioavailability Data in Humans

Mohr *et al.* (1992) examined the effects of coenzyme Q₁₀ supplementation on plasma and lipoprotein ubiquinol-10 concentrations. Within 6 hours of consumption of a single oral dose of 100 or 200 mg coenzyme Q₁₀, total plasma coenzyme Q₁₀ content was increased by 80% or 150%, respectively, in a single subject. Long-term supplementation, consisting of oral doses of 100 mg coenzyme Q₁₀ three times daily for 11 days resulted in a 4-fold increase of ubiquinol (CoQH₂) in the plasma and LDL of 3 normolipidemic male subjects. The proportion of coenzyme Q₁₀ in the reduced state [percent CoQH₂ = 100 CoQH₂ / (coenzyme Q₁₀ + CoQH₂)] was reportedly unchanged by treatment and remained constant throughout the monitored period, with approximately 80% of the coenzyme present as CoQH₂. The authors suggested that this finding indicated that efficient reduction of coenzyme Q₁₀ to CoQH₂ must occur either during absorption or rapidly after the appearance of coenzyme Q₁₀ in the blood (Mohr *et al.*, 1992).

In light of the approximate 4-fold increase in CoQH₂ and the potential role of CoQH₂ as an LDL antioxidant, Mohr *et al.* (1992) also examined whether *in vivo* supplemented LDL was correspondingly less susceptible to radical oxidation. The authors reported that incubation of LDL (isolated from a single subject before and after long-term supplementation) with 2,2'-azobis(2-amidinopropane hydrochloride) [AAPH, 20 or 40 μL of 100 mM in 0.156 M NaCl] resulted in immediate formation of lipid hydroperoxides; formation initially occurred at very low rates, however, a marked increase in the rate of lipid oxidation was noted with the disappearance of 80 to 90% CoQH₂. Results also demonstrated that the cumulative radical dose required to reach a "break point" in lipid hydroperoxide formation (*i.e.*, on a radical/LDL particle basis, ROO[•]/LDL ≈ 3 for non-supplemented versus ≈ 11 for the supplemented LDL) was nearly proportional to the 4-fold increase observed in LDL-[CoQH₂]. On this basis, Mohr *et al.* (1992) concluded that oral supplementation with coenzyme Q₁₀ increases CoQH₂ in the plasma and all lipoproteins, thereby increasing the resistance of LDL to radical oxidation.

4.4 SAFETY OF UBIQUINOL

KANEKA QH™ was used as the test material in all ubiquinol safety studies in Section 4.4.1 (4.4.1.1, 4.4.1.2, 4.4.1.3). As previously discussed (see Section 4.3.1.1.1), pharmacokinetic studies in humans indicate that, following oral supplementation with the oxidized form of Coenzyme Q10 (ubiquinone), this compound is reduced almost completely to ubiquinol *in vivo*. Therefore, information in the published scientific literature related to non-clinical toxicity, genotoxicity, and clinical safety of coenzyme Q₁₀ are also included in this notification to support the safety of ubiquinol. The following table provides an overview of the test materials used in the safety studies described herein.

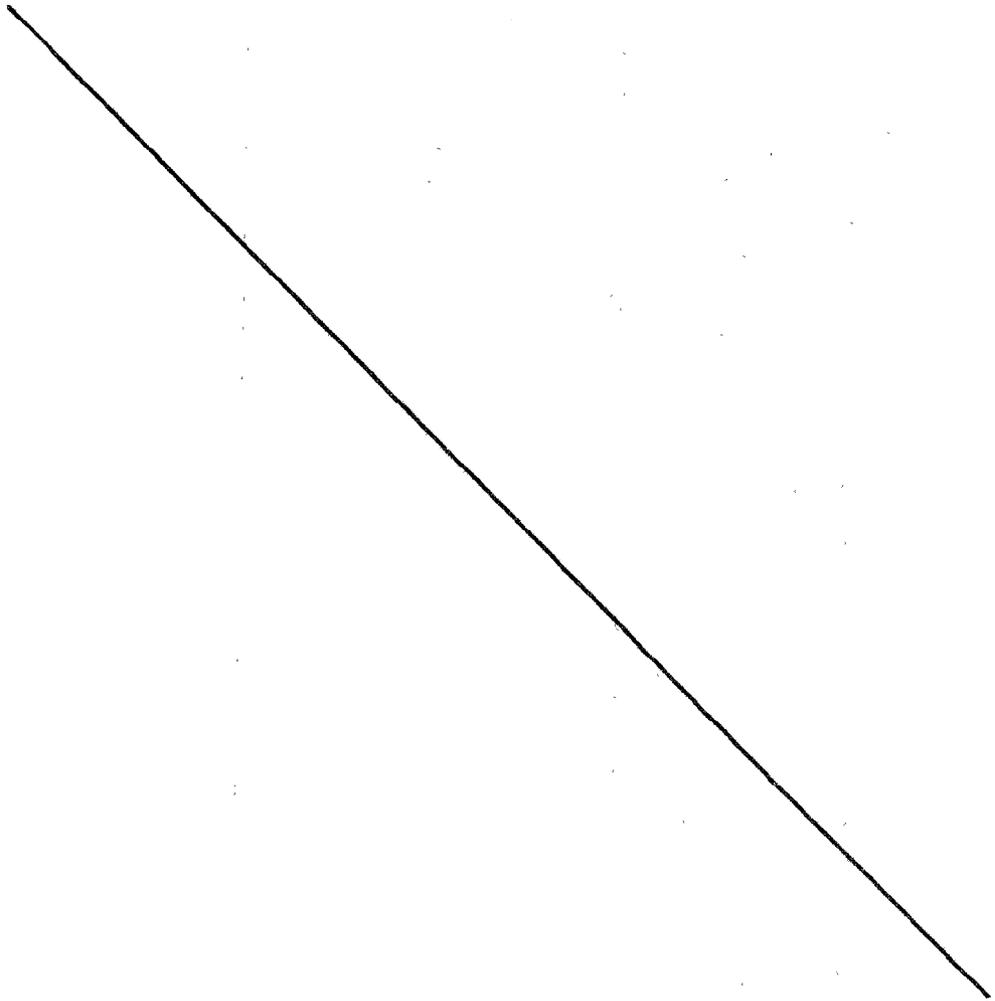
Test	Test Substance	Notes
4.4.1.1 Acute Toxicity 4.4.1.2 Subchronic Toxicity 4.4.1.3 Genotoxicity	Kaneka QH™	The sample name, method, and the result are described as ubiquinol
4.4.2 Non-clinical & Genotoxicity	Degraded Kaneka QH™*	-
4.4.3 Clinical Safety	Coenzyme Q10	Published scientific literature
4.4.4 Supporting Safety studies	Coenzyme Q10	Published scientific literature

4.4.1 Non-clinical and Genotoxicity Studies Conducted with KANEKA QH™

4.4.1.1 Acute Toxicity

4.4.1.2 Subchronic Toxicity

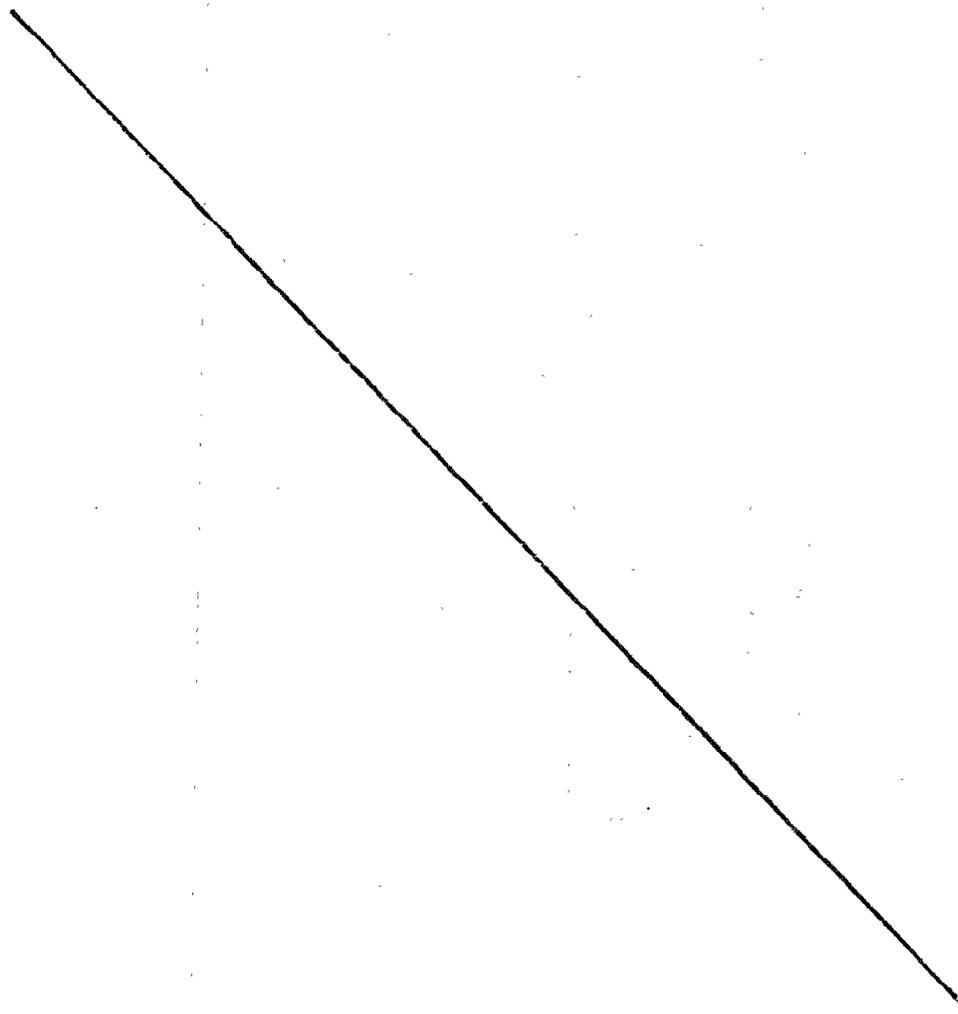
4.4.1.2.1 13-Week Study in Male and Female Dogs



PAGES 25 THROUGH 31

REDACTED IN ITS
ENTIRETY
CONTAINS
TRADE SECRET
CONFIDENTIAL
COMMERICAL
INFORMATION

4.4.1.2.2 *13-Week Study in Male and Female Rats*



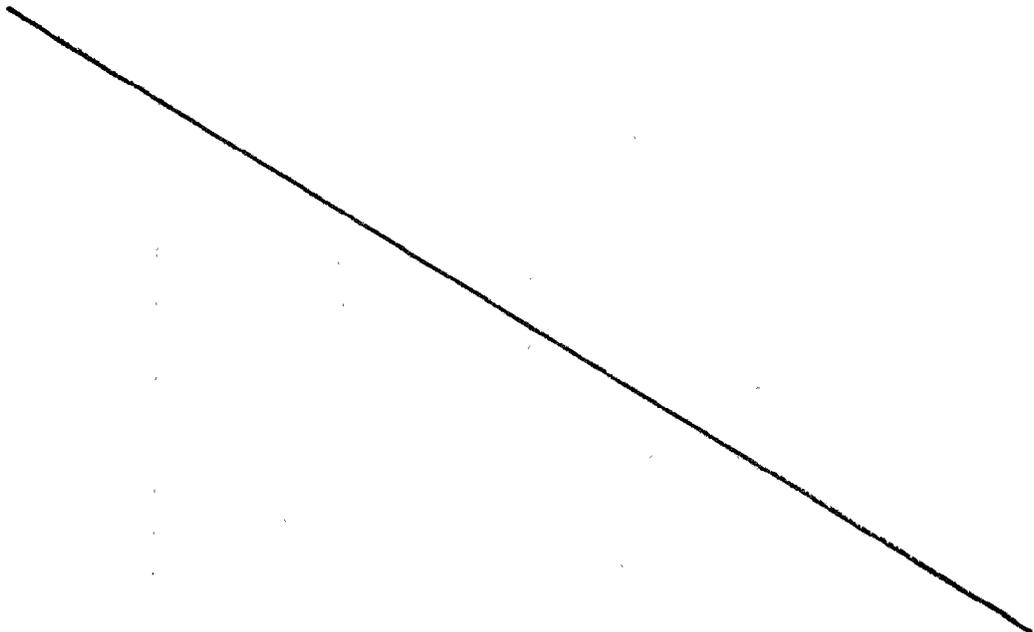
PAGES 33 THROUGH 41

REDACTED IN ITS
ENTIRETY
CONTAINS
TRADE SECRET
CONFIDENTIAL
COMMERICAL
INFORMATION

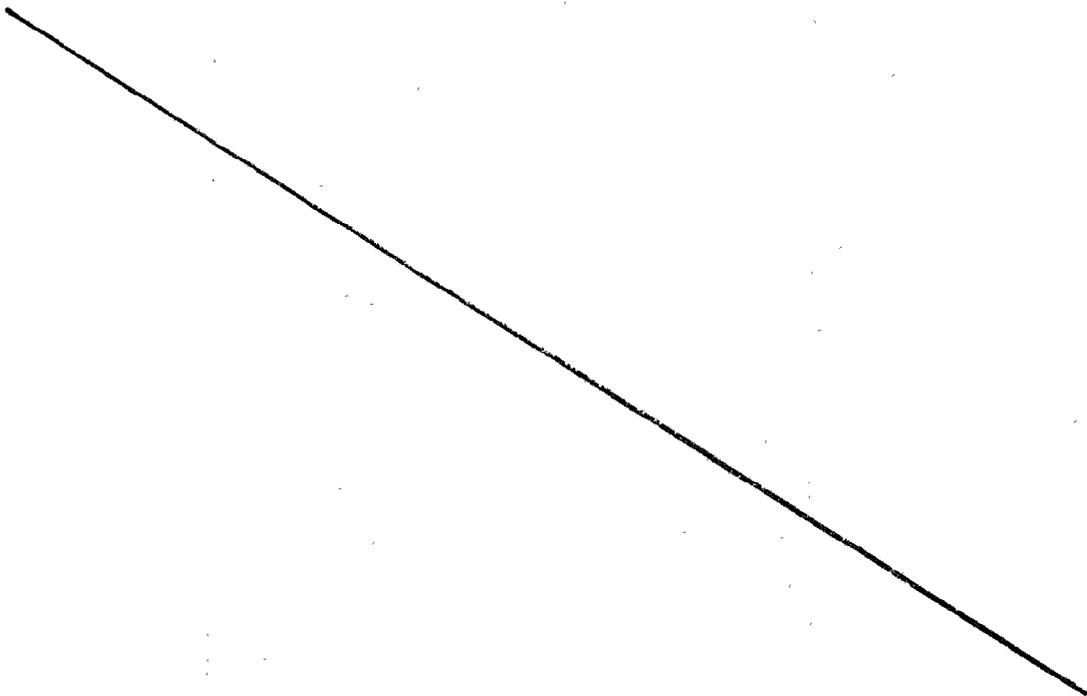
4.4.1.2.3 *Follow up 13-Week Oral Toxicity Study with Ubiquinol in Female Rats*

PAGES 43 THROUGH 48

REDACTED IN ITS
ENTIRETY
CONTAINS
TRADE SECRET
CONFIDENTIAL
COMMERCIAL
INFORMATION



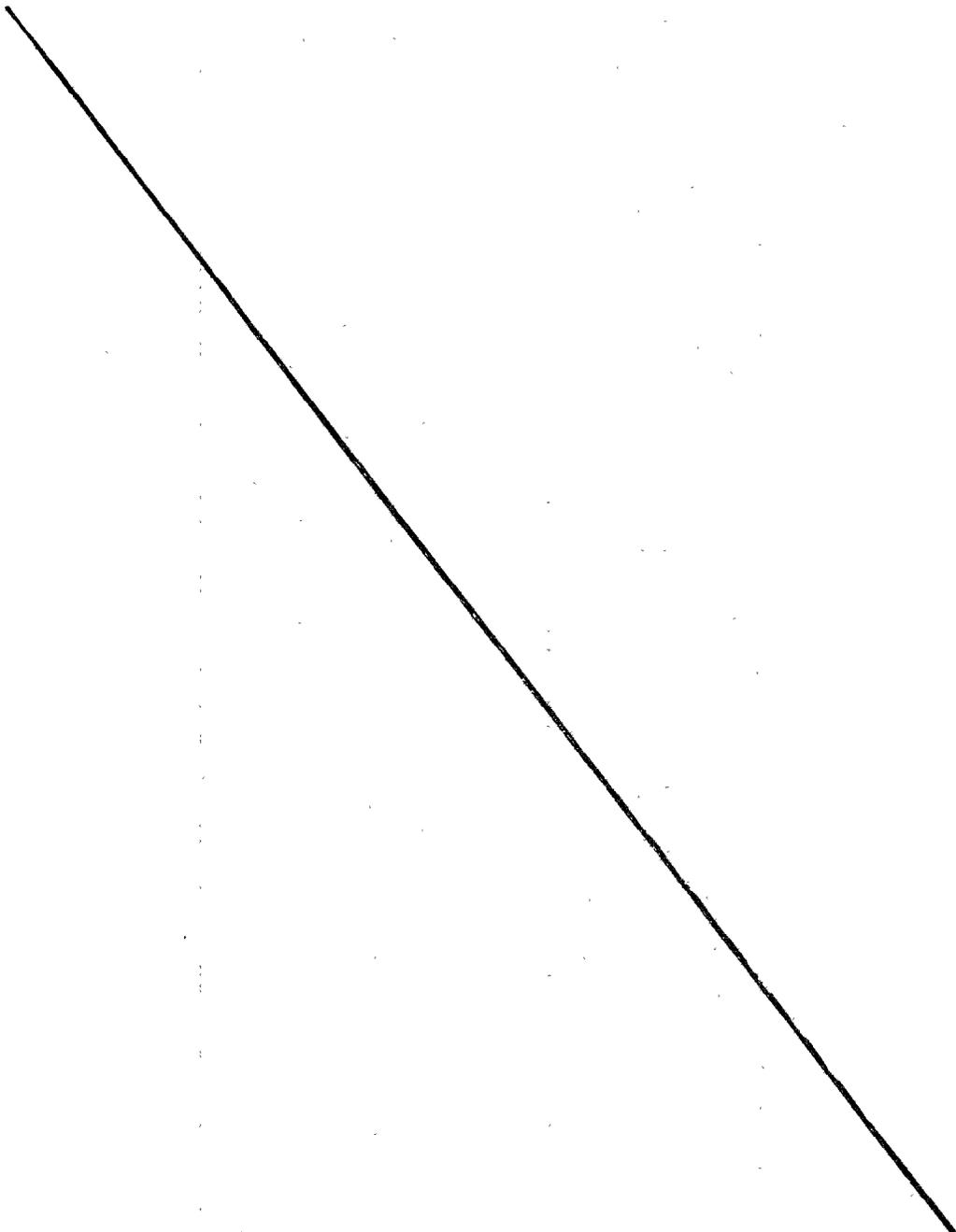
4.4.1.3 Genotoxicity



PAGES 50 THROUGH 59

REDACTED IN ITS
ENTIRETY
CONTAINS
TRADE SECRET
CONFIDENTIAL
COMMERICAL
INFORMATION

**4.4.2 Non-clinical and Genotoxicity Studies Conducted with Degraded
KANEKA QH™**



PAGES 61 THROUGH 66

REDACTED IN ITS
ENTIRETY
CONTAINS
TRADE SECRET
CONFIDENTIAL
COMMERICAL
INFORMATION

4.4.3 Clinical Safety

Pharmacokinetic studies in humans indicate that, following oral supplementation with the oxidized form of Coenzyme Q₁₀ (ubiquinone), this compound is reduced almost completely to ubiquinol *in vivo*. On this basis, clinical studies examining the safety of coenzyme Q₁₀ were considered supportive of KANEKA QH™ (ubiquinol) safety. A brief summary of the available safety data for coenzyme Q₁₀ is provided below.

Weber *et al.* (1994) investigated the effect of coenzyme Q₁₀ supplementation on antioxidative status by exposing healthy subjects (n=22) to coenzyme Q₁₀ supplementation before and after induction of oxidative stress by fish oil supplementation, and subsequently monitoring markers of plasma oxidative status [levels of antioxidants (α -tocopherol, ascorbic acid), lipid peroxidation products (thiobarbituric acid reacting substances; TBARS), and the total amount and redox status of coenzyme Q₁₀]. Subjects consumed 90 mg/day of coenzyme Q₁₀ for 6 weeks; during weeks 3 and 4, α -tocopherol (10 mg/day) was added to the treatment regimen, while during weeks 5 and 6, subjects also consumed 3 g/day fish oil (in capsules furnishing 1.1 g/day EPA, 0.8 g/day DHA, and 10 mg/day vitamin E) to increase oxidative stress. Results pertaining to the effect of coenzyme Q₁₀ supplementation on antioxidant plasma levels and TBARS are not discussed herein since they were considered outside the scope of the current document; results pertaining to the effect of coenzyme Q₁₀ supplementation on the total amount and redox status of coenzyme Q₁₀ are summarized below.

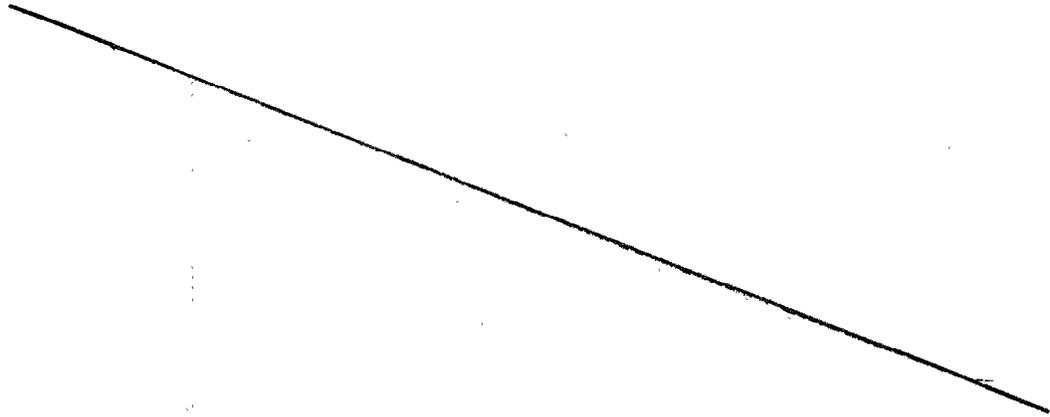
The level of total coenzyme Q₁₀ increased significantly after coenzyme Q₁₀ supplementation and remained constant throughout the supplementation period. In addition, the redox status of coenzyme Q₁₀ (reduced coenzyme Q₁₀/total coenzyme Q₁₀) was unchanged by coenzyme Q₁₀ supplementation. The authors suggested that this finding indicated that exogenously supplied coenzyme Q₁₀ is reduced when appearing in plasma. Furthermore, Weber *et al.* (1994) suggested that the constant redox status implies the existence of a reducing capacity for coenzyme Q₁₀ large enough to maintain the majority of plasma coenzyme Q₁₀ in the reduced form, even when the total coenzyme Q₁₀ level increased 2-fold. With respect to the effect of increased oxidative stress, the redox level of coenzyme Q₁₀ was reportedly unaffected by the addition of fish oil to the treatment regimen (Weber *et al.*, 1994).

4.4.4 Supporting Safety Studies Conducted With Coenzyme Q₁₀

As noted previously, pharmacokinetic studies in humans indicate that, following oral supplementation with the oxidized form of Coenzyme Q₁₀ (ubiquinone), this compound is reduced almost completely to the reduced form, ubiquinol *in vivo*. Mohr *et al.* (1992) and Weber *et al.* (1994) reported that following dietary supplementation with coenzyme Q₁₀, efficient reduction of coenzyme Q₁₀ to ubiquinol (CoQH₂) occurs either during absorption or rapidly after the appearance of coenzyme Q₁₀ in the blood. Furthermore, ubiquinol-10 has been identified as

the most common form of coenzyme Q₁₀ *in vivo* (Frei *et al.*, 1990), representing more than 80% of the total ubiquinol-10 + coenzyme Q₁₀ pool in human plasma, intestine and liver (Edlund, 1988; Okamoto *et al.*, 1989; Åberg *et al.*, 1992). On this basis, studies examining the safety of coenzyme Q₁₀ were considered supportive of ubiquinol safety, and a brief summary of the available safety data for coenzyme Q₁₀ is provided below.

4.4.4.1 Non-Clinical Safety of Coenzyme Q₁₀



4.4.4.2 Clinical Safety of Coenzyme Q₁₀

Coenzyme Q₁₀ deficiency has been reported in patients with various cardiovascular diseases (*e.g.*, congestive heart failure, angina pectoris, coronary artery disease, cardiomyopathy, hypertension, mitral valve prolapse), therefore, much of the available clinical data for coenzyme Q₁₀ was obtained from studies examining its possible therapeutic effect in these conditions. Although in most instances the safety of coenzyme Q₁₀ supplementation was not the focus of clinical trials, the absence of major adverse effects in subjects, as reported by several authors (Shults *et al.*, 2002, The Huntington Study Group, 2001, Baggio *et al.*, 1994; Hofman-Bang *et al.*, 1995; Langsjoen *et al.*, 1990), does offer support for safety. Adverse effects associated with coenzyme Q₁₀ therapy were reportedly rare and included nausea (0.16%), decreased appetite (0.23%), epigastric discomfort (0.39%), vomiting (rare), and diarrhea (0.12%). Increased lactic dehydrogenase and serum glutamic oxalotransferase levels have also been observed in rare instances, at coenzyme Q₁₀ doses above 300 mg/day; however, serious hepatotoxicity has not been reported (Singh *et al.*, 1998; Tran *et al.*, 2001). The use of coenzyme Q₁₀ is not recommended in patients with renal insufficiency, or during pregnancy and lactation (Micromedex, undated; Tran *et al.*, 2001).

4.4.5 Proposed Daily Intake

As mentioned in Section 3 of the current document, label directions of the dietary supplement containing KANEKA QH™ will suggest or recommend consumption of up to 6 servings per day, resulting in a maximum daily consumption of up to 300 mg KANEKA QH™ (equivalent to 6 mg/kg/day for a 50 kg body weight person).

Shapiro *et al.* (1995) and Mugford and Kedderis (1998) suggested that exaggerated gender differences in rat drug metabolism might hinder extrapolation to other species, including humans, in which gender-related differences are generally subtler.

Aside from gender-dependent differences noted in female rats, the selection of an appropriate experimental animal model was influenced by reported species-dependent differences. Specifically, as mentioned in Section 4.3.1.1.1, coenzyme Q homologs consist of a redox active quinoid moiety, and a hydrophobic side chain comprised of 6 to 10 isoprenoid units, depending on the species (Ibrahim *et al.*, 2000; Matthews *et al.*, 1998; Lenaz, 2001). In humans and most mammals, including dogs, the predominant form of coenzyme Q is coenzyme Q₁₀ (CoQ₁₀), which consists of 10 isoprenoid units in the side chain (Ramasarma, 1985). In rats and mice the primary form is coenzyme Q₉, which contains 9 isoprenoid units (Battino *et al.*, 1992).

SUMMARY

The information presented herein shows that:

- (i) The chemical composition of KANEKA QH™ is well characterized, and the manufacturing process yields a product demonstrated to reproducibly meet compositional specifications.
- (ii)
- (iii)
- (iv) Numerous studies in the scientific literature have demonstrated the safety of coenzyme Q₁₀. Given that efficient reduction of coenzyme Q₁₀ to ubiquinol (CoQH₂) occurs either during absorption or rapidly after the appearance of coenzyme Q₁₀ in the blood, studies conducted with coenzyme Q₁₀ were considered supportive of the safety of KANEKA QH™.
- (v) Small quantities of KANEKA QH™ will be consumed as dietary supplements.
- (vi) Ubiquinol is a vital nutritive substance naturally present in human tissues and a common component of the human diet.

CONCLUSION

Based on the evidence above, including results of chronic safety studies, the absence of mutagenic and reproductive activity, the presence of a safety factor in excess of 100-fold for human exposure compared to the lowest effect levels in safety studies, and substantial clinical experience indicating ample tolerance as well as potential benefit, Kaneka concludes that the chronic use of KANEKA QH™ in dietary supplements at a level of up to 300 mg KANEKA QH™ (equivalent to 6 mg/kg/day for a 50 kg body weight person), will be reasonably expected to be safe.

REFERENCES

- Åberg, F.; Appelkvist, E.L.; Dallner, G.; Ernster, L. 1992. Distribution and redox state of ubiquinones in rat and human tissues. *Arch Biochem Biophys* 295:230-234. Cited In: Podda *et al.*, 1996.
- Arroyo, A.; Kagan, V.E.; Tyurin, V.A.; Burgess, J.R.; de Cabo, R.; Navas, P. 2000. NADH and NADPH-dependent reduction of coenzyme Q at the plasma membrane. *Antioxidants & Redox Signaling* 2(2):251-262.
- Baggio, E.; Gandini, R.; Plancher, A.C.; Passeri, M.; Carmosino, G. 1994. Italian multicenter study on the safety and efficacy of coenzyme Q10 as adjunctive therapy in heart failure. *Molec. Aspects Med.* 15(Supplement): S287-S294.
- Battino, M.; Ferri, E.; Gorini, A.; Villa, R.F.; Huertas, J.F.R.; Fiorella, P.; Genova, M.L.; Lenaz, G.; Marchetti, M. 1992. Natural distribution and occurrence of coenzyme Q homologues. *Membrane Biochemistry* 9:179-190.
- Bertelli, A.; and Ronca, G. 1990. Carnitine and coenzyme Q10: biochemical properties and functions, synergism and complementary action. *Int J Tissue React* 12(3):183-186.
- Budavari, S.; O'Neil, M.J.; Smith, A.; Heckelman, P.E.; Kinneary, J.F. (Eds.). 1996. *The Merck Index. An Encyclopedia of Chemicals, Drugs and Biologicals.* Twelfth Edition. Merck & Co., Inc., New Jersey, p. 1679.
- Chiba, T.; Watanabe, T.; Kume, Y.; Sugiyama, K.; Shiojiri, H. 1972a. Toxicological studies of ubiquinone-10 (I) Acute toxicity test in rats and mice and subacute and chronic toxicity tests in rats. *Oyo Yakuri* 6:769-779. Abstract in English. Also cited in Folkers and Morishita, 1987.
- Chiba, T.; Sugiyama, K.; Kume, Y.; Shiojiri, H.; Watanabe, T.; Ozeki, M. 1972b. Toxicological studies of ubiquinone-10 (II) Subacute toxicity test in rabbits. *Oyo Yakuri* 6:781-786. Abstract in English. Also cited in Folkers and Morishita, 1987.
- Crane, F.L.; Sun, I.L.; Sun, E.E. 1993. The essential functions of coenzyme Q. *Clin Investig* 71:S55-S59. Cited In: Kontush *et al.*, 1999.
- Czerniak, R. 2001. Gender-based differences in pharmacokinetics in laboratory animal models. *Int J Toxicol* 20(3):161-163.
- Edlund, P.O. 1988. Determination of coenzyme Q₁₀, α -tocopherol and cholesterol in biological samples by coupled-column liquid chromatography with coulometric and ultraviolet detection. *J Chromatogr* 425:87. Cited In: Kontush *et al.*, 1997.

Ernster, L.; and Dallner, G. 1995. Biochemical, physiological and medical aspects of ubiquinone function. *Biochimica et Biophysica Acta* 1271:195-204.

Ernster, L.; and Forsmark-Andree, P. 1993. Ubiquinol: An endogenous antioxidant in aerobic organisms. *Clin Invest* 71:S60. Cited In: Kontush *et al.*, 1997.

Folkers, K.; and Morishita, M. 1987. Critique of the metabolism, pharmacokinetics, and toxicology of Coenzyme Q10. *Chemiker-Zeitung* 111 (4):139-143.

Forsmark-Andree, P.; Lee, C.-P.; Dallner, G.; Ernster, L. 1997. Lipid peroxidation and changes in the ubiquinone content and the respiratory chain enzymes of submitochondrial particles. *Free Radic Biol Med* 22:391-400. Cited In: Ibrahim *et al.*, 2000.

Frei B.; Kim, M.C.; Ames, B.N. 1990. Ubiquinol-10 is an effective lipid-soluble antioxidant at physiological concentrations. *Proc Natl Acad Sci* 87:4879-4883.

Hofman-Bang, C.; Rehnquist, N.; Swedberg, K., *et al.* 1995. Coenzyme Q10 as an adjunctive in the treatment of chronic congestive heart failure. *J Cardiac Failure* 1:101-107. Cited In: Tran *et al.*, 2001.

Hughes, K.; Lee, B.L.; Feng, X.; Lee, J.; Ong, C.N. 2002. Coenzyme Q10 and differences in coronary heart disease risk in Asian Indians and Chinese. *Free Radic Biol Med* 32(2):132-138.

Huntington Study Group, The. 2001. A randomized, placebo-controlled trial of coenzyme Q10 and remacemide in Huntington's disease. *Neurology* 57:397-404.

Ibrahim, W.H.; Bhagavan, H.N.; Chopra, R.K.; Chow, C.K. 2000. Dietary coenzyme Q10 and vitamin E alter the status of these compounds in rat tissues and mitochondria. *J Nutr* 130(9):2343-2348.

Kaikkonen, J.; Nyssönen, K.; Salonen, J.T. 1999. Measurement and stability of plasma reduced, oxidized and total coenzyme Q10 in humans. *Scand J Clin Lab Invest* 59:457-466.

Kelso, G.F.; Porteous, C.M.; Hughes, G.; Ledgerwood, E.C.; Gane, A.M.; Smith, R.A.; Murphy, M.P. 2002. Prevention of mitochondrial oxidative damage using targeted antioxidants. *Ann NY Acad Sci* 959(April):263-274.

Kitano, M.; Hosoe, K.; Fukutomi, N.; Hidaka, T.; Imai, N.; Kawabe, M. 2004. 28-Day repeated dose toxicity study of dried microorganism in rats. *Food Chem Toxicol* 42:1817-1824.

Kontush, A.; Schippling, S.; Spranger, T.; Beisiegel, U. 1999. Plasma ubiquinol-10 as a marker for disease: is the assay worthwhile? *BioFactors* 9:225-229.

Kontush, A.; Reich, A.; Baum, K.; Spranger, T.; Finckh, B.; Kohlschütter, A.; Beisiegel, U. 1997. Plasma ubiquinol-10 is decreased in patients with hyperlipidaemia. *Atherosclerosis* 129:119-126.

Legendijk, J.; Ubbink, J.B.; Delport, R.; Vermaak, W.J.H.; Human, J.A. 1997. Ubiquinol/ubiquinone ratio as a marker of oxidative stress in coronary artery disease. *Research Communications in Molecular Pathology and Pharmacology* 95(1):11-20.

Lansjoen, P.H.; Langsjoen, P.H.; Folkers, K. 1990. Long-term efficacy and safety of coenzyme Q10 therapy for idiopathic dilated cardiomyopathy. *Am J Cardiol* 65:521-523.

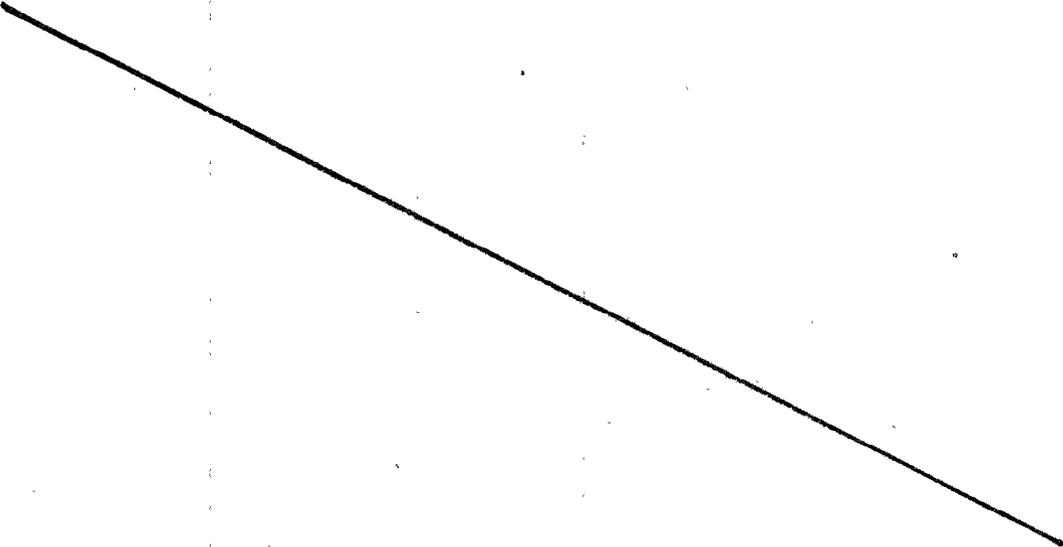
Lass, A.; and Sohal, R.S. 1999. Comparisons of coenzyme Q bound to mitochondrial membrane proteins among different mammalian species. *Free Radic Biol Med* 27(1-2):220-226.

Lenaz, G. 2001. A critical appraisal of the mitochondrial coenzyme Q pool. *FEBS Lett* 509(2):151-155.

Matthews, R.T.; Yang, L.; Browne, S.; Baik, M.; Beal, M.F. 1998. Coenzyme Q10 administration increases brain mitochondrial concentrations and exerts neuroprotective effects. *Proc Natl Acad Sci USA* 95(15):8892-8897.

Micromedex. Healthcare series. Ubidecarenone. Micromedex, Inc., Englewood, CO. Cited In: Tran *et al.*, 2001.

Miles, M.V.; Horn, P.S.; Morrison, J.A.; Tang, P.H.; DeGrauw, T.; Pesce, A.J. 2003. Plasma coenzyme Q₁₀ reference intervals, but not redox status, are affected by gender and race in self-reported healthy adults. *Clinica Chimica Acta* 332:123-132.



Mohr, D.; Bowry, V.W.; Stocker, R. 1992. Dietary supplementation with coenzyme Q10 results in increased levels of ubiquinol-10 within circulating lipoproteins and increased resistance of human low density lipoprotein to the initiation of lipid peroxidation. *Biochim Biophys Acta* 1126:247-254.

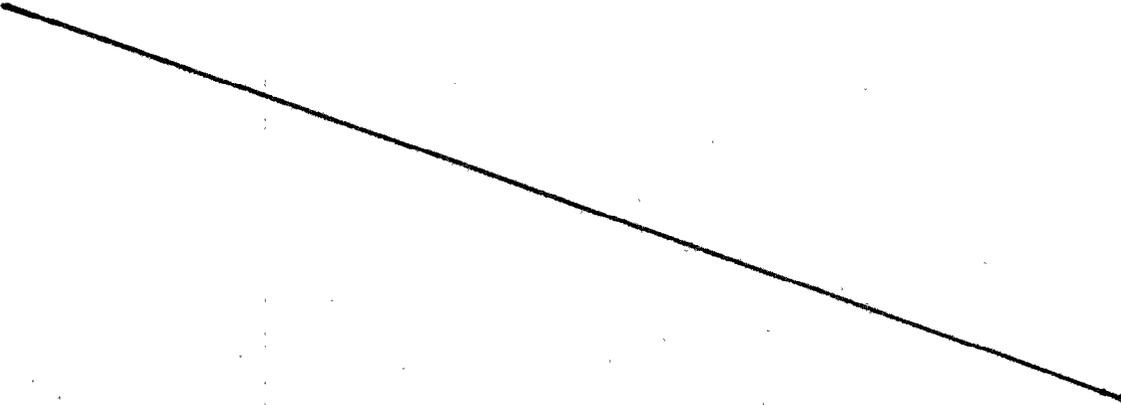
Mugford, C.A.; and Kedderis, G.L. 1998. Sex-dependent metabolism of xenobiotics. *Drug Metab Rev* 30(3):441-498.

Noack, H.; Kube, U.; Augustin, W. 1994. Relations between tocopherol depletion and coenzyme Q during lipid peroxidation in rat liver mitochondria. *Free Radic. Res* 20:375-386. Cited In: Ibrahim *et al.*, 2000.

Nohl, H.; Kozlov, A.V.; Staniek, K.; Gille, L. 2001. The multiple functions of coenzyme Q. *Bioorg Chem* 29(1):1-13.

Nohl, H.; Gille, L.; Staniek, K. 1998. The biochemical, pathophysiological, and medical aspects of ubiquinone function. *Ann NY Acad Sci* 854:394-409.

Notake, Y., Tamura, S., Toyoshima, S., Fujita, H., Suzuki, Y. and Chiba, T. 1972. Effects of coenzyme Q₁₀ on development of the fetuses and neonates in rats and mice. *Iyakuin Kenkyu* 3(3):306-315. Abstract in English. Also cited in Folkers and Morishita, 1987.



Okamoto, T.; Matsuya, T.; Fukunaga, Y.; Kishi, T.; Yamagami, T. 1989. Human serum ubiquinol-10 levels and relationship to serum lipids. *Int J Vit Nutr Res* 59:288.

Pampori, N.A.; and Shapiro, B.H. 1999. Gender differences in the responsiveness of the sex-dependent isoforms of hepatic P450 to the feminine plasma growth hormone profile. *Endocrinology* 140(3):1245-1254.

Pepping, J. 1999. Coenzyme Q10. *Am J Health Syst Pharm* 56(6):519-521.

Podda, M.; Weber, C.; Traber, M.G.; Packer, L. 1996. Simultaneous determination of tissue tocopherols, tocotrienols, ubiquinols, and ubiquinones. *Journal of Lipid Research* 37:893-901.

Ramasarma, T. 1985. Natural occurrence and distribution of coenzyme Q. In: Lenaz G. (Ed.). *Coenzyme Q: Biochemical, bioenergetics and clinical applications of ubiquinone*. John Wiley & Sons, New York, pp.67-81.

Runquist, M.; Parmryd, I.; Thelin, A.; Chojnacki, T.; Dallner, G. 1995. Distribution of branch point prenyltransferases in regions of bovine brain. *J Neurochem* 65:2299-2306. Cited In: Turunen *et al.*, 2004.

Schoepp, G. 1997. Is coenzyme Q10 (ubiquinone) effective for the treatment of heart failure? *Pharmacy Practice (Canada)* 13(March):31-32.

Schultz, J.R.; Ellerby, L.M.; Gralla, E.B.; Valentine, J.S.; Clarke, C.F. 1996. Autoxidation of ubiquinol-6 is independent of superoxide dismutase. *Biochemistry* 35:6595. Cited In: Villalba *et al.*, 2000.

Shapiro, B.H.; Agrawal, A.K.; Pampori, N.A. 1995. Gender differences in drug metabolism regulated by growth hormone. *Int J Biochem Cell Biol* 27(1):9-20.

Shults, C.W.; Oakes, D.; Kiebertz, K.; Beal, M.F.; Hass, R.; Plumb, S.; Juncos, J.L.; Nutt, J.; Shoulson, I.; Carter, J.; Kompoliti, K.; Perlmutter, J.S.; Reich, S.; Stern, M.; Watts, R.L.; Kurlan, R.; Molho, E.; Harrison, M.; Lew, N. 2002. Effects of coenzyme Q10 in early Parkinson disease: evidence of slowing of the functional decline. The Parkinson Study Group. *Arch Neurol* 59(10):1541-1550. Abstract only.

Singh, R.B.; Niaz, M.A.; Rastogi, V.; Rastogi, S.S. 1998. Coenzyme Q10 in cardiovascular disease. *JAPI* 46(3): 299-306.

Stocker, R.; and Frei, B. 1991. Endogenous antioxidant defenses in human blood plasma. In: Sies, H. (Ed.). *Oxidative Stress: Oxidants and Antioxidants*. Academic Press, San Diego, pp. 213. Cited In: Villalba *et al.*, 2000.

Stocker, R.; and Suarna, C. 1993. Extracellular reduction of ubiquinone-1 and -10 by human Hep G2 and blood cells. *Biochim Biophys Acta* 1158:15-22. Cited In: Kontush *et al.*, 1999 and Villalba *et al.*, 2000.

Takahashi, T.; Okamoto, T.; Mori, K.; Sayo, H.; Kishi, T. 1993. Distribution of ubiquinone and ubiquinol homologues in rat tissues and subcellular fractions. *Lipids* 28:803-809.

Tang, P.H.; Miles, M.V.; Miles, L.; Quinlan, J.; Wong, B.; Wensch, A.; Bove, K. 2004. Measurement of reduced and oxidized coenzyme Q₉ and coenzyme Q₁₀ levels in mouse tissues by HPLC with coulometric detection. *Clinica Chimica Acta* 341:173-184.

Thomas, S.R.; Witting, P.K.; Stocker, R. 1999. A role for reduced coenzyme Q in atherosclerosis? *BioFactors* 9:207-224.

Thomas, S.R.; Neuzil, J.; Stocker, R. 1996. Co-supplementation with coenzyme Q prevents the pro-oxidant effect of α -tocopherol and increases the resistance of low-density lipoprotein towards transition metal-dependent oxidation initiation. *Arterioscler Thromb Vasc Biol* 16:687-696. Cited In: Thomas *et al.*, 1999.

Tran, M.T.; Mitchell, T.M.; Kennedy, D.T.; Giles, J.T. 2001. Role of coenzyme Q10 in chronic heart failure, angina, and hypertension. *Pharmacotherapy* 21(7):797-806.

Turunen, M.; Olsson, J.; Dallner, G. 2004. Metabolism and function of coenzyme Q. *Biochimica et Biophysica Acta* 1660:171-199.

Villalba, J.M.; López-Lluch, G.; Santos-Ocaña, C.; Rodríguez-Aguilera, J.C.; Navas, P. 2000. Extramitochondrial functions of coenzyme Q. In: Kagan, V.E. and Quinn, P.J. (Eds.). *Coenzyme Q: Molecular Mechanisms in Health and Disease*. CRC Press, New York, pp. 83-98.

Weber, C.; Jakobsen, T.S.; Mortensen, S.A.; Paulsen, G.; Hølmer, G. 1994. Effect of dietary coenzyme Q10 as an antioxidant in human plasma. *Molec Aspects Med* 15:s97-s102.

Williams, K.D., Maneke, J.D., AbdelHameed, M., Hall, R.L., Palmer, T.E., Kitano, M. and Hidaka, T. 1999. 52-Week oral gavage chronic toxicity study with ubiquinone in rats with a 4-week recovery. *J Agric Food Chem* 47:3756-3763.

Wittenstein, B.; Klein, M.; Finckh, B.; Ullrich, K.; Kohlschütter, A. 2002. Plasma antioxidants in pediatric patients with glycogen storage disease, diabetes mellitus, and hypercholesterolemia. *Free Radical Biology & Medicine* 33(1):103-110.

Yamamoto, Y.; and Yamashita, S. 1999. Plasma ubiquinone to ubiquinol ratio in patients with hepatitis, cirrhosis, and hepatoma, and in patients treated with percutaneous transluminal coronary reperfusion. *Molec Aspects Med* 18(Supplement):s79-s84.

Yamamoto, Y.; and Yamashita, S. 1997. Plasma ratio of ubiquinol and ubiquinone as a marker of oxidative stress. *Molec Aspects Med* 18(Supplement):s79-s84.

Yamashita, S.; and Yamamoto, Y. 1997. Simultaneous detection of ubiquinol and ubiquinone in human plasma as a marker of oxidative stress. *Anal Biochem* 250:66-73.

**New Dietary Ingredient Notification
for
KANEKA QH™**

APPENDIX 1: USP Monograph for Ubidecarenone