

## CLINICAL PROTOCOL COVER PAGE

**Protocol Title:** A Randomized, Double-blind, Crossover Trial Comparing the Bioavailability of Three CoQ10 Formulations over 72 Hours

**Protocol Number:** 09CBHE

**Protocol Date:** November 12, 2008

**Amendment(s):**

**Study Phase:** Phase I

**Study Design:** Randomized, double-blind, crossover, 72 hour, bioavailability study

**Sponsor:** Enerex Botanicals Ltd.  
305-6741 Cariboo Road  
Burnaby, British Columbia V3N 4A3  
Canada

**Sponsor Contact:** Barrie Carlsen  
CEO  
Enerex Botanicals Ltd.  
604-422-8777

**CRO:** KGK Synergize Inc.  
Suite 1440, One London Place  
255 Queens Ave  
London Ontario N6A 5R8  
Canada  
519-438-9374

**Medical Directors:** David Crowley, MD and Dale Wilson, MD  
KGK Synergize Inc.  
519-438-9374 ext 250

## LIST OF ABBREVIATIONS

AE	adverse event
ALT	alanine transaminase
AST	aspartate aminotransferase
AUC	area under the concentration-time curve
BUN	blood urea nitrogen
CBC	complete blood count
Cl	chloride
C <sub>max</sub>	maximum concentration
CoQ10	coenzyme Q10
EDTA	diaminoethanetetraacetic acid
g	gram
GCP	Good Clinical Practices
GGT	gamma glutamyl transferase
GMP	Good Manufacturing Practice
g/L	grams per liter
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
IRB	Institutional Review Board
K	potassium
LDL	low density lipoprotein
mg/dL	milligrams per deciliter
ml	milliliter
mmHg	millimeter of mercury
mmol/L	millimoles per liter
Na	sodium
QOL	Quality of Life
SAE	serious adverse event
SST	serum separating tube
t <sub>1/2</sub>	half life
T <sub>max</sub>	time at maximum concentration
ULN	upper limit of normal
umol/L	micromole

## TABLE OF CONTENTS

<b>CLINICAL PROTOCOL COVER PAGE</b> .....	<b>1</b>
<b>LIST OF ABBREVIATIONS</b> .....	<b>2</b>
<b>TABLE OF CONTENTS</b> .....	<b>3</b>
<b>1. INTRODUCTION</b> .....	<b>4</b>
<b>2. STUDY OBJECTIVES</b> .....	<b>5</b>
<b>3. STUDY DESIGN</b> .....	<b>5</b>
<b>4. SELECTION OF STUDY POPULATION</b> .....	<b>6</b>
<b>4.1 INCLUSION CRITERIA</b> .....	<b>6</b>
<b>4.2 EXCLUSION CRITERIA</b> .....	<b>7</b>
<b>4.3 CONCOMITANT MEDICATIONS</b> .....	<b>8</b>
<b>4.4 EARLY WITHDRAWAL</b> .....	<b>8</b>
<b>5. INVESTIGATIONAL PRODUCT</b> .....	<b>9</b>
<b>5.1 MANUFACTURING AND STORAGE</b> .....	<b>9</b>
<b>5.2 LABELING AND CODING</b> .....	<b>9</b>
Sample Label.....	10
<b>5.3 CoQ10 30MG</b> .....	<b>10</b>
<b>5.4 Co-Q10 30MG; DIN 02231736</b> .....	<b>10</b>
<b>5.5 COENZYME Q10 30MG; NPN 02176955</b> .....	<b>10</b>
<b>5.6 DIRECTIONS</b> .....	<b>10</b>
<b>6. STUDY ASSESSMENTS</b> .....	<b>11</b>
<b>6.1 SCREENING (VISIT 1)</b> .....	<b>11</b>
<b>6.2 ON STUDY</b> .....	<b>11</b>
<b>6.2.1 Randomization/First Test Period (Visit 2)</b> .....	<b>11</b>
<b>6.2.2 Second Test Period (Visit 3)</b> .....	<b>11</b>
<b>6.2.3 End of Study/Third Test Period (Visit 4)</b> .....	<b>12</b>
<b>6.3 ADVERSE EVENTS</b> .....	<b>12</b>
<b>6.4 LABORATORY ANALYSIS</b> .....	<b>13</b>
<b>7. STATISTICAL EVALUATION</b> .....	<b>13</b>
<b>7.1 DETERMINATION OF SAMPLE SIZE</b> .....	<b>13</b>
<b>7.2 ANALYSIS PLAN</b> .....	<b>13</b>
<b>7.2.1 Statistical Analysis</b> .....	<b>13</b>
<b>7.2.2 Study Population Description</b> .....	<b>14</b>
<b>7.2.3 Premature Discontinuation Description</b> .....	<b>14</b>
<b>7.2.4 Safety</b> .....	<b>14</b>
<b>7.2.5 Protocol Deviation Description</b> .....	<b>14</b>
<b>8. DATA COLLECTION AND STORAGE</b> .....	<b>14</b>
<b>9. POTENTIAL RISKS AND PROCEDURES TO MINIMIZE RISK</b> .....	<b>14</b>
<b>10. REFERENCES</b> .....	<b>14</b>
<b>11. APPENDICES</b> .....	<b>16</b>
<b>11.1 APPENDIX 1 SCHEDULE OF ASSESSMENTS</b> .....	<b>16</b>

## 1. INTRODUCTION

Clinical and experimental studies indicate that coenzyme Q10 deficiency may be associated with hypertension, hyperlipidaemia, coronary artery disease (CAD) and congestive heart failure. Coenzyme Q10 (CoQ10) is an endogenously produced co-factor in oxidative respiration for the Krebs cycle and the electron transport chain. It also acts as a fat soluble antioxidant. Highest levels of CoQ10 are in tissues that are the most metabolically active, such as the heart and immune system.<sup>1</sup> In the blood, CoQ10 is associated with lipoproteins; primarily low density lipoprotein, LDL.<sup>2</sup> The reduced form of CoQ10 (CoQ10H<sub>2</sub>) functions as an antioxidant to reduce oxidative stress on LDL. Early work suggested that CoQ10 levels were modified with age, disease (such as cardiovascular and neuromuscular diseases), medications (statins), and impaired synthesis.<sup>1</sup> More recent work suggests that total CoQ10 may not be as important as reduced CoQ10 or ratio of reduced CoQ10 to total CoQ10 for some conditions. CoQ10H<sub>2</sub> (reduced CoQ10) may be lower in relation to total CoQ10 in individuals with various types of cancer, heart disease, and neuromuscular diseases.<sup>3</sup> In metabolic syndrome, levels of CoQ10H<sub>2</sub> may increase as an adaptive response to oxidative stress.<sup>4</sup> Further, the ratio of reduced CoQ10 to oxidized CoQ10 decreases with age.<sup>5</sup> In animals, tissue content of reduced CoQ10 and the ratio of reduced CoQ10 to total CoQ10 have been used as a marker of disease.<sup>6</sup> CoQ10 is a central rate limiting constituent of the mitochondrial respiratory chain which generates most of the adenosine triphosphate within the cell (Mitchell, 1991). As it can be synthesized by the body, CoQ10 is not considered an essential nutrient. However, as indicated above total CoQ10 levels are modified with age, disease, or medication, and changes in reduced CoQ10 or CoQ10 ratios may also occur.

Evidence from human trials suggests that CoQ10 supplementation may be beneficial for individuals with cardiovascular disease; chronic heart failure, and hypertension.<sup>1</sup> CoQ10 supplementation may also be beneficial for individuals using statin therapy due to statin-induced reduction in plasma CoQ10. Supplemental CoQ10 has been investigated in individuals with neurological disorders, cancer, diabetes, migraine, asthma, and many other clinical conditions.<sup>1,7</sup> CoQ10 is a popular oral supplement due to its clinical potential. In general, CoQ10 supplements are considered to be bioavailable. Peak levels of CoQ10 occur five to ten hours following administration, with a half-life of 34 hours.<sup>8-</sup><sup>10</sup> A second peak may occur at approximately 24 hours, likely due to enterohepatic recirculation.<sup>11</sup> Oil suspended or solubilized CoQ10 may increase bioavailability of CoQ10 due to its fat solubility.<sup>12</sup> Non-linear CoQ10 absorption has been suggested from a few human studies.<sup>11</sup> It is not clear what effect, if any, age, gender, or diet have on

bioavailability of CoQ10. Information from animal models suggests that CoQ10 is taken up by all tissues following oral administration.<sup>10</sup>

CoQ10 is absorbed in the gastrointestinal tract in a similar manner as lipids as CoQ10 is a lipophilic substance. Absorption is enhanced in the presence of lipids by inducing the release of bile in turn promoting emulsification to promote absorption.<sup>13</sup> Lecithin is a naturally occurring emulsifier and has been demonstrated to significantly increase the bioavailability of a phytosterol compound, sitostanol.<sup>13</sup> Additionally, phosphatidylcholine from soybean lecithin has been demonstrated to increase the absorption of another fat soluble compound, lycopene.<sup>14</sup>

The active form of Vitamin B6, Pyridoxal 5'-phosphate (also known as PLP and P5P), is essential to the internal synthesis of coenzyme Q10 and it has been shown that individuals with low plasma CoQ10 status also have low plasma P5P levels.<sup>15</sup>

It is therefore believed that a Coenzyme Q10 supplement formulated with P5P and phosphatidylcholine may increase plasma CoQ10 levels over currently marketed forms of CoQ10 with CoQ10 as the sole ingredient. Furthermore, this formulation uses an enteric coating process (cellulose acetate phthalate) which may enhance the delivery of the active ingredient, CoQ10 to the small intestine, bypassing the digestion process of the stomach.

## **2. STUDY OBJECTIVES**

This study will compare the bioavailability of CoQ10 from three CoQ10 formulations in healthy adult volunteers. The objective of this study is to evaluate the bioavailability of CoQ10 from a single dose of product containing 30 mg of CoQ10. Each subject will act as their own control as subjects will consume a single dose of each formulation on three separate treatment days. These visits will be separated by a minimum of two weeks. Blood sampling will be conducted under similar dietary conditions. The endpoints will be the determination of CoQ10 at each time point, as well as area under the concentration-time curve (AUC), half life ( $t_{1/2}$ ), time at maximum concentration ( $T_{max}$ ) and maximum concentration ( $C_{max}$ ) for CoQ10.

## **3. STUDY DESIGN**

This study is a randomized, double-blind, crossover, 72 hour, bioavailability study with three arms. The study will be conducted at a single site in London, Ontario. Twenty-one subjects will be enrolled and randomized to treatment sequence after undergoing a screening visit and passing eligibility criteria. Subjects will receive a single dose of either CoQ10 formulation on three test days separated by a minimum of two weeks. To evaluate bioavailability, blood samples will be taken pre-dose and again at 2, 4, 5, 6, 8,

12, 24, 48 and 72 hours post-dose for determination of CoQ10 levels. Standardized meals (breakfast, lunch and dinner) will be provided immediately after the dose, following the 4 hour blood sample and following the 8 hour blood sample, respectively. As there are no known interaction of CoQ10 with food, subjects will not be restricted in their choice of foods. However, subjects will be provided with the same meals in the clinic on each test day (hours 0-12). The period from screening to study completion will be approximately five weeks. Adverse events will be assessed at each study visit.

### SEQUENCE GROUPS

Dosing Sequence	Subject Number
CoQ10 with P5P and Phosphatidylcholine → Co-Q10 (NPN 02176955) → Coenzyme Q10 (DIN 02231736)	N = 7
Co-Q10 (NPN 02176955) → Coenzyme Q10 (DIN 02231736) → CoQ10 with P5P and Phosphatidylcholine	N = 7
Coenzyme Q10 (DIN 02231736) → CoQ10 with P5P and Phosphatidylcholine → Co-Q10 (NPN 02176955)	N = 7
<b>Total</b>	<b>N = 21</b>

#### 4. SELECTION OF STUDY POPULATION

This study will include 21 healthy adult volunteers. Each subject will have to fulfill the inclusion criteria and will not be allowed to meet any of the exclusion criteria as described in sections 4.1 and 4.2.

##### 4.1 Inclusion Criteria

1. Male or female age 18 years or older
2. If female, subject is not of child bearing potential. Defined as females who have had a hysterectomy or oophrectomy, bilateral tubal ligation or are post-menopausal (natural or surgically with > 1 year since last menstruation).

OR

Female subject of childbearing potential must agree to use a medically approved method of birth control and have a negative urine pregnancy test result.

Acceptable methods of birth control include:

- Double-barrier method (condoms with spermicide or diaphragm with spermicide)
- Hormonal contraceptives including oral contraceptives, hormone birth control patch (Ortho Evra), vaginal contraceptive ring (NuvaRing),

- injectable contraceptives (Depo-Provera, Lunelle), or hormone implant (Norplant System)
- Intrauterine devices
  - Vasectomy of partner
  - Abstinence
3. Healthy as determined by laboratory results and medical history
  4. BMI 18-30 kg/m<sup>2</sup>
  5. Screening CoQ10 levels of 0.8 ± 0.2 mg/L
  6. Non-smoker or ex-smoker for more than three months
  7. Voluntary, written, informed consent to participate in the study

#### 4.2 Exclusion Criteria

1. Women who are pregnant, breastfeeding, or planning to become pregnant during the course of the trial
2. Alcohol use >2 standard alcoholic drinks per day
3. Unstable cardiac condition
4. Use of Coumadin (Warfarin)
5. History of or current diagnosis of any cancer (except for successfully treated basal cell carcinoma) diagnosed less than 5 years prior to screening. Subjects with cancer in full remission more than 5 years after diagnosis are acceptable.
6. Uncontrolled hypertension defined as untreated systolic blood pressure > 160 mmHg and/or diastolic blood pressure > 100 mmHg
7. Unstable renal and/or liver disease
8. Alcohol or drug abuse within the past year
9. Unstable psychiatric disorder
10. Immunocompromised individuals such as subjects that have undergone organ transplantation or subjects diagnosed with human immunodeficiency virus (HIV)
11. History of hemoglobinopathies such as sickle cell anemia or thalassemia, sideroblastic anemia
12. Participation in a clinical research trial within 30 days prior to randomization
13. Use of products containing CoQ10 and/or other natural health products other than vitamins or minerals within 2 weeks of randomization
14. Use of acute medication 72 hours of study supplement dose
15. Unstable medications (Dosage must be stable for 90 days prior to randomization)
16. Significant abnormal liver function as defined as AST and/or ALT > 2 x the upper limit of normal (ULN), and/or bilirubin > 2 x the ULN
17. Serum creatinine  $\geq$  130 umol/L for males and  $\geq$  125 umol/L for females
18. Anemia of any etiology defined as hemoglobin  $\leq$  120 g/L for males and  $\leq$  110 g/L for females
19. Allergy or sensitivity to study supplement ingredients
20. Individuals who are cognitively impaired and/or who are unable to give informed consent.
21. Any other condition which in the Investigator's opinion may adversely affect the subject's ability to complete the study or its measures or which may pose significant risk to the subject

### **4.3 Concomitant Medications**

Subjects taking a prescription medication for an acute condition (i.e. antibiotics for an infection), will be required to washout for 72 hours prior to randomization. Subjects will not be asked to discontinue any medication but may be randomized 72 hours after the end of their prescribed regimen. Subjects will also be required to washout for 14 days after taking any natural health products other than vitamins or minerals before they can be randomized if the investigator determines that this will not negatively affect the subject's health. Subjects requiring medications for chronic conditions will be allowed to participate in this trial provided that they have been on a stable dosage of the medication for at least 90 days prior to randomization. If acute medication is necessary during the trial, the subject must wait a minimum of 72 hours after medication use before they can receive the test product. Birth control is allowed during the study. Subjects who are currently taking prescribed birth control must agree to maintain their current method and dosing regimen during the course of the study.

### **4.4 Early Withdrawal**

Subject discontinuation should be considered at the discretion of the principal investigator. The circumstances of any discontinuation have to be documented in detail. If possible, the evaluations planned for the end of study will be carried out at the time when the subject is withdrawn from the study. A subject leaving the study prematurely will NOT be replaced by another. Criteria for removal of subjects from the study will include:

### **Personal reasons**

As stated in the Informed Consent Form, a subject may withdraw from the study for any reason at any time.

### **Clinical judgment of physician**

A subject may be withdrawn from the study if, in the opinion of the principal investigator, it is not in the subject's best interest to continue. This includes but is not limited to adverse events or serious adverse events related to the investigational product causing clinically significant illness; the need for prohibited concomitant medication; female subject who becomes pregnant during the course of the trial.

### **Protocol violation**

Any subject found to have entered this study in violation of the protocol will be discontinued from the study at the discretion of the principal investigator. This will include any subject found to have been inappropriately enrolled (did not meet eligibility criteria). Subject non-compliance includes either not showing up for study visits, not taking investigational product as directed, or refusing to undergo study visit procedures. Subjects who are found to be less than 70% compliant with test article usage at any study visit will be withdrawn. Subjects who are found to be taking prohibited medications or supplements without the knowledge of the principal investigator will also be withdrawn.

## **5. INVESTIGATIONAL PRODUCT**

### **5.1 Manufacturing and Storage**

The investigational product will be provided by the sponsor. The manufacturing process will be done under GMP requirements and control. The investigational product will be carefully stored at the study site in a lockable, limited access area, in compliance with pertinent regulations. Only authorized persons will have access to the investigational product. The bottles should be stored at room temperature between 17 to 25 degrees Celsius and should not be exposed to direct sunlight or heat. All unused investigational product will be returned to the study sponsor (at the sponsor's expense) at study closeout (within one month of last subject visit).

Manufactured by:

Canadian Phytopharmaceuticals Corp.  
Richmond, BC

Date of Manufacture: May 2008

### **5.2 Labeling and Coding**

The investigational product will be labeled according to the requirements of ICH-GCP guidelines and applicable local regulatory guidelines. Investigational product will be randomized and coded by the sponsor. The investigator will be provided with a randomization list indicating the order of randomization. Each subject will be assigned a randomization code according to the order of the randomization list. The investigator

will be provided with sealed envelopes for each randomization code. These envelopes are to remain sealed except in the event of an emergency. In the event that it is necessary to unblind a subject's treatment, the envelope labeled with the subject's randomization code will be opened. Notification of unblinding must be reported to the study sponsor within 24 hours.

### **Sample Label**

Randomization Code: \*\*\*\*\* Protocol No.: 09CBHE  
CoQ10 (30mg) with P5P and Phosphatidyl Choline Lot# TQ01642; Expiry Date May 2011 or  
CoQ10 (30mg) DIN 02231736 or CoQ10 (30mg) NPN 02176955  
Investigational Natural Health Product  
To be used under the supervision of a qualified investigator.  
Sponsor: Enerex Botanicals Ltd. Manufacturer: Canadian Phytopharmaceuticals  
Burnaby, British Columbia Corp.  
Richmond, BC

For investigational use only. Keep out of reach of children.  
Store at room temperature.

### **5.3 CoQ10 30mg with P5P and Phosphatidyl Choline**

CoQ10 – 30mg

Non medicinal ingredients:

Vitamin B6 (10mg), Phosphatidyl Choline (250mg), Dicalcium phosphate, magnesium stearate, microcrystalline cellulose, silicon dioxide, vegetable stearin, cellulose acetate phthalate.

### **5.4 Co-Q10 30mg; DIN 02231736**

CoQ10 – 30mg

Non medicinal ingredients:

Cellulose, gelatin

### **5.5 Coenzyme Q10 30mg; NPN 02176955**

CoQ10 (Ubiquinone) – 30mg

Non medicinal ingredients:

Black iron oxide, gelatin, glycerin, red iron oxide, soy lecithin, soybean oil, yellow iron oxide, yellow beeswax.

### **5.6 Directions**

Subjects will be instructed to take 1 capsule/caplet in the clinic after the pre-dose blood sample has been obtained. The time of dose will be recorded and the timing of blood draws will be based on the dose time.

## **6. STUDY ASSESSMENTS**

See Appendix 1 for schedule of assessments.

### **6.1 Screening (Visit 1)**

At screening, a Subject Information and Consent Form will be given to the potential subject. The subject will read the information carefully and will be given the opportunity to seek more information if needed. The subject will also be provided with the option of taking the consent form home to review prior to making his or her decision. If agreeable, the subject will sign the consent form and receive a duplicate. Once consent has been obtained, the screening visit will proceed. Eligibility will be determined based on the inclusion and exclusion criteria. Medical history and concomitant therapies will be reviewed. Height, weight, heart rate and blood pressure will be measured. BMI will be calculated. Peripheral blood will be collected to determine CBC, electrolytes, glucose, creatinine, AST, ALT, GGT, bilirubin and total CoQ10. A urine pregnancy test will be performed on all females of childbearing potential.

### **6.2 On Study**

#### **6.2.1 Randomization/First Test Period (Visit 2)**

Concomitant therapies and inclusion/exclusion criteria will be reviewed. Subjects who have passed all eligibility criteria will be randomized to treatment sequence. Fasting blood samples will be taken pre-dose for CoQ10 analysis. The subject will then be given 1 capsule(s), at time 0 with 125 ml of water and breakfast will be provided immediately after the dose. Blood samples will be taken again at 2 and 4 hours post-dose, lunch will be provided following the 4 hour sample. Blood samples will be taken again at 5, 6 and 8 hours post-dose with dinner provided after the 8 hour sample. A blood sample will be taken at 12 hours post-dose. Subjects will remain in the clinic during the study visit from pre-dose until the 12 hour samples are collected. The food consumed at each meal time will be recorded. Subjects will be allowed to watch television, use computers/laptops, read, talk, play video or board games, or sleep. Subjects will be allowed to leave the clinic after the 12 hour post-dose blood sample and are to return to the clinic for the 24 hour, 48 hour, and 72 hour post-dose collection of blood. Subjects will return to the clinic a minimum of two weeks after dosing to begin the second test period.

#### **6.2.2 Second Test Period (Visit 3)**

Visits 2 and 3 will be separated by a minimum of two weeks. Concomitant therapies and adverse events will be reviewed. Fasting blood samples will be taken pre-dose for total CoQ10 analysis. The subject will then be given 1 capsule(s), at time 0 with 125 ml of water and breakfast will be provided immediately after the dose. Blood samples will be taken again at 2 and 4 hours post-dose, lunch will be provided following the 4 hour sample. Blood samples will be taken again at 5, 6 and 8 hours post-dose with dinner provided after the 8 hour sample. A blood sample will be taken at 12 hours post-dose. Subjects will remain in the clinic during the study visit from pre-dose until the 12 hour samples are collected. The food consumed at each meal time will be recorded. Subjects will be allowed to watch television, use computers/laptops, read, talk, play video or board games, or sleep. Subjects will be allowed to leave the clinic after the 12 hour post-dose

blood sample and are to return to the clinic for the 24 hour, 48 hour, and 72 hour post-dose collection of blood. Subjects will return to the clinic a minimum of two weeks after dosing to begin the third and final test period.

### **6.2.3 End of Study/Third Test Period (Visit 4)**

Visits 3 and 4 will be separated by a minimum of two weeks. Concomitant therapies and adverse events will be reviewed. Fasting blood samples will be taken pre-dose for total CoQ10 analysis. The subject will then be given 1 capsule(s), at time 0 with 125 ml of water and breakfast will be provided immediately after the dose. Blood samples will be taken again at 2 and 4 hours post-dose, lunch will be provided following the 4 hour sample. Blood samples will be taken again at 5, 6 and 8 hours post-dose with dinner provided after the 8 hour sample. A blood sample will be taken at 12 hours post-dose. Subjects will remain in the clinic during the study visit from pre-dose until the 12 hour samples are collected. The food consumed at each meal time will be recorded. Subjects will be allowed to watch television, use computers/laptops, read, talk, play video or board games, or sleep. Subjects will be allowed to leave the clinic after the 12 hour post-dose blood sample and are to return to the clinic for the 24 hour, 48 hour, and 72 hour post-dose collection of blood.

### **6.3 Adverse Events**

An adverse event (AE) is any untoward medical occurrence in a clinical investigational subject who has been administered an investigational product and which does not necessarily have a causal relationship with this treatment. An AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a product, whether or not it is considered related to that product. During the study, subjects will record adverse effects in their subject diary. At each visit the subject will be asked "Have you experienced any difficulties or problems since I saw you last"? Any adverse events will be documented and recorded in the study record and will be classified according to the description, duration, severity, frequency, and outcome. The investigator will assess any AE's and decide causality.

A serious adverse event (SAE) is any AE that results in any of the following outcomes:

- Death
- A life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability or incapacity
- A congenital anomaly/birth defect in the offspring of a subject who received the study treatment
- Important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the outcomes listed above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or the development of drug dependency or drug abuse.

Notification of any serious adverse events must be made in writing to the study sponsor within 24 hours of learning of the event. The IRB will be notified of all SAE's.

The sponsor must notify the Natural Health Products Directorate (NHPD) of any serious adverse or unexpected adverse reaction to a natural health product as follows:

- a. If it is neither fatal or life threatening, within 15 days after the day on which the sponsor becomes aware of the information; and
- b. If it is fatal or life threatening, within seven days after the day on which the sponsor becomes aware of the information.

#### **6.4 Laboratory Analysis**

Peripheral blood will be collected by veinpuncture using SST, EDTA (lavender), and Heparin vacutainer tubes. At screening, approximately 15 ml will be used to determine CBC, electrolytes (Na, K, Cl), glucose, creatinine, AST, ALT, GGT, bilirubin and total CoQ10. Approximately 5 ml of blood will be collected in Heparin tubes at each time point (pre-dose and 2, 4, 5, 6, 8, 12, 24, 48 and 72 hours post-dose) for total CoQ10 analysis.

Whole blood will be collected into 4 ml EDTA tubes for CBC analysis. Serum will be generated from blood collected into 5 ml SST tubes for electrolytes, glucose, creatinine, AST, ALT, GGT, and bilirubin analysis. These samples will be analyzed by LifeLabs Medical Laboratory Services in London, Ontario.

Whole blood will be collected into 6 ml Heparin tubes for total CoQ10 analysis. The total CoQ10 analysis will be done by HPLC with UV detection. These samples will be analyzed by KGK Synergize Laboratory in London, Ontario.

All samples will be alphanumerically coded. The persons performing the laboratory analysis will be unaware of the identity of the subject.

### **7. STATISTICAL EVALUATION**

#### **7.1 Determination of sample size**

No formal sample size calculation was performed as this is an exploratory study to determine bioavailability. A 3x3 latin square design will be used so a total of 21 subjects will be randomized to three treatment sequence groups with 7 subjects per group.

#### **7.2 Analysis Plan**

##### **7.2.1 Statistical Analysis**

Descriptive statistics will be provided for patient demographic and baseline characteristics.

Graphs showing the mean concentrations of total CoQ10 over time will be shown for each formulation. Bioavailability parameters, including the area under the curve (AUC), the maximum observed concentration ( $C_{max}$ ), time at maximum concentration ( $T_{max}$ ), and the half life ( $t_{1/2}$ ), for total CoQ10 levels for the three study formulations will

be calculated. Statistical analysis will be performed on both the measured plasma total CoQ10 values and on the level of increase post dose (post dose minus pre-dose for each time point measured). Descriptive statistics, including means and standard deviations will be calculated for each formulation and repeated measures analysis of variance will be used to compare the two formulations.

### **7.2.2 Study Population Description**

Frequency counts and proportions will be used to describe categorical variables. The mean, standard deviation, minimal and maximal values, and median will be calculated for continuous variables.

### **7.2.3 Premature Discontinuation Description**

For each premature discontinuation, the following parameters will be listed: subject number, dates of treatment start and end of treatment, and the reason of premature discontinuation. Drop-outs during the treatment period will not be replaced.

### **7.2.4 Safety**

For adverse events, a descriptive analysis will be given. Adverse events will be presented in a frequency table, by body system, group and treatment. Furthermore, nature, incidence, severity and causality will be reported for each adverse event.

### **7.2.5 Protocol Deviation Description**

All protocol deviations will be listed in the final study report.

## **8. DATA COLLECTION AND STORAGE**

All data collection and record storage will be done in compliance with ICH GCP Guidelines Current Step 4 version dated June 10, 1996 and applicable local regulatory guidelines.

## **9. POTENTIAL RISKS AND PROCEDURES TO MINIMIZE RISK**

All potential risks are disclosed to study participants prior to their participation. The potential risks associated with this study include venipuncture. Risks associated with venipuncture include pain, bruising, and infection at the site. Alcohol swabs and proper venipuncture procedure will be followed to minimize the risk of infection.

## **10. REFERENCES**

1. Anonymous. Coenzyme Q10. *Alt Med Rev* 2007; 12:159-168.
2. Alleva R, Tomasetti M, Bompadre S, Littarru P. Oxidation of LDL and their subfractions: kinetic aspects and CoQ10 content. *Mol Aspects Med* 1997; 18:s105-12.
3. Tang PH, Miles MV, DeGrauw A, Hershey A, Pesce A. HPLC Analysis of Reduced and Oxidized Coenzyme Q10 in Human Plasma. *Clin Chem* 2001; 47: 256-265.

4. Miles MV, Morrison JA, Horn PS, *et al.* Coenzyme Q10 changes are associated with metabolic syndrome. *Clin Chim Acta* 2004; 344:173-179.
5. Miles MV, Horn PS, Tang PH, *et al.* Age-related changes in plasma coenzyme Q10 concentrations and redox state in apparently healthy children and adults. *Clin Chim Acta* 2004; 347:139-144.
6. Miles L, Miles MV, Tang PH, *et al.* Ubiquinol: a potential biomarker for tissue energy requirements and oxidative stress. *Clin Chim Acta* 2005; 360:87-96.
7. Miles MV, Patterson BJ, Schapiro MB, *et al.* Coenzyme Q10 absorption and tolerance in children with Downs' syndrome: a dose-ranging trial. *Ped Neurol* 2006; 35:30-37.
8. Greenberg S, Frishman WH. Co-enzyme Q10: a new drug for cardiovascular disease. *J Clin Pharmacol* 1990; 30:596-608.
9. Tomono Y, Hasegawa J, Seki T, Motegi K, Morishita N. Pharmacokinetic study of deuterium-labelled coenzyme Q10 in man. *Int J Clin Pharmacol Ther Toxicol* 1986; 24:536-541.
10. Bhagavan HN, Chopra RK. Coenzyme Q10: absorption, tissue uptake, metabolism and pharmacokinetics. *Free Rad Res* 2006; 40:455-453.
11. Miles MV. The uptake and distribution of coenzyme Q10. *Mitochondrion* 2007; Suppl:S72-7.
12. Weis M, Mortensen SA, Rassing MR, Moller-Sonnergaard J, *et al.* Bioavailability of four oral coenzyme Q10 formulations in healthy volunteers. *Mol Aspects Med* 1994; 15 Suppl:s273-s280.
13. Ostlund RE Jr, Spilburg CA, Stenson WF. Sitostanol administered in lecithin micelles potently reduces cholesterol absorption in humans. *Am J Clin Nutr.* 1999; 70 (5): 826-831.
14. Nishimukai M and Hara H. Enteral Administration of Soybean phosphatidylcholine enhances the lymphatic absorption of lycopene, but reduces that of alpha-tocopherol in rats. *J Nutr* 2004; 134(8): 1862-6.
15. Willis R, Anthony M, Sun L, Honse Y, Qiao G. Clinical implications of the correlation between coenzyme Q10 and vitamin B6 status. *Biofactors* 1999; 9 (2-4): 359-63.

Protocol 09CBHE  
A Randomized, Double-blind, Crossover Trial Comparing the Bioavailability of Three CoQ10  
Formulations Over 72 Hours

**11. APPENDICES**

**11.1 Appendix 1 Schedule of Assessments**

Procedure/assessment	Visit 1 Screen -3 to -28 days	Visit 2 Test Period 1 Randomization Days 1-3				Visit 3 Test period 2 Days 15-17 (min)			
		0-12 hours	24 hours	48 hours	72 hours	0-12 hours	24 hours	48 hours	72 hours
Informed consent	X								
Review inclusion/exclusion criteria	X	X							
Review medical history	X								
Review concomitant therapies	X	X	X	X	X	X	X	X	X
Height, weight,	X								
Heart rate, blood pressure	X								
Urine pregnancy test	X								
Laboratory tests: CBC, electrolytes (Na, K, Cl), glucose, creatinine, AST, ALT, GGT, and bilirubin	X								
Laboratory tests: Total CoQ10 Screening, pre-dose, and 2, 4, 5, 6, 8, 12, 24, 48 and 72 hours post dose	X	X	X	X	X	X	X	X	X
Randomization		X				X			
Dispense one dose of test article (Time 0)		X				X			
Review adverse events		X	X	X	X	X	X	X	X

Protocol 09CBHE  
A Randomized, Double-blind, Crossover Trial Comparing the Bioavailability of Three CoQ10  
Formulations Over 72 Hours

Procedure/assessment	Visit 4 Test Period 3 End of Study Days 29-31 (min)			
	0-12 hours	24 hours	48 hours	72 hours
Informed consent				
Review inclusion/exclusion criteria				
Review medical history				
Review concomitant therapies	X	X	X	X
Height, weight,				
Heart rate, blood pressure				
Urine pregnancy test				
Laboratory tests: CBC, electrolytes (Na, K, Cl), glucose, creatinine, AST, ALT, GGT, and bilirubin				
Laboratory tests: Total CoQ10 Screening, pre-dose, and 2, 4, 5, 6, 8, 12, 24, 48 and 72 hours post dose	X	X	X	X
Randomization	X			
Dispense one dose of test article (Time 0)	X			
Review adverse events	X	X	X	X