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VIA HAND DELIVERY

May 31, 2006

Office of Food Additive Safety (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835



7361 Calhoun Place,
Suite 500
Rockville, Maryland 20855-2765
301.838.3120
fax: 301.838.3182

RE: Submission of GRAS Notification – Polyoxyethanyl-a-tocopheryl
sebacate (PTS) for use as a solubilizer

Dear Sir/Madame:

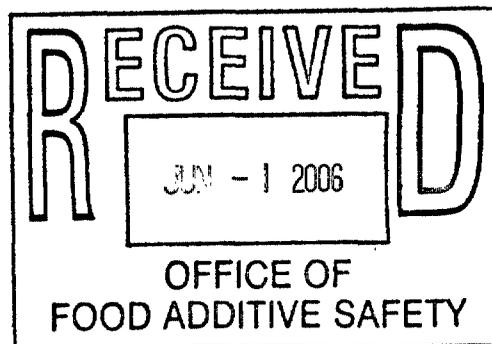
In accordance with proposed 21 CFR § 170.36 (Notice of a claim for exemption based on a GRAS determination) published in the Federal Register (62 FR 18939-18964), I am submitting in triplicate, as the agent to the notifier, Zymes, LLC, a GRAS Notification of Polyoxyethanyl-a-tocopheryl sebacate (PTS) for use as a solubilizer. Also enclosed is a GRAS panel report setting forth the basis for the GRAS determination.

Please let me know if you have any questions.

Sincerely,

Edward A. Steele
President

Enclosures



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I. GRAS Exemption Claim

A. Claim of Exemption From The Requirement for Premarket Approval Requirements Pursuant to Proposed CFR § 170.36(c)(1)

Polyoxyethanyl- α -tocopheryl sebacate (PTS), for use as a solubilizer, has been determined to be generally recognized as safe, and therefore, exempt from the requirement of premarket approval, under the conditions of its intended use as described below. The basis for this finding is described in the following sections.

Signed,

Edward A. Steele / Date 5/31/06

Agent for:

Zymes LLC
777 Terrace Avenue
Hasbrouk Heights, NJ 07604
201-727-1520

B. Name and Address of Notifier

Randi Fain, M.D.
Zymes LLC
777 Terrace Avenue
Hasbrouk Heights, NJ 07604

C. Common or Usual Name of the Notified Substance

Polyoxyethanyl- α -tocopheryl sebacate (PTS)

D. Conditions of Use

The intended use of PTS is as a nutritive solubilizer for the dietary supplement ingredient Coenzyme Q₁₀ (CoQ₁₀) in either the fully oxidized form as ubiquinone or in the reduced form as ubiquinol.

The proposed product uses of PTS as a water solubilizer for CoQ₁₀ dietary supplementation are:

- dietary supplements sold as tablets or capsules,
- dietary supplement drinks
- dietary supplement sports drinks.

CoQ₁₀ will be added to dietary supplements at levels up to 200 mg in a single tablet or capsule and up to 30 mg in dietary supplement drinks or sports beverages per serving. The optimum ratio of PTS to CoQ₁₀ is about 3:1 on a w/w basis. We are proposing to use the solubilizer up to 200 mg CoQ₁₀ for dietary supplements and 100 mg CoQ₁₀ in dietary supplement drinks or sports beverages per serving. Such applications and use therefore correspond to PTS doses of up to 600 mg in dietary supplements and up to 300 mg in dietary supplement drinks and supplemented sports drinks

E. Basis for the GRAS Determination

Pursuant to 21 CFR § 170.30, polyoxyethanyl- α -tocopheryl sebacate (PTS), has been determined to be GRAS by scientific procedures. A comprehensive search of the scientific literature was also utilized for this review.

F. Availability of Information

The data and information that serve as a basis for this GRAS are available for the Food and Drug Administration's review and copying during reasonable business hours at the offices of:

PTS Solubilizer

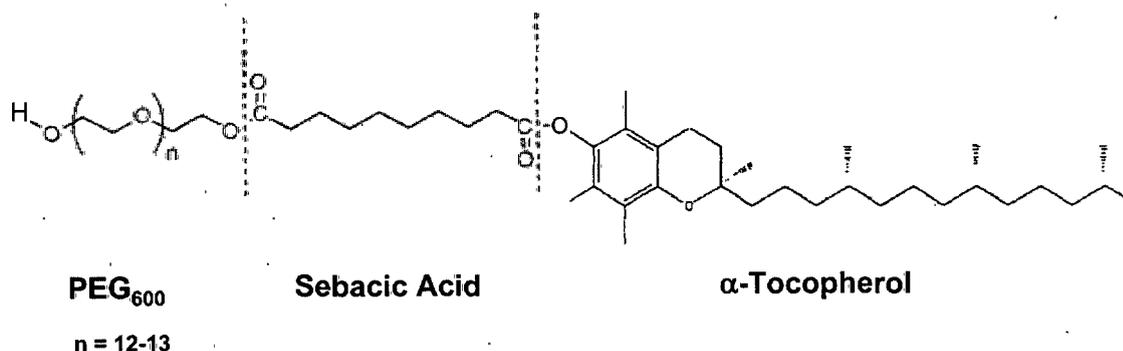
Edward A. Steele, President
AAC Consulting Group
7361 Calhoun Place, Suite 500
Rockville, MD 20855-2765
Telephone: 301-838-3120
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II. Detailed Information About the Identity of the Substance

The substance that is the subject of this notification is polyoxyethanyl- α -tocopheryl sebacate or PEG α -tocopheryl sebacate (PTS). The structural formula is presented in Figure 1 below.

PTS has a melting point of 25⁰ C. Below its melting point, PTS is a yellow-orange wax. Above its melting point, PTS is a yellow-orange viscous fluid which dissolves in water, alcohols, dichloromethane and tetrahydrofuran, but not hexane. PTS is hygroscopic. PTS is stable at or below room temperature, over long periods of time (up to a year) in aqueous solution or in native form.

Figure 1. Structural Formula of PTS



B. Method of Manufacture

PTS is synthesized by two consecutive esterification reactions. α -Tocopherol is linked to polyethylene glycol (mol. wt. 600-1000) using sebacyl dichloride, by esterification at its hydroxy terminus. The resultant α -tocopheryl mono-ester of sebacyl chloride is subsequently esterified with PEG₆₀₀ to form PTS. Reactions were performed under strictly anhydrous conditions in dry ethylacetate with triethylamine. PTS is prepared by sequential esterification, carried out in one pot. The reaction mixture is subsequently purified with an acetonitrile/hexane hot-cut to remove the major impurity, di- α -tocopheryl-sebacate which is formed in the first esterification as a by-product of sebacyl chloride diester formation with α -tocopheryl. As shown in Table 1, the resulting product consists of approximately 94% PTS, 2.9% unreacted residual PEG₆₀₀, 3.4% di- α -tocopheryl sebacate and trace amounts (0.1%) of (di α -tocopheryl sebacate) PEG₆₀₀.

Identity	Percent weight
PEG ₆₀₀	2.88
PTS	93.62
Di- α -tocopheryl sebacate	3.37
(Di- α -tocopherol-sebacate) PEG	0.14
Total	100

C. Specifications for PTS

The specifications for the final product are given in Table 2 below.

Parameter	Method	Specification
Appearance	Visual	Light yellow wax/oil
Identification	IR	Conforms to standard
Purity	HPLC	> 90.0%
Impurities	HPLC	Total impurities < 10% No single impurity > 5%
Melting Point	Melting Point	25°C +/- 2°C
Moisture Content	KF	Not more than 1%
Residual Solvents	GC	Meets ICH Standards* Ethylacetate < 500 ppm Triethylamine < 100 ppm Cyclohexane < 400 ppm Acetonitrile < 40 ppm

III. Self-Limiting Levels of Use

With typical amounts of CoQ₁₀ in dietary supplements ranging from 100-200 mg/day, the amount of PTS used for solubilization would be up to 600 mg in tablets or capsules and 300 total intake mg/serving in drinks. Thus, if a consumer ingested one tablet and two sports drinks/day, the intake of PTS is estimated to be 1200 mg/day or 20 mg/kg/day from the proposed uses. Thus, intake would be limited to amounts normally taken for supplementation and it is unlikely that consumers would consume excessive amounts given the limited benefits and cost of excessive intake.

4. Summary of the Basis for the Notifier's Determination that PTS Solubilizer is GRAS

An independent panel of recognized experts, qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was requested by Zymes, LLC to determine the Generally Recognized As Safe (GRAS) status of PTS

PTS Solubilizer

solubilizer with CoQ10 intended for use in dietary supplement tablets and dietary supplement drinks. A comprehensive search of the scientific literature was also utilized for this review.

Based on a critical evaluation of the pertinent data and information summarized above, the Expert Panel members have individually and collectively determined by scientific procedures that addition of PTS solubilizer with CoQ10, meeting the specifications cited above and manufactured accordance with current good manufacturing practice, is generally recognized as safe (GRAS) under the conditions of intended use in dietary supplements and dietary supplement drinks, as specified herein.

In coming to its decision that is GRAS, the Expert Panel relied upon the conclusions neither PTS nor any of its degradation products, that are likely to be systemically absorbed, pose any toxicological hazards or safety concerns at the proposed levels of addition for solubilization of the active ingredients CoQ₁₀ in dietary supplementation, published toxicology studies and other articles relating to the safety of the end product and of the components of the product which were considered to collectively demonstrate the safety of the end product. It is also their opinion that other qualified and competent scientists, reviewing the same publicly available toxicological and safety information, would reach the same conclusion.

EXPERT PANEL STATEMENT

DETERMINATION OF THE GRAS STATUS OF PTS SOLUBILIZER FOR ADDITION TO DIETARY SUPPLEMENTS AND SUPPLEMENTED BEVERAGES

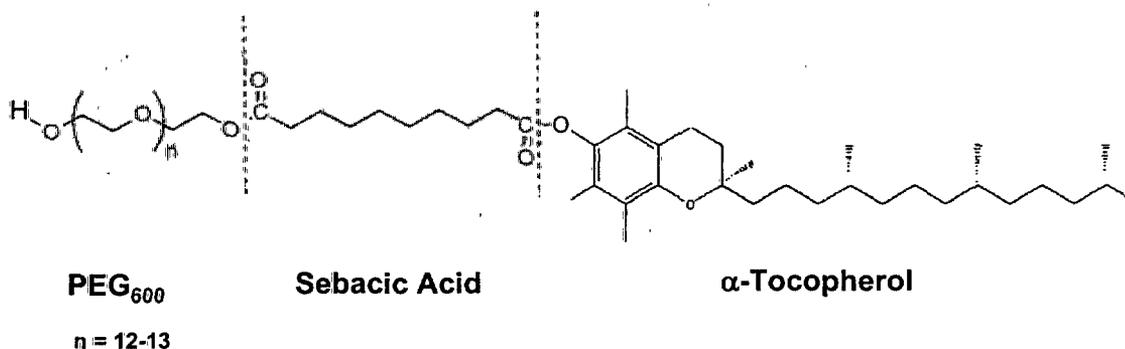
The undersigned, an independent panel of recognized experts (hereinafter referred to as the Expert Panel), qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was requested by Zymes LLC (Zymes) to determine the Generally Recognized as Safe (GRAS) status of polyoxyethanyl- α -tocopheryl sebacate (PTS) for use as a solubilizer for Coenzyme Q₁₀ for addition to specified dietary supplement foods and supplemented beverages. A comprehensive search of the scientific literature for safety and toxicity information on PTS and its components was conducted through May 2006 and made available to the Expert Panel. The Expert Panel independently evaluated materials submitted by Zymes and other materials deemed appropriate or necessary. Following independent, critical evaluation, the Expert Panel conferred and unanimously agreed to the decision described herein.

Identity and Composition

The substance that is the subject of this notification is polyoxyethanyl- α -tocopheryl sebacate or PEG α -tocopheryl sebacate (PTS). The structural formula is presented in Figure 1 below. PTS has no geometric isomers formed through cis-trans isomerization during synthesis and is an optically pure material based on the chirality of α -tocopheryl.

PTS has a melting point of 25⁰ C. Below its melting point, PTS is a yellow-orange wax. Above its melting point, PTS is a yellow-orange viscous fluid which dissolves in water, alcohols, dichloromethane and tetrahydrofuran, but not hexane. PTS is hygroscopic. PTS is stable at or below room temperature, over long periods of time (up to a year) in aqueous solution or in native form.

Figure 1. Structural Formula of PTS



The empirical formula for PTS is C₆₅H₁₁₈O₁₈ when n=12 and C₆₇H₁₂₂O₁₉ when n=13. The molecular weight of PTS was determined by MALDI-TOF mass spectrometry to be a distribution centered around 1209.6 g/mol.

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Specifications of PTS Solubilizer

The specifications for the PTS product are given in Table 1.

Table 1. Specifications of PTS		
Parameter	Method	Specification
Appearance	Visual	Light yellow wax/oil
Identification	IR	Conforms to standard
Purity	HPLC	> 90.0%
Impurities	HPLC	Total impurities < 10% No single impurity > 5%
Melting Point	Melting Point	25°C +/- 2°C
Moisture Content	KF	Not more than 1%
Residual Solvents	GC	Meets ICH Standards* Ethylacetate < 500 ppm Triethylamine < 100 ppm Cyclohexane < 400 ppm Acetonitrile < 40 ppm

*ICH guidelines assumes intake up to 10 g/day.

Manufacturing Process and Purity

PTS is synthesized by two consecutive esterification reactions. α -Tocopherol is linked to polyethylene glycol (mol. wt. 600–1000) using sebacoyl dichloride, by esterification at its hydroxy terminus. The resultant α -tocopheryl mono-ester of sebacoyl chloride is subsequently esterified with PEG₆₀₀ to form PTS. Reactions were performed under strictly anhydrous conditions in dry ethylacetate with triethylamine. PTS is prepared by sequential esterification, carried out in one pot. The reaction mixture is subsequently purified with an acetonitrile/hexane hot-cut to remove the major impurity, di- α -tocopheryl-sebacate which is formed in the first esterification as a by-product of sebacoyl chloride diester formation with α -tocopheryl. The PEG₆₀₀ impurity is unreacted PEG residue. The minor impurity, di- α -tocopherol-sebacate PEG, results from the reaction of two of these monoesters with PEG. The final product is isolated with a purity around 94%.

Chromatographic analysis of the resulting PTS product is presented in Attachment 1. As shown in Table 2, the resulting product consists of approximately 94% PTS, 2.9% unreacted residual PEG₆₀₀, 3.4% di- α -tocopheryl sebacate and trace amounts (0.1%) of (di α -tocopheryl sebacate) PEG₆₀₀.

Table 2. Analytical Composition of PTS	
Identity	Percent weight
PEG ₆₀₀	2.88
PTS	93.62
Di- α -tocopheryl sebacate	3.37
(Di- α -tocopherol-sebacate) PEG	0.14
Total	100

Proposed Use and Intake/Exposure to PTS

PTS, also a source of water-soluble vitamin E, will be used as a nutritive solubilizer for the dietary supplement ingredient Coenzyme Q₁₀ (CoQ₁₀) in either the fully oxidized form as ubiquinone or in the reduced form as ubiquinol. Upon ingestion and absorption, ubiquinone is almost completely converted in the body to the "active" antioxidant ubiquinol. CoQ₁₀ is practically insoluble in water. PTS exerts its technical effect as a water solubilizer by complexing with the water-insoluble dietary supplement ingredient without chemical bonds or altering the chemical structure of the CoQ₁₀ active ingredient.

The manufacturer has developed a novel method for water-solubilization and delivery of lipophilic compounds by using a polyethylene glycol (PEG)-derivatized natural compound, namely tocopherol, as a carrier of water-insoluble active ingredients such as CoQ₁₀. The CoQ₁₀ molecules form a non-covalent complex with the solubilizing carrier at the optimum molar ratio of 1:2 mol/mol (Figure 2). Although the physical structure of these complexes has not been elucidated, it is likely they form spherical nanomicelles with a hydrophobic interior and a hydrophilic outer shell of PEG. Particle size analysis has measured such nanomicelles at 20 nm. Such complexation facilitates ease of addition of the dietary supplement ingredient into water matrices.

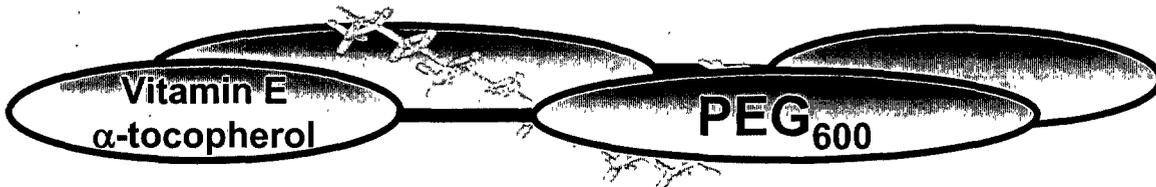


Figure 2: Representation of active ingredient and carrier non-covalent complex

The proposed product uses of PTS as a water solubilizer for CoQ₁₀ dietary supplementation are:

- dietary supplements sold as tablets or capsules,
- dietary supplement drinks
- dietary supplement sports drinks.

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CoQ₁₀ is typically added to dietary supplements at levels up to 200 mg in a single tablet or capsule and up to 30 mg in dietary supplement drinks or sports beverages per serving. The optimum ratio of PTS to CoQ₁₀ is about 3:1 on a w/w basis. We are proposing to use the solubilizer up to 200 mg CoQ₁₀ for dietary supplements and 100 mg CoQ₁₀ in dietary supplement drinks or sports beverages per serving. Such applications and use therefore correspond to PTS doses of up to 600 mg in dietary supplements and up to 300 mg in dietary supplement drinks and

supplemented sports drinks as detailed below. By weight, PTS is approximately 35% α -tocopherol, 45% PEG₆₀₀ and 20% sebacate.

For its application as a solubilizer, PTS is added to the dietary ingredient at about 3-fold the dietary ingredient concentration to form water soluble complexes. Because PTS contains about 35% α -tocopherol by weight, the amount of α -tocopherol in the required solubilizer would be roughly equivalent to the amount of the dietary ingredient in the formulation (i.e. with 33 mg of CoQ₁₀ ingredient and a maximum of 100 mg PTS containing 35mg equivalents of Vitamin E). Therefore, with typical amounts of CoQ₁₀ in dietary supplements ranging from 100-200 mg/day, the amount of PTS used for solubilization would be up to 600 mg in tablets or capsules and 300 total intake mg/serving in drinks. Thus, if a consumer ingested one tablet and two sports drinks/day, the intake of PTS is estimated to be 1200 mg/day or 20 mg/kg/day from the proposed uses.

The recommended dietary reference intake (DRI) of vitamin E (α -tocopherol) is in the range of 15-19 mg/day for adolescents, adults and lactating females. The National Academy of Sciences (NAS) has determined that the upper limit (UL) of α -tocopherol intake as a dietary supplement was 1000 mg/day in adults and 800 mg/day in adolescents and pregnant women. According to NAS, the UL is a maximal level of nutrient intake that is likely to pose no risks of adverse effects. The UL was based on animal studies where the no-observed-adverse-effect-level (NOAEL) was 500 mg/kg for hemorrhagic effects. Because PTS contains about 35% α -tocopherol by mass, the acceptable upper intake of PTS would be approximately 2400-3000 mg/day if it was the only source of dietary supplement intake of α -tocopherol.

Other sources likely for vitamin E intake are from the diet, dietary supplements containing vitamin E and multivitamin supplements. US national surveys indicate the dietary intake is at or below the DRI or about 15 mg/day. Typical supplement intake of vitamin E is 400 IU or about 266 mg α -tocopherol equivalents. Multivitamins contain low amounts of vitamin E, typically 30 IU or about 20 mg α -tocopherol equivalents. So the amount of vitamin E intake from both dietary supplementation and dietary intake in a population using dietary supplements is estimated to be approximately 300 mg/day.

Thus, if a consumer took one tablet or capsule containing 600 mg PTS and drank 2 servings of a dietary supplement drink daily containing 300 mg PTS, the added vitamin E intake would be approximately 420 mg vitamin E. In combination with the dietary intake and other possible forms of vitamin E supplementation, the total vitamin E intake for consumers associated with a reasonable estimate of PTS solubilization usage with CoQ₁₀ is approximately 700 mg/day. This figure is approximately 70% of the UL for vitamin E established recently by National Academy of Sciences, Institute of Medicine (IOM) and should therefore be considered acceptable.

PEG₆₀₀ comprises 45% of PTS by weight, so a comparable intake if a consumer took one tablet or capsule containing 600 mg PTS and drank 2 servings of a dietary supplement drink daily containing 300 mg PTS would be 270 mg and 270 mg, respectively, or a total of 540 mg/day PEG₆₀₀.

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Similarly, with sebacate being 20% of PTS by weight, estimated sebacate intake would be 120 mg from dietary supplement tablets and 120 mg from dietary supplement beverage intake or a total intake of 240 mg/day.

Regulatory Status of PTS Components

PTS is considered to be safe for human consumption based on the safety summary below and expected metabolism of PTS into its component substances. These components have GRAS, multiple food additive use or indirect food additive approvals in 21 CFR as noted in Table 3 below.

Table 3. Regulatory Status of PTS Components	
α -Tocopherol	<ul style="list-style-type: none"> • Essential nutrient; approved under fortification of foods at §104.50 • α-Tocopherol is a natural component in wheat germ, corn, sunflower seed, rapeseed, soybean oils, alfalfa and lettuce. • α-Tocopherol acetate has GRAS Status §182.8892 • JECFA ADI 0.25-2 mg/kg
Sebacate	<ul style="list-style-type: none"> • Multiple approvals of dibutyl and diethyl esters of sebacate as an indirect food additive. • Dibutyl and diethyl sebacate approved for use as synthetic flavors or adjuvants at §172.515.
PEG ₆₀₀	<ul style="list-style-type: none"> • PEG (200-9250) has approval as multipurpose food additive at §172.820 including adjuvant use in dispersing vitamin and mineral preparations and as a coating, plasticizer, lubricant and/or binder in tablets used for food; multiple other approvals as color diluents, fruit coating and in indirect additive uses. • JECFA ADI 0-10 mg/kg (2004)

Safety Studies

Studies of Absorption and Bioavailability

The National Academy of Sciences' Institute of Medicine (NAS IOM) report on vitamin E provides a useful summary of the absorptive processes occurring in the gastrointestinal tract for fat soluble vitamins such as vitamin E. The absorption of vitamin E in the GI tract is represented graphically in Figure 3. Vitamin E absorption from the intestinal lumen is dependent upon biliary and pancreatic secretions, micelle formation, uptake into enterocytes, and chylomicron secretion. Vitamin E acetate, the most often used analogue in food supplements and cosmetic products, is more stable due to its esterification and consequent protection from oxidation. In the gut, vitamin E esters are cleaved to their unesterified forms under the action of intestinal esterases.

In humans, vitamin E is taken up together with nutritional lipids in the proximal part of the intestine and released in the lymph within chylomicrons (Fig. 3). All forms of vitamin E are equally absorbed, which suggests the absence of selectivity at this level. After passing through the lymphatic pathway, the chylomicrons reach the systemic circulation, and are progressively hydrolyzed under the action of the endothelial lipoprotein lipase present in the target tissues. During this process, a part of vitamin E is released in the plasma and taken up by the cells.

In contrast to vitamins A and D, vitamin E does not seem to have any specific plasma carrier protein. Incorporated into plasma lipoproteins, it is non-specifically transported to the tissues. In the liver, the tocopherols are taken up from chylomicron remnants mainly via LDL receptor, and the α -tocopherol transfer protein (α -TTP) channels α -tocopherol specifically to organelles such as the rough endoplasmic reticulum and Golgi apparatus where very low density lipoproteins (VLDL) are synthesized.

Intestinal esterases are found within the intestinal lumen ((pancreatic lipase and bile salt activated lipase) (Borel, 2003; Miled *et al.*, 2000; Lowe, 1997; Hernell and Blackberg, 1994) and within the cytosolic compartment of the enterocytes (Mahan *et al.*, 2001; Mathias *et al.*, 1981). . Thus, it is likely that the PTS nanomicelles undergo extensive esterase hydrolysis within the gut lumen, thus releasing its component PEG, α -tocopherol and sebacate subunits for systemic absorption into circulation. It has been demonstrated that the uptake of α -tocopherol is mediated by its solubilization in micelles (Pearson and Legge, 1972). The actual uptake may involve both passive as well as active process involving the SR-BI transporter (Reboul *et al.*, 2006). Polyethylene glycol enters the circulation from the gut lumen through passive diffusion (Lloyd, 1998). Sebacic acid is a dicarboxylic acid (with 10 carbon chain length) which is likely absorbed like fatty acids by the small intestine (Tso *et al.*, 2004).

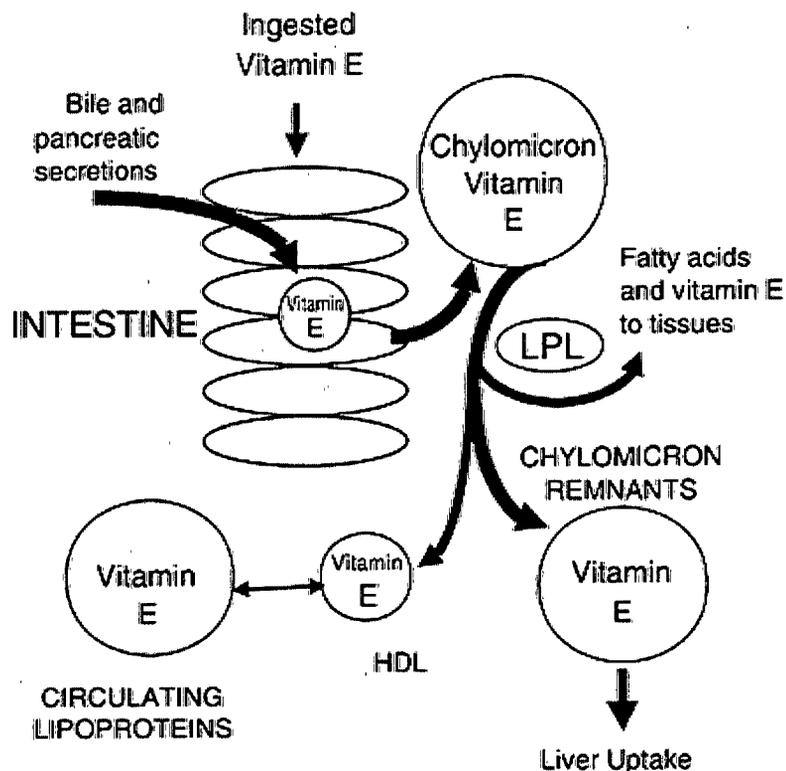


Figure 3 .Vitamin E secretion in chylomicrons and distribution to circulating lipoproteins. (From NAS, 2000)

Borel *et al.* (2001) have conducted investigations into the processing of vitamin A and E in the human gastrointestinal tract. Eight healthy men received intragastrically two lipid formulas differing in their fat-globule median diameter (0.7 vs. 10 micron). Formulas provided 28 mg vitamin A as retinyl palmitate and 440 mg vitamin E as all-rac- α -tocopherol. Vitamins were measured in gastric and duodenal aspirates, as well as in chylomicrons, during the postprandial period. The gastric emptying rates of lipids and vitamin A and E were similar. The free retinol/total vitamin A ratio was not significantly modified in the stomach, whereas it was dramatically increased in the duodenum. A significant fraction (42%) of retinyl palmitate ester was digested to free retinol in the duodenum lumen by 4 hrs, probably by pancreatic lipase. The authors concluded as follows: 1) there is no significant metabolism of vitamin A and E in the human stomach, 2) the enzyme(s) present in the duodenal lumen is significantly involved in the hydrolysis of retinyl esters, and 3) the size of emulsion fat globules has no major effect on the overall absorption of vitamin A and E. This study indicates that esterified fat-soluble vitamins such vitamin A and E undergo esterase-catalyzed hydrolysis in the duodenum and small intestine by pancreatic lipases to release their free retinol and tocopherol forms for further absorption. This was further supported by the finding that tetrahydrolipostatin, a gut lipase inhibitor, inhibits the absorption of retinyl palmitate, but not that of retinol.

A bioavailability study conducted by Borowy-Borowski *et al* (2004) clearly suggests that the weak ester linkages between PEG, sebacate and α -tocopherol in PTS are readily and completely degraded in the body by enzymatic hydrolysis by esterases into its individual components for subsequent systemic absorption separately. To establish that PTS is systemically metabolized to release active vitamin E, the plasma content of vitamin E was measured following both oral and intravenous delivery of PTS into rats. Elevated levels of vitamin E were detected in blood with concentrations 2 to 2.5 times higher than endogenous levels 3 hours after oral administration and nearly 5 times higher after intravenous injection as shown in Figure 4.

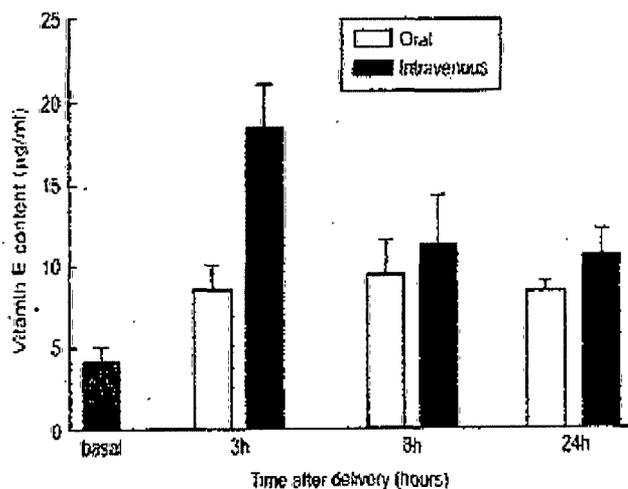


Figure 4: Release of Vitamin E after oral and intravenous administration of PTS

In addition, the bioavailability of CoQ₁₀ and vitamin E following oral dosing of PTS formulated as a non-covalent complex with CoQ₁₀ with a 2:1 molar ratio (PTS to CoQ₁₀) to rats has been investigated by Sikorska *et al.* (2003). Rats were given CoQ₁₀ (3 mg/kg body weight) and PTS (9 mg/kg body weight) by gavage. Blood samples were collected at 1, 3, 6, 12, 15, 18, 21 and 24 hrs and analyzed for vitamin E and CoQ₁₀ content. An elevated plasma level of vitamin E and CoQ₁₀ were clearly detectable over a period of 24 hours, peaking at 3 hrs for CoQ₁₀ and 9 hrs for vitamin E. Concentrations 3 to 4-times higher than the endogenous vitamin E content were measured 3-9 hrs after the ingestion, from approximately 2.44 µg/ml of plasma to 8.6 µg/ml at 3 hrs and 9.7 µg/ml at 9 hrs. Elevated plasma content was observed even after 24 hr post-ingestion.

A high plasma level of CoQ₁₀ was also detected over a period of 24 hrs post-ingestion. Moreover, the kinetics of its uptake were much more rapid than that of vitamin E. Within 1 hr post-ingestion, its plasma content already reached values 8 times higher than its endogenous level. At 24 hrs after dosing, the plasma CoQ₁₀ level significantly dropped, but it was still as high as that at 1 hr. Taken together, the data indicated that following oral administration, both of the active components of the formulation were bioavailable. PTS was metabolized to α -tocopherol and CoQ₁₀ was released and adsorbed rapidly through the gastrointestinal tract, after which both compounds appeared increasingly in the blood from early as the first sample at 1 hr.

Safety Studies on PTS and Other PEGylated Vitamin E Carriers

Screening studies were conducted on a series of pegylated lipophilic compounds to assess their suitability for use as carrier molecules for drugs (Borowy-Borowski *et al.*, 2004). Compounds of the following formula were synthesized and tested: X-OOC-(CH₂)_n-COO-Y, where X was a sterol, tocopherol or tocotrienol and Y was a polyethylene glycol or its derivative. They were produced by linking α -tocopherol, β -sitosterol, cholesterol or tocotrienols via ester linkages to polyethylene glycol (mol. wt. 600-1000) or methoxypolyethylene glycol (mol. wt. 750) using adipoyl, suberoyl, azelaoyl, sebacoyl or odecanedioyl dichlorides. One of the compounds synthesized was PTS, for which the results of these screening studies are presented below. However, because the likely metabolism of PTS will result in systemic exposure to its degradation components of α -tocopherol, sebacate and PEG₆₀₀ as separate compounds following hydrolysis and esterase action, these studies are considered supportive, but not fully determinative, of the safety of PTS. The safety of PTS primarily relies on the proven safe and non-hazardous character of its major components for food and dietary supplement use.

In vitro toxicity of PTS was tested on human immortalized NT2 cells grown under tissue culture conditions (Borowy-Borowski *et al.*, 2004). Cells were grown to 70% confluence in DMEM medium supplemented with 10% fetal bovine serum (FBS). PTS at the concentration range of 0-200 µg/ml was added directly to the tissue culture media for 16 hr overnight. Cell viability was measured by Trypan blue exclusion assay. The cells were also stained with Hoechst dye (DNA staining dye) to assess effects on the cell genome. PTS did not have any effects on NT2 cell viability nor was any alteration in either cellular or nuclear morphology found.

Acute cardiotoxicity was assessed in four male Sprague-Dawley rats (Borowy-Borowski *et al.*, 2004). The rat hearts were excised and arrested in ice-cold buffer solution. A water filled compliant balloon was inserted into the left ventricle and was connected to a pressure transducer to permit monitoring of the left ventricular pressure and heart rate. Mechanical function was

assessed as the heart rate, end diastolic pressure, developed pressure and the rate pressure product (RPP), the product of heart rate times left ventricular developed pressure and the flow. PTS was prepared as a concentrated working solution of 3 mM and delivered by aortic cannula, resulting in an effective concentration at the heart of 50 μ M. PTS was infused for a period of 30 minutes. Mechanical function was determined at baseline and at 5-minute intervals throughout the infusion of test compound. PTS infusion had no adverse effect on either cardiac function or flow; therefore, the compound demonstrated no acute functional cardiotoxicity in this screening assay.

The repeat dose toxicity of PTS was tested on 6 male Sprague Dawley rats (Sikorska and Borowy-Borowski, 2002). The animals were divided into two groups of 3 per group and groups received a solution of PTS via intra-peritoneal (ip) injection, of either 20 mg or 40 mg/kg body weight, every second day for a total of 15 treatments over a 30 day period. Brain, liver, kidney, spleen and skeletal muscle tissues from two rats/group were collected at necropsy and used for histopathological examination. All animals were reported to be in good condition during dosing and no significant external or internal abnormalities were noted. The results of the gross and histopathology examinations indicated that PTS did not exert pathological effects in the tissues examined.

The toxicity of PTS-CoQ₁₀ was also tested in 6 male Sprague Dawley rats (Sikorska and Borowy-Borowski, 2002). The animals were divided into two groups of 3 per group and groups received either 7 mg CoQ₁₀ or 20 mg PTS or 14 mg CoQ₁₀ and 40 mg PTS/kg body weight via ip injection, every second day, for a total of 15 treatments over a 30 day period. Brain, liver, kidney, spleen and skeletal muscle tissues from two rats/group were collected at necropsy and used for histopathological examination. All animals were reported to be in good condition during dosing and no significant external or internal abnormalities were noted. The results of the gross and histopathology examinations indicated that PTS-CoQ₁₀ did not exert pathological effects in these animals in any of the tissues examined.

In summary, PTS did not produce acute toxic effects in cell culture. Acute treatment in infused rat hearts did not affect cardiac function. Repeat dose ip administration to rats up to 14 mg CoQ₁₀ and/or 40 mg PTS/kg for 30 days on alternate days did not produce gross pathology or histopathological effects in the limited tissues evaluated. These results support the safety of the PTS product as a solubilizer of water-insoluble CoQ₁₀.

Further, safety data on d- α -tocopherol polyethylene glycol₁₀₀₀ succinate (TPGS), a very similar PEGylated molecule to PTS, has been reported in the literature. An extensive safety assessment and toxicological characterization of TPGS was conducted by Krasavage and Terhaar (1977). This water soluble form of α -tocopherol differs from PTS in that the linking molecule between α -tocopherol and the PEG adduct is the 4 carbon dicarboxylic acid succinate versus the 10 carbon dicarboxylic acid sebacate and has a slightly higher molecular weight addition of PEG addition. Even with these differences, the compounds are considered sufficiently similar structurally to draw meaningful direct comparisons. The similarities with PTS in both its ester linkages between a dicarboxylic acid linkage, PEGylation and probable micellar formation in combination with α -tocopherol are sufficient to be informative regarding the safety of the analogous compound PTS which is the subject of this notice.

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The acute oral LD₅₀ values for TPGS was 7000 mg/kg for young adult Charles River CD rats of both sexes.

In a subchronic toxicity study, groups of 30 Charles River CD rats of each sex were fed diets containing TPGS at dietary concentrations of 0, 0.002, 0.2, or 2% (approximately 1000 mg/kg/day) for 90 days. Hematological and clinical chemistry examinations were made on 15 rats of each sex in the control and high-dose groups at 42 and 84 days. At terminal necropsy, organ weights were determined for liver, spleen, brain, pituitary, kidneys, gonads, adrenals, and thyroids, and a histopathological examination was performed. TPGS up to 1000 mg/kg/day in the diet of rats had no effect on body-weight gain, food consumption, hematology, organ weights, serum chemistry, or histopathology (Krasavage and Terhaar, 1977).

At the end of the 90-day subchronic study on TPGS, half the rats from each dose group were maintained on their respective diets and used for a reproduction study. The dietary concentrations of TPGS were 0, 0.002, 0.2, and 2% (approximately 1000 mg/kg/day). The animals were mated on day 112 of treatment to produce the F_{1a} generation and on day 175 to produce the F_{1b} generation. The F₀ animals were maintained on their respective diets up to 265 days of treatment, then sacrificed and examined histopathologically. Reproductive indices (mean gestation period, litter size, sex ratio, and mortality of pups or parents) were unaffected by treatment. Clinical chemistry and hematological parameters were normal in the F₀ generation 10 days before terminal sacrifice. No effects on reproductive parameters or development of offspring were observed over two generations of rats dosed up to 1000 mg/kg/day in the diet (Krasavage and Terhaar, 1977).

Groups of 15 pregnant Charles River CD rats were given TPGS in the diet at concentrations of 0, 0.002, 0.2, or 2% on days 6 to 16 of gestation. On day 20 of gestation, the dams were sacrificed, the uteri excised, and the number of implantation sites (live fetuses, dead fetuses, or resorption sites) were counted. All the fetuses were examined for gross anomalies, soft-tissue abnormalities and skeletal defects. No differences were observed between controls and any of the treatment groups with respect to the parameters studied; therefore it was concluded that there were no adverse effects on fetal development or evidence for teratogenicity at doses up to 1000 mg/kg/day during the majority of gestation period (Krasavage and Terhaar, 1977).

In summary, TPGS, a compound similar to PTS, did not exhibit any adverse effects in reproductive, developmental, or subchronic toxicity studies when given orally to rats at doses up to approximately 1000 mg/kg/day, which was the highest dose tested in these studies. Therefore, the true NOAEL may actually be higher than 1000 mg/kg/day.

Safety Studies on Vitamin E

Given the common natural food presence, fortification as an essential nutrient and approval for multipurpose direct food addition of α -tocopherol, the safety of direct food addition of this component of PTS is considered well established at moderate intake levels. As with most essential vitamins and minerals, health concerns may be associated with excessive intake. The National Academy of Sciences' Institute of Medicine (NAS IOM) is an authoritative body tasked by FDA and other agencies to recommend dietary reference intakes (DRI) for essential nutrients. NAS IOM also recommends Tolerable Upper Intake Levels (UL), the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects in almost all individuals.

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NAS IOM evaluated vitamin E in 2000 (NAS, 2000). In its summary, they concluded the following:

“A large and growing body of experimental evidence suggests that high intakes of vitamin E may lower the risk of some chronic diseases, especially heart disease. However, the limited and discordant clinical trial evidence available precludes recommendations at this time of higher vitamin E intakes to reduce chronic disease risk. The Tolerable Upper Intake Level (UL) for adults is set at 1,000 mg (2,325 μ mol)/day of any form of supplemental α -tocopherol based on the adverse effect of increased tendency to hemorrhage.”

Animal studies show that α -tocopherol is not mutagenic, carcinogenic, or teratogenic (Abdo *et al.*, 1986; Dysmsza and Park, 1975; Krasavage and Terhaar, 1977). Animals fed extremely high doses of α -tocopherol or α -tocopheryl acetate have been shown to experience a variety of adverse effects, but the relevance of some of this information to the human situation was considered by NAS to be questionable.

α -Tocopherol can cause hemorrhage, increased prothrombin time, and interrupt blood coagulation in experimental animals, albeit very high doses are required. The hemorrhagic toxicity of α -tocopherol has been observed in rats (Abdo *et al.*, 1986; Takahashi *et al.*, 1990; Wheldon *et al.*, 1983). However, high doses of 500 mg/kg/day or more of *RRR*- α -tocopheryl acetate were necessary to induce the effects.

In the absence of human data pertaining to dose-response relationships, the data sets used by NAS IOM to identify a no-observed-adverse-effect level (NOAEL) for α -tocopherol include studies showing hemorrhagic toxicity in rats (Abdo *et al.*, 1986; Takahashi *et al.*, 1990; Wheldon *et al.*, 1983). A lowest-observed-adverse-effect-level (LOAEL) of 500 mg/kg body weight/day can be identified based on a critical evaluation of the study by Wheldon *et al.* (1983). They fed α -tocopheryl acetate to Charles River CD strain rats at levels of 500, 1,000, or 2,000 mg/kg body weight/day for 104 weeks. Hemorrhages from the gut, the urinary tract, the orbit and meninges, and the claws were observed in male rats only by week 15 in the highest-dose group, by week 16 in the intermediate-dose group, and by week 18 in the low-dose group

Takahashi *et al.* (1990) supplemented the diets of male Sprague-Dawley rats with 600 or 1,000 mg/kg body weight/day *RRR*- α -tocopheryl acetate for 7 days. Despite the short duration of the feeding trial, a dose-dependent increase in prothrombin time and partial thromboplastin time was noted in rats receiving 600 and 1,000 mg/kg/day. The number of animals with hemorrhages was similar in both dose groups. This study would yield a LOAEL of 600 mg/kg/day.

Abdo *et al.* (1986) conducted a 13-week study administering *RRR*- α -tocopheryl acetate in corn oil by gavage to Fischer 344 rats at doses of 125, 500, and 2,000 mg/kg body weight/day. In males, high levels of *RRR*- α -tocopheryl acetate (2,000 mg/kg/day) caused prolongation of both prothrombin time and activated partial thromboplastin time (APTT), reticulocytosis, and a decrease in hematocrit values and hemoglobin concentrations. However, no adverse hemorrhagic effects, other than a minimal increase in activated partial thromboplastin time at a dose of 500 mg/kg/day, were observed.

While some differences were encountered among the results of these three key studies, they could be attributed to the dosage approach (gavage versus diet), the time period of dosing, the

strain of rats, and possibly the level of vitamin K supplementation. The data of Wheldon *et al.* (1983) demonstrate that the hazard posed by excessive dietary intake of α -tocopheryl acetate can be overcome by administration of additional vitamin K. The Wheldon *et al.* (1983) study is considered the most definitive estimate because of the long exposure period of the dosage via diet. The LOAEL in this study was 500 mg/kg/day, the lowest dose tested. Thus, a precise NOAEL cannot be determined from the experiment. However, this LOAEL is consistent with the results of the shorter-term feeding study of Takahashi *et al.* (1990) with a LOAEL at 600 mg/kg/day, the lowest dose tested, and the gavage study of Abdo *et al.* (1986) with no adverse hemorrhagic effects at 125 mg/kg/day and only a minimal increase in activated partial thromboplastin time at 500 mg/kg/day of RRR- α -tocopherol acetate.

When determining an uncertainty factor (UF) for α -tocopherol, several sources of uncertainty were considered and combined into the final UF. A UF of 2 was used to extrapolate the LOAEL to a NOAEL. The severity of hemorrhagic effects justifies a UF greater than 1; however, the results of Abdo *et al.* (1986) showing no adverse effects at 125 mg/kg/day for hemorrhagic effects justify a UF of 2 to extrapolate from the LOAEL of 500 mg/kg body weight/day to the NOAEL of 250 mg/kg body weight/day. A UF of 2 was selected to extrapolate from subchronic to chronic intake, and a UF of 3 was selected to extrapolate from experimental animals to humans because of data showing some similarities between the animal and human responses. Another UF of 3 was selected to account for inter-individual variation in sensitivity. This value was deemed appropriate based on pharmacokinetic data showing plasma saturation of α -tocopherol concentrations with increasingly higher intakes in humans. The various UFs are combined to yield an overall UF of 36 to extrapolate from the LOAEL in animals to derive a UL for humans.

The LOAEL of 500 mg/kg/day was divided by the overall UF of 36 to obtain a UL value of 14 mg/kg/day for adult humans. The value of 14 mg/kg/day was multiplied by the average of the reference body weights for male and female adults, 68.5 kg. The resulting UL for adults by NAS IOM's analysis is 959 mg/day, which was rounded to 1,000 mg/day.

Safety Assessment of Sebacic Acid

Several safety studies have been reported on the disodium salt of sebacic acid. These studies are considered applicable to the safety evaluation of sebacic acid as it is likely that the disodium compound will ionize and dissociate in the gastrointestinal tract to sodium ions and sebacic acid.

The toxicology of disodium sebacate has been extensively evaluated by Greco *et al.* (1990). The acute toxicity of disodium sebacate was investigated after oral, i.p. and i.v. administration to 220 Wistar rats (110 males and 110 females) and 204 New Zealand rabbits (102 males and 102 females). No oral acute toxicity by lethality was found up to 6000 mg/kg. The i.p. LD₅₀'s were 5500 mg/kg in rats and 6000 mg/kg in rabbits. When sebacate was given i.v., the median lethal dose was 560 mg/kg for rats and 1400 mg/kg for rabbits. Similar results were obtained in corresponding groups of animals (in total 220 rats and 204 rabbits) given oral, i.p. and i.v. saline solutions with added glucose in order to obtain the same value of osmolarity and sodium ion concentration. The above results appear indicative of relatively low toxicity of disodium sebacate, even suggesting that the toxic effects found could be due to the sodium content of the compound administered.

Chronic toxicity was evaluated by feeding disodium sebacate to 10 rats or rabbits/sex/group in the diet to achieve 500 or 1000 mg/kg/day in rats and 750 or 1000 mg/kg/day in rabbits for six

months. When compared to the control animals, no significant differences in biological parameters (clinical chemistry and hematological values, growth curves and histological findings for the different organs) were observed in the test groups during the treatment period.

Fetal toxicity, teratogenicity and neonatal toxicity were investigated in twenty female rats by feeding of 500 mg/kg bw in diet on gestation days 10-19) and twenty female rabbits at 1000 mg/kg bw in diet on gestation days 10-25. Sebacic acid did not show any teratogenic effect and the development of the fetuses was normal. Neonatal toxicity was also evaluated using ten female rats treated with 500 mg/kg in diet for 90 days through weaning of pups and ten female rabbits with 1000 mg/kg in diet for 90 days through weaning of offspring, followed by histopathological examination of organs. The no-observed-adverse-effect-level (NOAEL) in these studies in rats and rabbits was 1000 mg/kg bw/day in the diet, with no adverse effects on fetal or neonatal development or organ pathology in offspring observed.

Sebacic acid was not mutagenic in multiple *Salmonella* tester strains with and without activation up to 5000 µg/plate nor was it mutagenic in *E. coli* WP2 UVRA strain (Shimuzu *et al.*, 1985).

The pharmacokinetics of disodium sebacate was studied in Wistar rats of both sexes. Sebacate was administered either as intra-peritoneal (i.p.) bolus (six doses ranging from 10 mg to 320 mg) or as oral bolus (two doses: 80 and 160 mg) (Favuzzi *et al.* 1999). The sebacate half-life was 31.5 min. The tissue elimination rate was 0.0122 min^{-1} . The overall volume of distribution was found to be 26.817 ml/100 g bw. The renal clearance was 0.291 ml/min/100 g of bw, suggesting the presence of reabsorption from the ultrafiltrate. The value of sebacate renal clearance was found to be a concentration-independent function, also suggesting the presence of a passive back-diffusion. The relative bioavailability of the oral form compared to the i.p. form was 69.09%, showing a good absorption.

Another study investigated the metabolic disposition of radiolabelled sebacic acid in rats. Three groups of experimental animals received different doses of disodium sebacate with 25 uCi of ^{14}C -labeled molecule by intravenous injection (Tataranni *et al.*, 1992). In the first group, radioactivity plasma elimination curves were examined for two administered doses (80 and 160 mg). In the second group, expired $^{14}\text{CO}_2$, urine tracer and feces tracer were counted after intravenous administration of 160 mg of sebacate. The animals of the third group were sacrificed at different times after intravenous administration of 160 mg of sebacate, and tracer elimination curves were obtained for several organs. The plasma half-life of sebacate is 38.71 min; about 35% of the administered tracer was excreted in the urine as unchanged sebacate and about 25% was eliminated as $^{14}\text{CO}_2$ in expired air. Disposition of sebacate was complete within 4 hr of administration. The sebacate half-life is longest in adipose tissue (135 min) and in liver (74 min), sites of likely transformation. In all other organs examined, the sebacate half-life is similar to that in plasma.

Dicarboxylic acids have been proposed as an alternate lipid energetic substrate for total parenteral nutrition. A human study has examined the effect of a continuous intravenous infusion of the sodium salt of the 10-carbon atom aliphatic dicarboxylic acid, sebacate, on insulin-dependent glucose metabolism in four control subjects, four patients with insulin-dependent diabetes mellitus, and four obese subjects (Raguso *et al.*, 1994). All subjects received a constant 5-hour infusion of saline or sebacate (6.6 g/h), in a randomized order on two different

days. After 3 hours of infusion, a 120-minute euglycemic, hyperinsulinemic clamp procedure was performed (insulin infusion rate = 40 mU/m² per minute). Glucose uptake, plasma sebacate, insulin, glucagon, C-peptide, and ketone bodies were measured. No significant differences in insulinemia were found among groups either during the saline infusion or the sebacate infusion. On the contrary, glucose uptake (molar) was significantly reduced during the sebacate vs. the saline day in all three groups: 6.7 vs. 3.7 in control subjects ($p < 0.001$), 4.6 vs. 2.5 in patients with insulin-dependent diabetes mellitus ($p < 0.001$), and 4.8 vs. 2.7 mg/kg per minute in obese subjects ($p < 0.001$). In conclusion, sebacate administration was associated with a glucose-sparing effect as shown by the reduced glucose uptake in all patients studied. Sebacate did not stimulate insulin secretion, inasmuch as no modification of C-peptide plasma levels was observed after 3 hours of sebacate infusion.

In order to better ascertain its possible use as an alternative fuel substrate in total parenteral nutrition, sebacate metabolism was studied in seven overnight-fasting healthy male volunteers, who received a constant iv infusion (99 mmoles over 8 hours) of disodium sebacate (Mingrone *et al.*, 1991). Sebacate oxidation rate was determined using an isotopic sebacate (disodium salt of ¹⁴C-sebacic acid) infusion (100 uCi from the fourth to the eighth hour of the cold sebacate infusion). Blood samples were collected during and after sebacate infusion at intervals of 30 minutes and sebacate serum concentrations were determined by high performance liquid chromatography. The sebacate serum level at the plateau phase was 4.54 umole/mL, the overall rate of tissue uptake was 180.89 mumole/min, and the percent oxidation was 6.14%. These data show that a relatively small amount of the sebacate infused is oxidized in human tissues.

From these animal and human studies, sebacic acid has been shown to be readily absorbed, has a short plasma half-life, and is widely distributed in the body where it is used as fuel substrate like other fatty acids. No adverse effects on glucose utilization or insulin response were noted in diabetic subjects given sebacic acid.

In summary, dicarboxylic fatty acids such as sebacic acid (C 10) would not pose any notable toxicological concerns. This is supported by the lack of microbial mutagenicity, practical non-toxicity in acute oral studies, and absence of toxicological effects in chronic toxicity and teratological evaluations. Based on the NOAEL in chronic toxicity studies reported by Greco *et al.* (1990) of 1000 mg/kg/day, and given that the likely intake of sebacic acid in PTS would be approximately 4 mg/kg/day for the proposed uses and levels, there is an ample 250-fold margin of safety from the chronic NOAEL in animals.

Safety Studies on Polyethylene Glycol

Polyethylene glycols (PEG) are condensation polymers of ethylene oxide (mw ~44 each subunit). The various grades of PEG are named by their molecular weight and may range from PEG₂₀₀ (10 subunits) to PEG₂₀₀₀₀. In PTS, PEG₆₀₀ is used with 12-13 subunits. The properties of the PEGs vary with molecular weight, with those under 700 being colorless liquids and over 1000 MW being white waxy solids.

The oral absorption of PEG is largely dependent on molecular weight. In one study, polyethylene glycols having average molecular weights of 4000 and 6000 showed no absorption from the rat intestine over a five-hour period, while polyethylene glycols of 1000 and 1540 molecular weights showed a slight absorption amounting to less than 2% of the total dose during the same period. In a more recent study, polyethylene glycols (PEG's) 600, 1000, and 2000 were

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used to study the molecular weight permeability dependence in the rat nasal and gastrointestinal mucosa by measuring the urinary excretion of PEGs over a 6 h period to quantitate absorption. The permeabilities of both the gastrointestinal and the nasal mucosa exhibited similar molecular weight dependencies. For PEG 600 and 1000 the mean absorption from the nasal cavity is about 14%, while that from the gastrointestinal tract is only 9% (Donovan *et al.*, 1990).

The toxicology of the polyethylene glycols was summarized in a Cosmetic Ingredient Review in 1993 (CIR, 1993). The PEG's with the closest molecular weights to PEG₆₀₀ in this report were identified as PEG-8 at MW 400 and PEG-32 with MW 1500.

The acute oral LD50's were generally > 30 g/kg in all species tested for PEG-8 and >50 g/kg for PEG-32.

PEG₄₀₀ was evaluated in a series of genetic toxicity assays in 1980 by Bushy Run Research Center of Union Carbide as cited in CIR (1993). These study reports were submitted to the Cosmetic, Toiletries and Fragrance Association (CTFA) for the CIR review. PEG₄₀₀ was negative for CHO mammalian cell point mutation, unscheduled DNA synthesis (UDS) in rat hepatocytes and sister chromatid exchange (SCE's) in CHO cells with and without metabolic activation as needed. PEG₄₀₀₀ was reported positive in the mouse lymphoma TK assay at 150 g/l, but not at concentrations of 125 g/l or lower, raising the potential confounding factor of high osmolarity of the test material (Wangenheim and Bolcsfoldi, 1988).

Various polyethylene glycols were fed to rats (5 males and 5 females per dose level at 0, 2, 4, 8, 16 and 24% w/w of the diet) for 90 days. Criteria studied were mortality, food consumption, body weight gain, liver weight, kidney weight and pathology of liver and kidney (Smyth *et al.*, 1955). For PEG₆₀₀, the NOAEL was 8% (~4 g/kg bw /day), with increased kidney weight at 16% in diet.

PEG₂₀₀ was administered orally to monkeys (*Macaca fascicularis*) and rats (Sprague-Dawley) for a 13-week period at dosage levels of 2 to 4 ml/kg bw (monkeys) and 2.5 to 5.0 ml/kg bw (rats) per day (Prentice and Majeed, 1978). Pathological lesions were encountered only in monkeys and these consisted of intratubular deposition of small numbers of oxalate crystals in the renal cortex. These lesions were not associated with other clinical or pathological findings.

Dosages of 0.016 to 1.6 g/kg/day of PEG 6-32 did not cause any significant adverse effects in mortality, frequency of infection, life-span, fluid consumption, body weight gain, kidney and liver weights, frequency of size of litters, blood cytology, urinary albumen and sugars, occurrence of neoplasms, and micropathology in albino rats when administered in the drinking water over a two-year period (Smyth *et al.*, 1947, 1950).

When fed to rats for two years as a part of their diet, PEG 1540 and 4000 had no effect at a level of 4% and PEG₄₀₀ had no effect at a level of 2% (1000 mg/kg bw /day). In these animals, higher levels of polyethylene glycols produced small, nonspecific effects upon growth or minor cloudy swelling of the liver (Smyth *et al.*, 1955).

Groups of 20 male and 20 female rats were fed 4.0, 2.0, 1.0, 0.5 and 0% w/w of PEG₂₀₀ in their diet for two years and observed for food consumption, mortality rate, number of infections, life-span, growth rate, liver and kidney weights, gross pathological condition of organs, blood

hematocrit values, and incidence of neoplasms. The results indicated that, even at 4.0% dose level (2000 mg/kg bw /day), PEG₂₀₀ produced no significant effects differing from the control rats during the two-year feeding study (Weil and Smyth, 1956).

Polyethylene glycols 400, 1540 and 4000 cause no adverse effect upon dogs when fed at 2% of the diet for one year (Smyth *et al.*, 1955).

The potential teratogenicity of PEG₂₀₀ was investigated in CD-1 female mice orally dosed on gestation days 6-17 with 0.5 and 0.7 mL/animal/day (~15 and 21 g/kg bw /day) of undiluted PEG₂₀₀ (Vannier *et al.*, 1989). At sacrifice on day 18 live fetuses were examined for external, visceral and skeletal malformations. No signs of maternal toxicity were noted except for the death of one high-dosed female on sacrifice day. Some slight teratogenic effects were in evidence at 0.5 mL/animal/day and were more marked at 0.7 mL/animal/day. Fetal loss was slightly increased and mean fetal bodyweight was lower in treated groups compared with controls. Severe malformations of the skull (exencephaly, fissure in the median facial line), of the paws (dysgenesis of long bones and digits) and of the thoracic skeleton (joined ribs and vertebrae) were noted. PEG₂₀₀ administered at extremely high dose levels was considered teratogenic in the mouse. Sprague Dawley female rats were also orally dosed on gestation days 6-14 or 11-16 with 1.5 to 5 mL/animal/day (~5-15 g/kg bw /day) (Vannier *et al.*, 1989). Some maternal deaths occurred at all dosages. Fetal loss and fetal body weight remained within normal limits. In rats, even at extremely high dose levels, no evidence of teratogenicity was observed, even with evident maternal toxicity.

In another set of teratology studies, timed pregnant Sprague-Dawley rats and New Zealand white rabbits were randomly assigned to four dose groups (10/group) (Gupta *et al.*, 1996). The animals were dosed between gestational days 6-17 (rats) and 6-18 (rabbits) by oral gavage at dose volumes of 1 mL/kg bw (rats) and 2 mL/kg bw (rabbits) with either 0.5% methylcellulose, PEG₄₀₀, cremophor, or 0.1% carboxymethylcellulose. Body weights and food consumption were recorded daily. Cesarean sections were performed on gestational days 21 and 28 for the rats and rabbits, respectively. Reproductive parameters, numbers of corpora lutea, implantation sites, and resorptions were recorded and the fetuses were examined for external, visceral and skeletal malformations. There were no treatment-related mortalities. Body weights, food consumption and reproductive parameters were comparable between the groups. Some differences were noted in the incidences of minor anomalies between groups, but none were biologically significant.

Fischer-344 rats (10/group/sex) were administered PEG₄₀₀ by gavage at 1.0, 2.5 or 5.0 ml/kg (1.1, 2.8 and 5.6 g/kg, respectively) bw /day 5 days/wk for 13 wk (Hermansky *et al.*, 1995). An additional 10 rats/sex/group were assigned to the control and high-dose groups for a 6-wk recovery period. There was no mortality or changes in hematology or clinical chemistry measurements attributed to PEG₄₀₀ toxicity. Increased urinary concentration and decreased urinary pH were at least partially attributed to absorption, possible metabolism, and urinary excretion of PEG₄₀₀. Small increases in absolute and/or relative kidney weights observed in many dose groups were attributed to the osmotic effect of the test substance and/or metabolites in the urine. The significance of a slight increase in relative kidney weights in female rats following the recovery period was unknown. Although no microscopic changes were observed in the kidneys or urinary bladder, a slight, reversible renal toxicity may have resulted in male rats treated by gavage with 2.5 ml/kg/day and rats of both sexes treated by gavage with 5.0 ml PEG₄₀₀ kg/day. This was based on the increased concentration of protein and bilirubin, urinary

vascular cell findings and N-acetyl-beta-D-glucosaminidase activity. The authors concluded that PEG₄₀₀ in mid to high dose levels may have caused slight, reversible renal toxicity in rats.

Rats given dosages of 0.016 to 1.6 g/kg/day of PEG 6-32 administered in the drinking water over a two-year period were allowed to breed and produce F₁ and F₂ generations (Smyth *et al.*, 1947). No adverse reproductive effects or other notable changes were found in these two generations of offspring.

In summary, PEG's in the MW range of 400 to 1500 are poorly absorbed by the oral route (<10%) and are practically non-toxic in acute oral studies. PEG₄₀₀ was non-genotoxic in multiple mammalian cell assays for mutagenicity, DNA interaction and clastogenicity. At high dose levels 2-4 g/kg/day, the kidney is the apparent target organ for PEG's, but the observed histopathological changes at effect levels were mild and reversible. In subchronic and/or chronic toxicity studies in rats, monkeys and dogs, the NOAEL for adverse effects was approximately 1000 mg/kg/day. At extremely high dose levels of 15 g/kg/day PEG₂₀₀, teratogenic effects were noted in mice, but not in rats. In rats and rabbits given 1 or 2 g/kg/day PEG₄₀₀ respectively, no morphological changes indicative of teratogenicity were observed. Breeding trials associated with long-term drinking water studies of PEG 6-32 with intake up to 1.6 g/kg/day did not show reproductive effects in rats. Therefore, based on the above, PEG₆₀₀ should not pose any notable toxicological concerns. Based on the NOAEL in subchronic and chronic toxicity studies in three species (dogs, rats and monkeys) of 1000 mg/kg/day, and given that the likely intake of PEG₆₀₀ in PTS would be approximately 9 mg/kg/day for the proposed uses and levels, there is an adequate 110 fold margin of safety from the chronic NOAEL in animals.

SAFETY ASSESSMENT

The proposed product uses of PTS as a water solubilizer for CoQ₁₀ dietary supplementation are in dietary supplements sold as tablets or capsules or dietary supplement drinks and sports drinks. The maximal amount of PTS to be used for solubilization would be up to 600 mg in tablets or capsules and 300 mg/serving in drinks. Thus, the total intake of PTS is estimated to be 1200 mg/day or 20 mg/kg/day from the proposed use.

Using a conservative estimate of potential supplement intake, where a consumer took one tablet or capsule containing 600 mg PTS with CoQ₁₀ and drank 2 servings of dietary supplement drinks daily containing 300 mg PTS each, the added vitamin E intake from this consumption would be approximately 420 mg vitamin E, 540 mg/day PEG₆₀₀ and 240 mg/day sebacate.

The discussion and data presented regarding the metabolism and bioavailability of PTS and its components strongly indicates that PTS is almost completely degraded to its major components by enzymatic hydrolysis via intestinal esterases in the gut lumen and brush border cells. Thus, systemic absorption and exposure would be to α -tocopherol, sebacate and, to a lesser extent, PEG₆₀₀ which is rather poorly absorbed (<10%).

PTS has been investigated in limited toxicological studies which have shown a lack of acute cardiotoxicity or adverse effects at doses up to 40 mg/kg/day ip given every other day for 30 days. A comprehensive toxicological evaluation has been conducted on an analogous compound, d- α -tocopherol polyethylene glycol₁₀₀₀ succinate (TPGS). TPGS, a compound which is expected to show a similar toxicological profile to PTS, did not exhibit any adverse effects in reproductive, developmental, or subchronic toxicity studies when given orally to rats at doses up

to approximately 1000 mg/kg/day in these studies, which was the highest dose tested, so the NOAEL may actually be substantially greater. The proposed intake of PTS is approximately 20 mg/kg/day, or 50 fold lower than the apparent NOAEL.

NAS IOM has set a UL of 1000 mg/day for α -tocopherol, based on animal studies indicating reduced clotting times and possible hemorrhagic effects. However, clinical studies in humans indicate that much higher levels may be taken safely for cardioprotective effects without hematological effects. A maximal estimate of probable α -tocopherol intake was used for the intake estimation, including normal dietary and other supplementation that totals approximately 300 mg/day. With the proposed use and intake of 420 mg/day of α -tocopherol in PTS associated with CoQ₁₀ and 300 mg/day from other sources, the estimated maximal intake of 720 mg/day is below the recommended UL of 1000 mg/day by NAS IOM. Therefore, the proposed use of PTS would not result in excessive α -tocopherol intake that would pose a safety concern.

As noted in the safety studies summary of sebacic acid, sebacic acid does not pose any notable toxicological concerns. This is supported by the lack of microbial mutagenicity, practical non-toxicity in acute oral studies, and absence of toxicological effects in chronic toxicity and teratological evaluations. Based on the NOAEL in chronic toxicity studies reported by Greco *et al.* (1990) of 1000 mg/kg/day, and given that the likely intake of sebacic acid in PTS would be approximately 4 mg/kg/day for the proposed uses and levels, there is an ample 250-fold margin of safety from the chronic NOAEL in animals.

As discussed in the safety study summary on PEGs, PEG₄₀₀ was non-genotoxic in multiple mammalian cell assays for mutagenicity, DNA interaction and clastogenicity. In subchronic and/or chronic toxicity studies in rats, monkeys and dogs, the NOAEL for adverse effects was approximately 1000 mg/kg/day. In rats and rabbits given 1 or 2 g/kg/day PEG₄₀₀ respectively, no morphological changes indicative of teratogenicity were observed. Breeding trials associated with long term drinking water studies of PEG 6-32 with intakes up to 1.6 g/kg/day did not show reproductive effects in rats. Therefore, PEG₆₀₀ should not pose any notable toxicological concerns. Based on the chronic NOAEL of 1000 mg/kg/day, and given that the likely intake of PEG₆₀₀ in PTS would be approximately 9 mg/kg/day for the proposed uses and levels, there is an adequate 110 fold margin of safety. This is also below the JECFA ADI of 10 mg/kg/day for PEGs. Therefore, the proposed intakes of PEG₆₀₀ have an adequate margin of safety and are not considered to pose any safety concerns.

In summary, neither PTS nor any of its degradation products, that are likely to be systemically absorbed, pose any toxicological hazards or safety concerns at the proposed levels of addition for solubilization of the active ingredients CoQ₁₀ in dietary supplementation.

CONCLUSION

Based on a critical evaluation of the pertinent data and information summarized above, the Expert Panel members, whose signatures appear below, have individually and collectively determined by scientific procedures that addition of PTS solubilizer with CoQ10, meeting the specifications cited above and manufactured accordance with current good manufacturing practice, is generally recognized as safe (GRAS) under the conditions of intended use in dietary supplements and dietary supplement drinks, as specified herein.

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5/29/06
Date

Sidney Green, Jr., Ph.D., ATS

5/24/06
Date

Stanley M. Tarka, Jr., Ph.D.

5/26/06
Date

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Attachment 1

Figure 1. Chromatographic Analysis of PTS

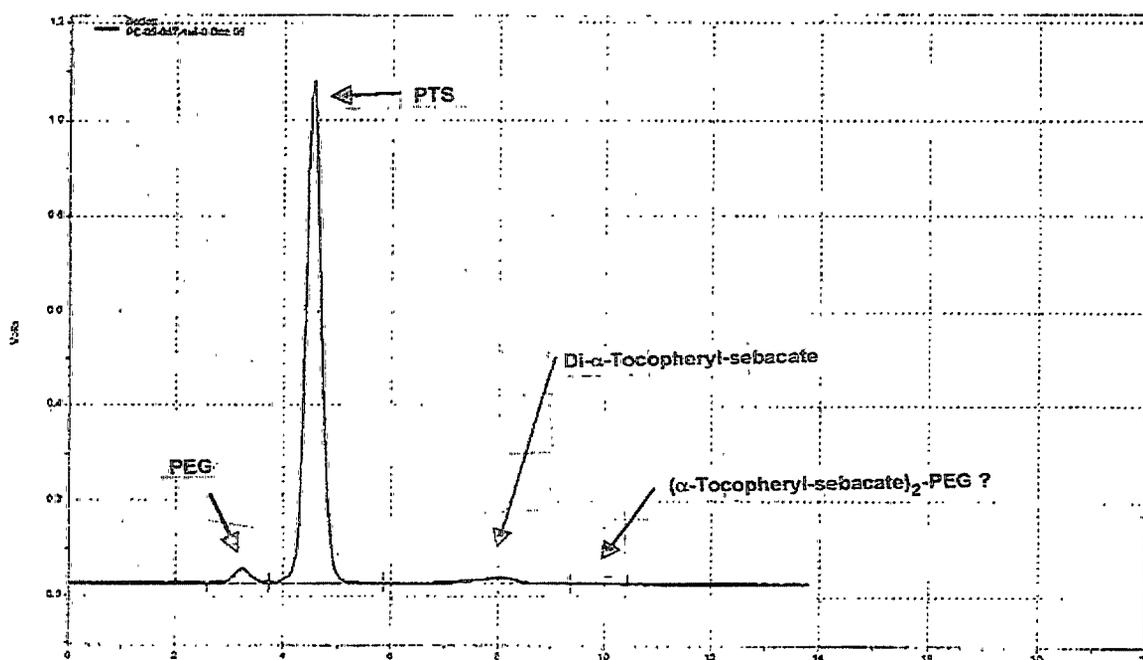
Figure 2. Mass Spectroscopic Analysis of PTS

Analysis of Product

PTS is analyzed using HPLC. A typical chromatogram (Figure 1) was obtained by injecting PTS Lot PC-05-047 into a Supelco reverse phase Supelcosil LC-18-DB column with a 1:1 mixture of CH_2Cl_2 :MeOH flowing at 1 mL/min. Light scattering detection was used. Integration data including impurities are supplied in Table 1. A typical mass spectrum, for Lot PC-05-047, is given in Figure 2. The spectrum is centered around a mass of 1210.

Identity	RT, min	Area	Area %
PEG ₆₀₀	3.242	659620	2.88
PTS	4.550	21441866	93.62
Di- α -tocopheryl sebacate	8.033	771382	3.37
(Di- α -tocopherol-sebacate) PEG	9.808	31338	0.14
Total			100

Table 1: Chromatographic Integration



SUBMISSION END

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