ORIGINAL SUBMISSION

Generally Recognized As Safe (GRAS) Determination for the Use of Palm Tocotrienol Rich Fractions (TRF) as Ingredients in Food

Prepared for :
Malaysian Palm Oil Board
Washington, D.C.

Prepared by:
NutraSource
Clarksville, Maryland

October 2009

NutraSource, Inc.
6309 Morning Dew Ct, Clarksville, MD 21029
(410)-531-3336 or (301) 875-6454

Office of Food Additive Safety (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5 100 Paint Branch Parkway
College Park, MD 20740

October 19, 2009

Subject: GRAS notice for palm tocotrienol rich fractions (TRF)

Dear Sir/Madam:

Pursuant to the proposed rule outlined at *Federal Register*, Vol. 62, No. 74, p. 18936 - 18937, April 17, 1997 (Sec. 170.36 of Part 21 of the *Code* of *Federal Regulations*), Malaysian Palm Oil Board (MPOB) hereby submits this notification that the use of palm tocotrienol rich fractions (TRF) as ingredients in foods is exempt from the premarket approval requirements of the Food, Drug, and Cosmetic Act because MPOB has determined that such use is generally recognized as safe (GRAS) based on scientific procedures.

On behalf of MPOB, NutraSource (an independent consulting firm) assembled a panel of experts highly qualified by scientific training and experience to evaluate the safety of the intended uses of palm TRF. The panel included Dr. Susan Cho at NutraSource (Clarksville, MD), Dr. Michael Falk at Life Science Research Organization (LSRO; Bethesda, MD), and Dr. George Fahey at the University of Illinois (Urbana, IL). Following independent critical evaluation of the available data and information, the panel has determined that the use of palm TRFs (that are manufactured by the industry members of MPOB, such as Carotech, Davos, Carotino, Sime Darby Bioganics, and SuperVitamins) described in the enclosed notification is GRAS based on scientific procedures.

Pursuant to the proposed 170.36 (c)(1), MPOB provides the following required information:

Notifier:	Malaysian Palm Oil Board (MPOB) TAS America
Mailing address:	3516 International Ct, N.W. Washington, D.C. 20008 (Attention: Ms. Rosidah Radzian, Regional Manager) e-mail: mpobtas@aol.com
GRAS substance:	Palm tocotrienol rich fractions (or palm tocotrienol concentrates)
Intended Use:	Fats or oils, such as salad dressing, mayonnaise, margarine, and spread; potato chips and salty snacks; bakery products; ready-to-eat cereals; meal replacement and other functional beverages; meatless soups and soup mix; meatless gravies, sauces, and pasta sauces; processed juices, processed fruits, juice drinks, and punch; frozen dairy desserts; yogurt; pudding;

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gelatin products; chocolate milk and flavored milk; soy milk and products; chocolate, candy, confectionary, and sweets; beverages; and chewing gum.

Basis for GRAS determination: Scientific procedures

The conclusions of these scientists and the summary of the information regarding the safety and GRAS status of the material in making this determination are enclosed. Also enclosed are three copies of the GRAS notification, an electronic copy, and additional information documents. All of the data and information that serve as the basis for this GRAS determination will be available to FDA upon request or is available for FDA's review and copying at the offices of MPOB located in the Malaysian Embassy Building in Washington, D.C. or at the office of NutraSource located in Clarksville, MD.

If you have any technical questions, please do not hesitate to contact me.

Sincerely,

Susan Cho, Ph.D.
sscho397@yahoo.com
Consultant to MPOB
(on the TRF GRAS notification project)

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Conclusion of the expert panel:
Generally recognized as safe (GRAS) determination for the addition of palm tocotrienol-rich fraction (TRF) to foods

August 2009

CONCLUSION

We, the undersigned expert panel members, Susan Cho, Ph.D., Michael Falk, Ph.D., and George Fahey, Ph.D., have individually and collectively critically evaluated the materials summarized in the palm TRF GRAS report, and conclude that palm TRF is safe and GRAS for its intended use in foods.

There is broad-based and widely disseminated knowledge concerning the chemistry of tocotrienols (T3) and tocopherols (T). Vitamin E is a generic name for the family of eight major compounds that have been found to possess vitamin E activity. These compounds are α-, β-, γ-, and δ-T; and α -, β-, γ-, and δ-T3, and are often called tocols. They cannot be synthesized in the body; thus, vitamin E must be obtained from the diet. Vitamin E deficiency results in serious health hazards. Symptoms caused by α -T deficiency can be alleviated by T or T3. Both T3 and T possess antioxidant activities, although T3 has much stronger antioxidant activities against lipid peroxidation than T. Palm oil and rice bran oil represent two major nutritional sources of natural T3. The TRF described in this self-affirmation is prepared from palm oil.

Palm TRF (a mixture of α -, β-, γ-, and δ-T3 with an average α-T content of 20-30% of total tocols) proposed for use in this food product is well characterized and free from chemical and microbial contamination. Palm TRF will be used as an ingredient in foods and beverages as a source of natural vitamin E and antioxidants.

Intended food applications include fats or oils, such as salad dressing, mayonnaise, margarine, and spread, potato chips and salty snacks, bakery products, ready-to-eat cereals, meal replacement and other functional beverages, meatless soups, soup mix meatless gravies, sauces, pasta sauces, processed juices, processed fruits, juice drinks and punch, frozen dairy desserts, yogurt, pudding, gelatin products, chocolate milk, flavored milk, soy milk and products, chocolate, candy, confectionary, sweets, beverages, and chewing gum.

Assuming 20% of food products will be used under the intended use, the 90th percentile daily consumption under proposed new food use of T3 would result in 9.4 mg/d (or 0.68 mg/kg body weight (BW)/d) in young children aged 1-3 yr and 17.4 mg/d (or 0.35 mg/kg BW/d) for the population aged 1 yr and older. These levels represent more than 100x below the no-observed-adverse-effect level (NOAEL) values that have been found from sub-chronic and chronic toxicity studies in rats. An assumption that 20 % of the population are consumers of TRF leads to dietary α-T exposure estimates of approximately 6.4 mg/d (or 0.49 mg/kg BW/d) for children aged 1-3 yr and 14.6 mg/d (or 0.27 mg/kg BW/d) for the population aged 1 yr and older at the 90th

percentile. These levels are far below the tolerable upper intake levels of α-T (200 mg/d for children aged 1-3 yr and 1000 mg/d for adults) established by the U.S. Institute of Medicine.

Currently, α-T (as a nutrient and as a chemical preservative), tocopherols, and α-T acetate (as a nutrient) are already listed as GRAS ingredients under the 21 CFR Part 184 and the 21 CFR Part 182. Dietary supplements containing T3 have been on the market in the U.S. as dietary supplements since 1999 under the *Dietary Supplements Health and Education Act of 1994* (DSHEA). Due to the current GRAS approval status of T, this GRAS self-affirmation is more focused on the review of T3.

Palm TRF has a long history of safe use as a natural vitamin E and as an antioxidant in foods. There are no indications of significant adverse effects related to palm TRF in the publicly available literature. The proposed food use results in exposure at levels significantly below those associated with any adverse effects. Therefore, not only is the proposed use of palm TRF safe within the terms of the Federal Food, Drug, and Cosmetic Act (meeting the standard of reasonable certainty of no harm), but because of this consensus among experts, it is also *Generally Recognized as Safe* (GRAS) according to Title 21 Code of Federal Regulations (21 CFR).

Susan Cho, PhD
President, NutraSource, Inc., Clarksville, MD 21029

Signature: _____ Date: _____

Michael Falk, Ph.D.
Executive Director, Life Science Research Organization (LSRO), Bethesda, MD 20814

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George C. Fahey, Jr., Ph.D.
Professor, University of Illinois, Urbana-Champaign, IL 61801

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Signature: (b) (6) [redacted] Date: 9/2/09

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Signature: (b) (6) [redacted] Date: 9/01/09

George C. Fahey, Jr., Ph.D.
Professor, University of Illinois, Urbana-Champaign, IL 61801

Signature: (b) (6) [redacted] Date: 8/31/09

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I. Identity of Substance

A. Common or trade name: Palm TRF or T3 concentrates are from palm oil sources (the content of alpha T is approximately 20-25% of the tocols) that are manufactured by the industry members of Malaysian Palm Oil Board (MPOB), such as Carotech, Davos, Carotino, Sime Darby Bioganics, and SuperVitamins. Specific trade names include, but are not limited to, the following:

1) Carotech products such as
   Tocomin® 30% - Palm TRF, 30% Oil Suspension,
   Tocomin® 50% - Palm TRF, 50% Oil Suspension,
   Tocomax™ 20% - Palm TRF,
   20% Cold Water Dispersible Powder,
   Tocobeads® 7.5% - Palm TRF,
   7.5% Beadlets, Tocosem™ 1.5% - Palm TRF,
   1.5% Water Soluble Emulsion,
   Tocomin® Suprabio™ 20% - Palm TRF,
   20% Bio-enhanced Suspension, and
   Tocomin® Suprabio™ 25% - Palm TRF, 25% Bio-enhanced Suspension;

2) Davos palm TRF products, such as
   Tocotrienol Concentrate DVL 30,
   Tocotrienol Concentrate DVL 50,
   Tocotrienol Concentrate DVL 70,
   Tocotrienol Powder DVP 7.5,
   Tocotrienol Powder DVP 10,
   Tocotrienol Powder DVP 20,
   Tocotrienol Powder DVP 30, and
   Water Soluble (WS) Tocotrienol 1.5%;

3) Carotino TRF oil suspension products, such as
   Carotino TRF 10%,
   Carotino TRF 20%,
   Carotino TRF 50%, and
   Carotino TRF 80%;

4) SD Bioganic TRF products, such as
   Gold Tri E30,
   Gold Tri E50,
   Gold Tri E70,
   Gold Tri E20 powder,

Gold Tri E30 powder,
Bio E Tri E 30 N (emulsion), and
Bio E Tri E 20 N (emulsion);

5) SuperVitamin TRF oil suspension products, such as
Super T25,
Super T30,
Super T50,
Super T80,
Super T Powder 20%, and
Super T Powder 25%.

B. Standards of identity: We note that an ingredient that is lawfully added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity that is located in Title 21 of the Code of Federal Regulations.

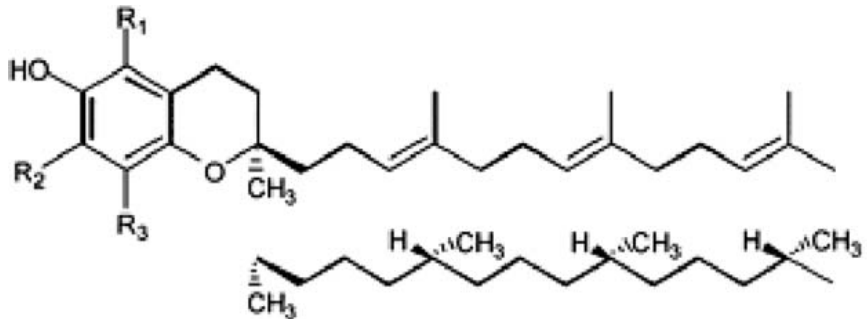
C. Background

Tocotrienols are the primary form of vitamin E in the seed endosperm of most monocots, including agronomically important cereal grains such as wheat, rice, and barley. Palm oil contains significant quantities of T3 and T (Sundram et al. 2003). Crude palm oil extracted from the fruits of *Elaeis guineensis* particularly contains a high amount of T3 (up to 800 mg/kg), mainly consisting of γ-T3, α-T3, and α-T. The distribution of vitamin E in palm oil is 20-30% T and 70-80% T3 (Sundram et al. 2003). The objective of this review is to highlight the safety and potential significance of the T3.

D. Chemistry and structure of T3

Tocopherols and T3 share common structural features of a chromanol ring and a side-chain at the C-2 position (Nesaretnam et al. 2008). The difference between T and T3 is that the former contains a saturated phytyl tail, whereas the latter bears an unsaturated isoprenoid side chain, i.e., three all trans double-bonds in the hydrocarbon tail (Figure 1; Tasaki et al. 2008). Each subgroup of vitamin E contains several isoforms. Chemical formulas and molecular weights (M.W.) of 8 isomers are summarized in Table 1. Palm TRF is a mixture of T3 and α-T.

Tocotrienol isoprenoid side chain



	R_1	R_2	R_3
α:	CH_3	CH_3	CH_3
β:	CH_3	H	CH_3
γ:	H	CH_3	CH_3
δ:	H	H	CH_3

Tocopherol phytylside chain

T3 isoforms

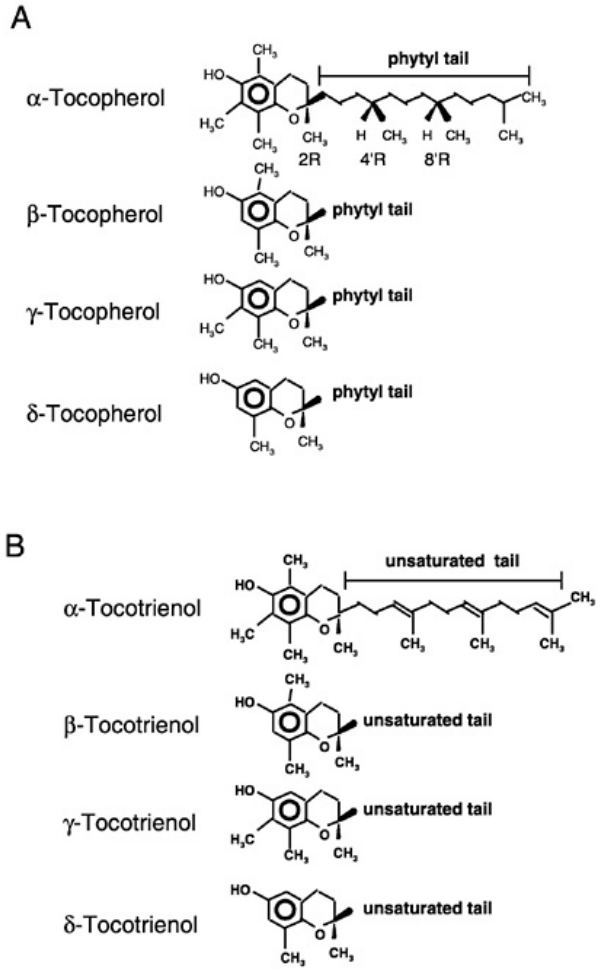


Figure 1. Structure of vitamin E stereoisomers. Tocotrienols consist of a chromanol nucleus and a lipophilic isoprenoid chain. Tocopherols have phytyl side chain. Methyl group placement in the naturally occurring isoforms α-, β-, γ-, and δ- is as indicated in the box (Adopted from Akoh and Min 2008).

Table 1. Chemical formulas, molecular weights, and CAS numbers of 8 forms of vitamin E

Compound	Chemical Formula	MW	CAS No.
α-tocotrienol	$C_{29}H_{44}O_2$	424	58864-81-6
β-tocotrienol	$C_{28}H_{42}O_2$	410	490-23-3
γ-tocotrienol	$C_{28}H_{42}O_2$	410	14101-61-2
δ-tocotrienol	$C_{27}H_{40}O_2$	396	25612-59-3
α-tocopherol	$C_{29}H_{50}O_2$	430	59-02-9
β-tocopherol	$C_{28}H_{48}O_2$	416	148-03-8
γ-tocopherol	$C_{28}H_{48}O_2$	416	7616-22-0
δ-tocopherol	$C_{27}H_{46}O_2$	402	119-13-1

Adopted from European Food Safety Authority (EFSA) 2008

E. Manufacturing Process

The industry members of MPOB describe their manufacturing process as follows:

1. TRF liquid (or TRF oil suspension):

(1) The starting raw material is crude palm oil, an edible vegetable oil extracted from the mesocarp of the oil palm. Crude palm oil is naturally reddish in color because it contains a high amount of natural mixed carotenoids. Palm TRFs (oil suspensions) are manufactured from crude palm oil using a trans-esterification reaction, followed by a multi-stage molecular distillation process.

(2)Extraction and Reaction Process: Crude palm oil contains the highest natural level of mixed carotenoids and palm TRF in nature (500 part per million (ppm) and 800 ppm of carotenoids and TRF, respectively). In addition to these two phytonutrients, there are other minor components naturally found in palm oil such as sterols, squalene, and Co-Q10. These phytonutrients (minor components) make up about 1% in total of the oil. The remaining 99% is basically the oil (triglycerides). A patented process (US Patent No. 5,157,132) extracts and concentrates this 1% of minor components in the fruits of oil palm. In the process, crude palm oil is converted to methyl esters and glycerol through

transesterification via the addition of methanol in the presence of sodium methoxide (catalyst). The minor components such as the mixed carotenoids, vitamin E, squalene, sterol, etc. are not involved in this reaction and are not changed chemically or structurally. The ratio, chemical structure, and characteristics remain the same in the starting natural crude palm oil. The reaction process targets and involves only the oil part (triglycerides).

(3) Molecular Distillation Process: Molecular distillation is a unique and well-known process for distilling heat-sensitive materials such as essential oils and antioxidants. It uses an extremely high vacuum and low temperature technology to distill materials such as methyl esters and antioxidants. In the molecular distillation process and with such an extreme vacuum, all the methyl esters will be distilled out to leave a concentrated residue of phytonutrients (including carotenoids and vitamin E). With the vigorous hot water wash, vacuum drying, and molecular distillation, any residue or unreacted compounds are taken out. The concentrated residue then is subjected again to molecular distillation (varying vacuum and temperature) to recover the individual components – mainly TRF, mixed carotenes, and phytosterols. To manufacture high concentrations and purities of T3, conventional high performance liquid chromatography (HPLC), involving an organic solvent mobile phase (a mixture of hexane, ethyl acetate, and methanol) and a stationary phase, silica, can be used. Then, ultra high vacuum rotary evaporation removes the solvents used in the manufacturing process.

Alternatively, the following process can be used: Crude palm oil is degummed by treating with food grade phosphoric acid to remove phospholipids. The degummed oil then is mixed with bleaching earth (activated calcium montmorillonite) to absorb pigments and particles as well as any metal contamination and oxidizing materials that

may be in the oil. The oil-earth mixture then is passed through filters where the spent earth is removed together with the precipitated materials from degumming. The bleached oil is first de-aerated and then collected separately as a process by-product. The T3 in the resultant material is concentrated using a multistage physical process without the use of solvents. To manufacture higher concentrations of T3, standard solvent-based chromatography may be used to further separate and fractionate the materials, thus increasing the concentration and purity.

2. Production of Palm TRF, Water Soluble Emulsion: TRF liquids (>50% tocols) are the starting materials for the formulation of water-soluble emulsions of palm TRF. Glycerine and a proprietary blend of emulsifiers, such as mono- and diglycerides, sorbitan (GRAS; 21CFR173.110 and 172.842), sugar esters, starch derivatives, and surfactants) are added into palm TRF liquids. The mixture then is homogenized into an emulsion.
3. Non-water Dispersible Powder: TRF liquids (>30% tocols) are the starting materials for the formulation of a cold water dispersible powder of palm TRF. A powder carrier (modified corn starch, maltodextrin, tapioca dextrin, corn starch, or sodium caseinate (GRAS; 21CFR182.1748)), with and without an anti-caking ingredient such as 5% calcium silicate), is added into palm TRF. The mixture is dried and milled or alternatively spray dried into a dry powder.
4. Production of Palm TRF, Beadlets: TRF liquids (>50% tocols) are the starting material for the formulation of palm TRF beadlets. Gelatin, corn starch, sucrose (GRAS; 21CFR184.1854), and ascorbyl palmitate (GRAS; 21CFR184.3149) are added into palm TRF liquids. The mixture is spray dried into dry beadlets.

5. Production of palm TRF, Bioenhanced Suspension: Palm TRF liquids (>50% tocols) are the starting material for the formulation of palm TRF, Bio-enhanced Suspension. A proprietary blend of emulsifiers (including glycerides and starch derivatives) are added into palm TRF liquids and undergo various physical processing such as high-speed stirring and homogenization to produce Tocomin® Suprabio™.

F. Specifications

Tables 2-1 to Table 2-31 list specifications of palm TRF. In general, specifications have the following common characteristics. (1) Palm TRF is a mixture of T3 and α-T and the content of α-T is 20-30% of the total tocols present in palm TRFs. For example, palm TRF of Carotech and Davos contain approximately 20% and 25-30% of α-T, respectively. (2) Any number in the product name indicates the concentration (as a percentage) of total tocols; for example, DVL 30 means the product contains 30% tocols and 70% (100%-30%) of other components. (3) The maximum moisture concentration is 0.5-1% in a liquid form of palm TRF. (4) Palm TRFs are available in many forms: liquid (oil suspension), powder, water-soluble emulsion, beadlets, and bio-enhanced suspension. (5) Among components other than T3-T, the liquid form contains mostly palm oil (GRAS; 21CFR184.1259). The powder form of palm TRF contains maltodextrin (GRAS; 21CFR184.1444), modified corn starch (GRAS; 21CFR172.892), cyclodextrin (GRAS; 21CFR170.36, GRN 000074), tapioca starch, or corn starch instead of palm oil. Water soluble TRF contains a mixture of water, sucrose (GRAS; 21CFR184.1854), propylene glycol (GRAS; 21CFR184.1666), glycerol (GRAS; 21CFR182.1320), palm oil, and sucrose monopalmitate (GRAS; 21CFR172.859), instead of palm oil. The emulsion form of palm TRF contains a mixture of glycerine (GRAS; 21CFR182.1320) and mono-and diglycerides instead of palm oil.

The beadlet form contains a mixture of gelatin, corn starch, sucrose, and ascorbyl palmitate (GRAS; 21CFR184.3149) instead of palm oil.

Palm TRFs also contain phytosterols and squalene as minor components. Vegetable oil sterol esters (including beta-sitosterol, campesterol, and stigmasterol; 21CFR170.36, GRN000048) and squalene (from olive oil and other oils; 21CFR170.36, GRN000048) are recognized as GRAS substances.

Unless noted otherwise, the palm TRFs have the following common specifications: peroxide value <10 meq/kg, lead <0.5 ppm, standard plate count <1000 CFU/g, yeast and mold <100 CFU/g, and *E. coli* and Salmonella-negative. Examples of analytical data for each T3 preparation are shown in the appendix.

Table 2-1. Specifications of Carotech Palm TRF - Tocomin® 30% (oil suspension)

Item	Minimum	Maximum
α-T3, %	6.5	9.0
β -T3, %	1.0	
γ-T3, %	12	14
δ-T3, %	3.3	5.0
α-T, %	6.0	9.0
Total T3 and α-T, %	30.0	
Palm oil, %		65.0
Moisture, %		1.0
Plant squalene, %	4.0	
Phytosterols, %	1.0	
Mixed carotene, %	0.03	

Table 2-2. Specifications of Carotech Palm TRF - Tocomin® 50% (oil suspension)

Item	Minimum	Maximum
α-T3, %	11.0	14.0
β -T3, %	1.5	
γ-T3, %	20.0	24.0
δ-T3, %	5.5	7.0
α-T, %	10.0	14.0
Total T3 and α-T, %	50.0	
Palm oil, %		39.0
Moisture, %		1.0
Plant squalene, %	8.0	
Phytosterols, %	3.0	
Mixed carotene, %	0.05	

Table 2-3. Specifications of Carotech Palm TRF - Tocomax™ 20% (powder)

Item	Minimum	Maximum
α-T3, %	4.5	6.0
β -T3, %	0.5	
γ-T3, %	8.0	10.0
δ-T3, %	2.0	3.0
α-T, %	4.0	6.0
Total T3 and α-T, %	20.0	
Palm oil, %	20.0	
Corn starch, %	52.0	
Moisture, %		8.0
Plant squalene, %	3.0	
Phytosterols, %	1.0	

Table 2-4. Specifications of Carotech Palm TRF - Tocomin Suprabio ™ 20% (bio-enhanced suspension)

Item	Minimum	Maximum
α-T3, %	4.4	6.0
β -T3, %	0.6	
γ-T3, %	8.0	9.6
δ-T3, %	2.2	2.8
α-T, %	4.0	5.6
Total T3 and α-T, %	20.0	
Palm oil, %	20.0	
Emulsifiers, plant-based, %	55.0	
Moisture, %		1.0
Plant squalene, %	3.0	
Phytosterols, %	1.5	
Mixed carotene, %	0.02	

Table 2-5. Specifications of Carotech Palm TRF - Tocomin Suprabio ™ 25% (bio-enhanced suspension)

Item	Minimum	Maximum
α-T3, %	5.5	7.0
β -T3, %	0.75	
γ-T3, 5	10.0	12.0
δ-T3, %	2.75	3.5
α-T, %	5.0	7.0
Total T3 and α-T, %	25.0	
Palm oil, %	25.0	
Emulsifiers, plant-based, %	44.0	
Moisture, %		1.0
Plant squalene, %	4.0	
Phytosterols, %	1.5	
Mixed carotene, %	0.02	

Plant based emulsifiers: Modified corn starch, maltodextrin, cyclodextrins, or corn starch.

Table 2-6. Specifications of Carotech Palm TRF - Tocosem ™ 1.5% (water-soluble emulsion)

Item	Minimum	Maximum
α-T3, %	0.3	0.36
β -T3, %	0.05	
γ-T3, %	0.5	0.52
δ-T3, %	0.35	0.36
α-T, %	0.28	0.29
Total T3 and α-T, %	1.48	
Glycerine, %	82.0	
Emulsifiers, plant-based, %	15.0	

Plant based emulsifiers: Modified corn starch, maltodextrin, cyclodextrins, or corn starch.

Table 2-7. Specifications of Carotech PalmTRF - Tocobeads® 7.5% (beadlets)

Item	Minimum	Maximum
α-T3, %	1.65	2.1
β -T3, %	0.15	
γ-T3, %	3.0	3.6
δ-T3, %	0.8	1.05
α-T, %	1.5	2.1
Total T3 and α-T, %	7.5	
Palm oil, %	7.5	
Alginate, %	40.0	
Gum Arabic, %	5.0	
Pea starch, %	34.0	
Moisture, %		8.0

Table 2-8. Specifications of Davos palm TRF - Tocotrienol Concentrate DVL 30 (oil suspension)

Item	Typical content	Minimum	Maximum
α-T3, %	8.3		
β -T3, %	0.6		
γ-T3, %	11.6		
δ-T3, %	3.4		
α-T, %	9.1		
Total T3 and α-T, %		30.0	49.9
Palm oil, %		60.1	70
Moisture, %			1.0
Peroxide value, meq/kg			5.0

Table 2-9. Specifications of Davos palm TRF - Tocotrienol Concentrate DVL 50 (oil suspension)

Item	Typical content	Minimum	Maximum
α-T3, %	13.4		
β -T3, %	1.4		
γ-T3, %	18.5		
δ-T3, %	5.7		
α-T, %	14.7		
Total T3 and α-T, %		50.0	59.9
Palm oil, %		40.1	50
Moisture, %			1.0
Peroxide value, meq/kg			5.0

Table 2-10. Specifications of Davos palm TRF – Tocotrienol Concentrate DVL 70 (oil suspension)

Item	Typical content	Minimum	Maximum
α-T3, %	18.8		
β -T3, %	2.0		
γ-T3, %	25.9		
δ-T3, %	8.0		
α-T, %	20.6		
Total T3 and α-T, %		70.0	79.9
Palm oil, %		20.1	30.0
Moisture, %			1.0
Peroxide value, meq/kg			5.0

Table 2-11. Specifications of Davos palm TRF powder- Tocotrienol Powder - DVL 7.5

Item	Typical content	Minimum	Maximum
α-T3, %	2.0		
β -T3, %	0.2		
γ-T3, %	2.8		
δ-T3, %	1.0		
α-T, %	2.0		
Total T3 and α-T, %		7.5	10.0
Moisture, %			5.0

Table 2-12. Specifications of Davos palm TRF powder- Tocotrienol Powder - DVL 10

Item	Typical content	Minimum	Maximum
α-T3, %	3.0		
β -T3, %	0.4		
γ-T3, %	4.1		
δ-T3, %	1.3		
α-T, %	3.5		
Total T3 and α-T, %		10.0	19.9
Moisture, %			5.0

Table 2-13. Specifications of Davos palm TRF powder - Tocotrienol Powder DVL 20

Item	Typical content	Minimum	Maximum
α-T3, %	5.5		
β -T3, %	0.6		
γ-T3, %	6.7		
δ-T3, %	2.1		
α-T, %	6.3		
Total T3 and α-T, %		20.0	29.9
Moisture, %			5.0

Table 2-14. Specifications of Davos palm TRF powder - Tocotrienol Powder DVL 30

Item	Typical content	Minimum	Maximum
α-T3, %	7.0		
β -T3, %	0.8		
γ-T3, %	9.5		
δ-T3, %	3.8		
α-T, %	9.6		
Total T3 and α-T, %		30.0	39.9
Moisture, %			5.0

Table 2-15. Specifications of Davos palm TRF – Water Soluble (WS) Tocotrienol 1.5%

Item	Typical content	Minimum	Maximum
α-T3, %	0.4		
β -T3, %	<0.1		
γ-T3, %	0.5		
δ-T3, %	0.2		
α-T, %	0.5		
Total T3 and α-T, %		1.5	
Moisture, %			5.0

Table 2-16. Specifications of Carotino Palm TRF 10% (oil suspension)

Item	Minimum	Maximum
Total T3 and α-T, %	10.0	
Palm oil, %		90
Moisture, %		1.0

Table 2-17. Specifications of Carotino Palm TRF 20% (oil suspension)

Item	Minimum	Maximum
Total T3 and α-T, %	20.0	
Palm oil, %		80
Moisture, %		1.0

Table 2-18. Specifications of Carotino Palm TRF 50% (oil suspension)

Item	Minimum	Maximum
Total T3 and α-T, %	50.0	
Palm oil, %		50
Moisture, %		1.0

Table 2-19. Specifications of Carotino Palm TRF 80% (oil suspension)

Item	Minimum	Maximum
Total T3 and α-T, %	80.0	
Palm oil, %		20
Moisture, %		1.0

Table 2-20. Specifications of SD Bioganic Palm TRF Bio Tri.E 20

Item	Average	Minimum	Maximum
A mixture of T3, %	15		
α-T, %	5 (25% of the total tocols)		
Total T3 and α-T, %	20	19.5	
Palm oil, %			80.5
Moisture, %			1.0

Table 2-21. Specifications of SD Bioganic Palm TRF Bio Tri.E 30 or Gold Tri.E 30 (amber liquid, bland taste)

Item	Average	Minimum	Maximum
A mixture of T3, %	22.5		
α-T, %	7.5		
Total T3 and α-T, %	30.0	29.5	
Palm oil, %			70.5
Moisture, %			1.0

Table 2-22. Specifications of SD Bioganic Palm TRF Gold Tri.E 50

Item	Average	Minimum	Maximum
A mixture of T3, %	37.5		
α-T, %	12.5 (25% of the total tocols)		
Total T3 and α-T, %	50.0	49.5	
Palm oil, %			50.5
Moisture, %			1.0

Table 2-23. Specifications of SD Bioganic Palm TRF - Gold Tri.E 70

Item	Average	Minimum	Maximum
A mixture of T3, %	52.5		
α-T, %	18.75 (25% of the total tocols)		
Total T3 and α-T, %	70.0	69.5	
Palm oil, %			30.5
Moisture, %			1.0

Table 2-24. Specifications of P SD Bioganic Palm TRF - Gold Tri.E 20 Powder

Item	Average	Minimum	Maximum
A mixture of T3, %	15		
α-T, %	5		
Total T3 and α-T, %	20	19.5	
Palm oil, %			30
Matodextrin, %			40

SD Bioganic Palm TRF powder products contain Na caseinate and plant-based emulsifiers (5%) and calcium silicate (5%) as an anticaking agent.

Table 2-25. Specifications of SD Bioganic Palm TRF - Gold Tri.E 30 Powder

Item	Average	Minimum	Maximum
A mixture of T3, %	22.5		
α-T, %	7.5		
Total T3 and α-T, %	30.0	29.5	
Palm oil, %			30
Matodextrin, %			30

Table 2-26. Specifications of SuperVitamins Palm TRF - Super T25

Item	Minimum	Maximum
α-T3, %	5.6	
β -T3, %	0.4	
γ-T3, %	7.9	
δ-T3, %	3.8	
α-T, %	6	
Total T3 and α-T, %	25.0	
Palm oil, %		75
Moisture, %		1.0

Table 2-27. Specifications of SuperVitamins Palm TRF - Super T30

Item	Minimum	Maximum
α-T3, %	5.6	
β -T3, %	0.4	
γ-T3, %	7.9	
δ-T3, %	3.8	
α-T, %	6	
Total T3 and α-T, %	30.0	
Palm oil, %		70
Moisture, %		1.0

Table 2-28. Specifications of SuperVitamins Palm TRF - Super T50

Item	Minimum	Maximum
α-T3, %	12.5	
β -T3, %	0.5	
γ-T3, %	17.0	
δ-T3, %	7.0	
α-T, %	12	
Total T3 and α-T, %	50.0	
Palm oil, %		50
Moisture, %		1.0

Table 2-29. Specifications of SuperVitamins Palm TRF - Super T80

Item	Minimum	Maximum
α-T3, %	20.0	
β -T3, %	1.0	
γ-T3, %	26.0	
δ-T3, %	12.5	
α-T, %	18.5	
Total T3 and α-T, %	80.0	
Palm oil, %		20
Moisture, %		1.0

Table 2-30. Specifications of SuperVitamins Palm TRF - Super T Powder 20%

Item	Minimum	Maximum
α-T3, %	5.0	
β -T3, %	0.2	
γ-T3, %	6.8	
δ-T3, %	2.8	
α-T, %	4.8	
Total T3 and α-T, %	20.0	
Moisture, %		8.0

Table 2-31. Specifications of SuperVitamins Palm TRF - Super T Powder 25%

Item	Minimum	Maximum
α-T3, %	6.2	
β -T3, %	0.2	
γ-T3, %	8.5	
δ-T3, %	3.5	
α-T, %	6.0	
Total T3 and α-T, %	25.0	
Moisture, %		1.0

G. Analytical methods

Tocopherols and T3 can be analyzed by high performance liquid chromatography (HPLC) with an ultraviolet (UV), liquid chromatography-mass spectrometry (LC-MS), or fluorescent detector (Yap et al. 1999; Sanagi et al. 2005; Schwartz et al. 2008). Appropriate sample preparation methods are to be employed depending on the various matrices. For example, oil samples are dissolved in heptane (50–1000 mg/10 ml depending on the expected concentration range), filtered in the case of highly concentrated or cloudy solutions, and injected into the HPLC system. Margarines and rolling fats are saponified in the presence of 100 mg of ascorbic acid as an antioxidant. The vials are flushed with nitrogen for 20 sec before and after addition of KOH. Subsequent to saponification and addition of 12 ml of water, analytes are extracted three times with 12 ml of heptane–diethyl ether (50:50, v/v). The combined organic extracts are washed with 5 ml of 10% aqueous sodium chloride solution (w/v), 10 ml of water and 10 ml of 0.01M hydrochloric acid, dried over 3 g of sodium sulfate, and evaporated at 30 ^{o}C. The solid residue can be taken up in 10 ml of heptane and the solution is syringe-filtered prior to HPLC analysis.

The following methods were used in the chemical and microbiological analysis: peroxide values by the AOAC 965.33 method; total plate count and yeast and mold by the USP 2021 method; *E.coli* and *salmonella* by the USP 2022 method; and lead by inductively coupled plasma mass spectrometry (ICP/MS) or atomic absorption (AA) spectrophotometry.

II. Natural occurrence and exposure to T3 and α-T.

A. Food sources of T3 and T

Table 3 lists the T3 and α-T content of foods eaten by Americans (Franke et al. 2007). Intakes of T3 were mainly associated with the consumption of cereal products, margarine, and oils (Chun et al. 2006; Franke et al. 2007; Schwartz et al. 2008). The relative abundance of the vitamin E homologues (T and T3) in a specific preparation depends on the species of plant and the extraction procedure used. Palm oil contains significant quantities of T3. Other sources of T3 are coconut oil, cereal grains including rice, barley, rye, wheat, and nuts. Palm, rice bran, oat, and barley contain vitamin E mostly as the T3 (Ong 1993). The common sources of commercial vitamin E such as soy, corn, cottonseed, canola, and sunflower oil distillates contain little or no T3. Unlike water-soluble vitamins, T is relatively stable during processing; vitamin E losses after bread making were close to 30% (Nandith and Prabhasankar 2009). During frying at 175 C, tocopherols have a slow degradation rate with a half-life over 72 h (Normand et al. 2001).

Table 3. Sources of T3 and α-tocopherol (α-T) in foods; mg/kg

Foods	T3	α -T
Nuts		
almonds, honey-roasted	8.3	185.3
macadamia nuts, dry-roasted	20.9	0.8 ± 1.1
mixed nuts, oil-roasted, with peanuts	9.5	69.6 ± 6.6
peanuts, dry-roasted	12.1	69.1 ± 0.8
peanuts, boiled	5.3	36.9
Oils and fats		
corn oil	81.3	222.1 ± 66.2
corn oil	25	
margarine, 60% fat	18.3	33.7 ± 6.7
margarine, 70% fat	20.7	63.4
palm oil	738	160.8 - 279
safflower oil	59.1	391.2 ± 190.9
sesame oil	<2	65.0 ± 2.4
sunflower oil	<2	541.8
Salad dressing		
fat-free mayonnaise	1.8	0.7
blue cheese salad dressing	4.0	16.3 ± 8.1
French salad dressing	4.7	19.3 ± 26.0
Italian salad dressing	15.1	40.7 ± 8.8
Oriental salad dressing	8.3	22.1 ± 25.1
ranch salad dressing	1.6	2.4 ± 0.7
sesame and miso salad dressing	15.6	57.9
sesame ginger salad dressing	28.7	9.0 ± 10.6
thousand island dressing	8.9	16.3 ± 12.0
Fish and meats		
ahi (block) raw	<0.04	6.4 ± 2.4
ahi (filet), raw	<0.04	1.6
meat ball	0.075	0.75
manapua (char siu)	7.8	1.2
manapua (pork)	2.6	2.8
salmon (filet), raw Atlantic	0.33	37.7 ± 13.3
salmon (steak), raw Atlantic	0.13	32.4

Table 3. Sources of T3 and α-tocopherol (α -T) in foods; mg/kg - continued

Foods	T3	α- T
Grain based products		
wheat	8.51	2.3 ± 1.0
cereals	11.9	7.7 ± 4.2
chocolate chip cookies	16	18.7 ± 11
fig bars	4.8	8.4 ± 3.5
fried noodles (chow mein)	5.2	9.2 ± 9.2
fried noodles (top ramen)	52.7	16.3 ± 4.8
spaghetti/marinara pasta sauce	4.2	10.9 ± 3.1
pie crust, Graham cracker	15.1	12.6
pie crust, frozen, baked	6.5	15.0
popcorn, Microwave	13.2	25.4 ± 2.4
potato chips, plain, salted	11.1	37.5 ± 14.3
potato chips, plain, salted, hydrogenated oils	18.6	76.5 ± 25.3
Fruits and products		
jack fruit	0.4	10.4
mango	<0.32	7.9 ± 0.9
papaya (green)	<0.33	0.7
papaya (red)	<0.33	1.1 ± 0.4
papaya (yellow)	2.6	6.8 ± 8
Tamarind, raw	0.33	<0.01
Vegetable and products		
bell pepper, red	1.13	20.8 ± 14.1
beet greens	4.2	13.9
broccoli, frozen	0.13	4.8
carrot juice	0.8	1.0
chili, hot pepper paste	3.2	12.1
Chinese cabbage	0.33	2.6 ± 0.8
collard, frozen	1.9	18.5
dandelion greens	1.2	7.0
spinach, canned, whole leaf	9.5	20.9 ± 3.7
spinach, fresh	1.7	11.4 ± 2.0
sweet potatoes	0.13	2.1
taro root	1.2	4.1 ± 14.1
tomato	1.4	5.5
tomato soup, canned	1.33	2.1 ± 0.2
turnip greens	1.9	19.6
turnip greens, frozen	2.1	19.2
tofu, fried	3.0	18.1
Miscellaneous foods		
dairy products	0.14	1.16
poi	0.23	2.0 ± 0.4

Adopted from Chun et al. 2006, Franke et al. 2007, Heinonen 1991, Ong 1993, and Schwartz 2008.

The α -T and T3 content of T3 and T of palm oil and rice bran oil. These oils have similar TRF composition profiles.

Table 4. Content of T3 and T present in palm oil and rice bran oil (mg/kg)

	α -T	T3				
		Alpha (α)	Beta (β)	Gamma (γ)	Delta (δ)	total
Palm oil 1	256	143	32	286	69	530
Palm oil 2	279	274	-	398	69	741
Rice bran oil	324	236		349		>909

Adopted from EFSA 2008

B. Intended use

Table 5 presents primary applications of palm TRF that include fats or oils, such as salad dressing, mayonnaise, margarine, and spread, potato chips and salty snacks, bakery products, ready-to-eat cereals, meal replacement and other functional beverages, meatless soups, soup mix meatless gravies, sauces, pasta sauces, processed juices, processed fruits, juice drinks and punch, frozen dairy desserts, yogurt, pudding, gelatin products, chocolate milk, flavored milk, soy milk and products, chocolate, candy, confectionary, sweets, beverages, and chewing gum. Intended use levels for each food category are expressed as mg/serving as well as % Daily Values (DV) of vitamin E (α-T). However, it is not possible to express the dosage levels of T3 as %DV as there is no established DV for T3.

Table 5. Intended use of palm TRF

	Recommended levels, mg/serving			Maximum levels, mg/serving			%DV of Vit. E (α-T)
Food category, serving size	tocols	T3	α-T	tocols	T3	α-T	
margarine and spread, 1 TSP=13.6 g	2.04	1.53	0.51	21.8	16.3	5.5	3-30% DV
designer fats and oils, 1 TSP=13.6 g	2.04	1.53	0.51	21.8	16.3	5.5	3-30%
salad dressing and mayonnaise, 27.2 g	1.36	1.02	0.34	13.6	9.8	3.4	2-20%
potato chips and salty snacks, 30 g	1.5	1.125	0.375	7.5	5.62	1.88	2-11%
bakery products (breads, rolls, croissants), 30-50 g	1.5	1.125	0.375	14.4	10.8	3.6	2-20%
Cookies, crackers, 25 g	1.25	0.94	0.31	7.5	5.62	1.88	2-11%
RTE cereals, cereal bars, granola bars, protein bars, and power bars, 30 g	7.5	5.6	1.9	75	56.25	18.75	10-100%
meal replacement and other functional beverage products (240 g) and mixes	7.2	5.4	1.8	72	54	18	10-100%
meatless meat products (soy based), 70 g	1.05	0.79	0.26	7.0	5.25	1.75	1.5-10%
meatless soups (240 g) and soup mix	2.7	2.02	0.68	5.4	4.04	1.36	3-8%
meatless gravies, sauces, and pasta sauces, 70 g	0.9	0.68	0.22	1.8	1.36	0.44	1-2.5%
processed juice, 240 g	7.2	5.4	1.8	72	54	18	10-100%
processed fruits, juice drinks, and punch, 240 g	3.6	2.7	0.9	14.4	10.8	3.8	5-20%
soft drinks, 240 g	2.4	1.8	0.6	7.2	5.4	1.8	3-10%
frozen dairy desserts and mixes, ½ C=68 g	1.36	1.02	0.34	2.04	1.53	0.51	2-3%
yogurt, pudding and jello, 240 g	4.8	3.6	1.2	7.2	5.4	1.8	7-10%
chocolate milk, flavored milk, soy milk and products, 240 g	7.2	5.4	1.8	14.4	10.8	3.6	10-20%
Chocolate, candy, confectionary and sweets, 43 g	2.4	1.8	0.6	7.2	5.4	1.8	3-10%
chewing gum, 1 stick	0.06	0.045	0.015	0.18	0.135	0.045	<1%

Daily Values of vitamin E are based on α –T values only and do not include any contribution from T3.

C. Current consumer intake levels

The Vitamin E intake status of the American population has been estimated using Recommended Daily Allowance (RDA) and Tolerable Upper Intake Levels (UL) outlined by the Institute of Medicine (IOM) of the U.S. National Academy of Sciences (IOM 2000; Table 6). Previous dietary recommendations for vitamin E intake were based on the α-tocopherol equivalent (TEV), which was derived by applying conversion factors to all 8 isomers of the vitamin E group. However, IOM recognized that α-T is the main form of vitamin E that exerts vitamin E activity and that other vitamin E isomers are poorly converted into α-T in humans. For dietary recommendation purposes, IOM (2000) has recently established RDAs, estimated average requirements (EARs), and ULs for α-T only. Thus, vitamin E intake refers to α-T intake in dietary recommendations unless noted otherwise, although other isomers also belong to the vitamin E family. The UL is defined as a safe upper level for consumption over a lifetime or the highest level of a daily nutrient intake that will most likely present no risk of adverse health effects in almost all individuals in the general population. The RDA is the intake level for a nutrient at which the needs of 97.5 % of the population will be met. The EAR is the intake level for a nutrient at which the needs of 50 percent of the population will be met; thus, the EAR is used (such as the percentage of people meeting EAR) when evaluating the nutrient adequacy of the population.

For adults, IOM recommends daily vitamin E (α -T) intake of 15 mg/d. Vitamin E intake (α -T) of up to1000 mg/d is considered as safe (IOM 2000). For T3, there are no established ULs.

Table 6. Vitamin E (α-T) intake recommendations (IOM 2000)

Age	RDA (mg/d)	UL (mg/d)
1-3	6	200
4-8	7	300
9-13	11	600
14-18	15	800
19+	15	1000

Based on food consumption data reported in the most recent National Health and Nutrition Examination Survey (NHANES; 2005-2006) compiled by the U.S. Department of Health and Human Services, National Center for Health Statistics and the Nutrition Coordinating Center, estimates of 2-d (d) average intakes of vitamin E (as α-T) and T3 were calculated from the food code list and the survey database of diet recalls. The usual intake estimation procedure requires multiple days of nutrient intake data for at least a representative subsample of the individuals in the sample in order to estimate variances (Goldman 2005). The NHANES provides the most current food consumption data available for the American population. The NHANES was conducted between 2005-2006 with non-institutionalized individuals in the U.S. In each of the two survey yrs, data were collected from a nationally representative sample of individuals of all ages.

The food and dietary supplement record for each individual includes the gram weight and nutrient data for all foods consumed during the day of the recall. All estimates were generated with USDA sampling weights to adjust for differences in representation of subpopulations.

The NHANES food code list includes the α-T content of each food, but not T3 values. Thus, the T3 content was developed by taking the α-T content for each food and multiplying it by a conversion factor to estimate the current consumption levels of T3. Based on published vitamin E composition tables (Chun et al. 2006, Franke et al. 2007, Heinonen 1991, Ong 1993,

and Schwartz 2008), the ratio of T3: α-T (i.e., a conversion factor) was determined for each food by category. The categories and conversion factors are listed in Table 7.

Table 7. Conversion factors of vitamin E

Food category	Conversion Factor: from α-T to T3
Breads, cookies, cereals, and cereal Bars	1.21358
Pasta	1.71
Desserts	0.7826
Nuts	0.1551
Popcorn	0.52
Potato chips and salty snacks	0.26
Margarine, fats, and oils	0.1357
Dressings, spreads, and mayonnaise	0.4723
Fruits and fruit products	0.1658
Vegetables, excluding sweet potatoes	0.1976
Sweet potatoes	0.062
Fried tofu	0.1657
Poi	0.115
Eggs and egg products	0.0238
Dairy products	0.1207
Mixed dish mostly grain	1.21
Mixed dish mostly beef or chicken	0.1
Fish products	0.00513
Pork products	0.9286
General foods (other foods)	0.55

The most up-to-date vitamin E (α-T) and T3 intake status for the U.S. population ages 1 yr and older are presented in Tables 8 and 9 (NHANES 2005-2006). The median intakes of T3 in the U.S. are estimated to be 2.2 mg/d for children aged 1-3 yr and 3.2 mg/d for the population aged 1 yr and above (the entire population)(Cho et al. 2009). The median intakes of vitamin E (α-T) are estimated to be 3.6 mg/d for children aged 1-3 yr and 5.8 mg/d for the population aged 1 yr and above; these intakes correspond to approximately 40-56% of RDAs. Over 98% of the U.S.

population did not meet the daily recommended intake of vitamin E and over 94% of the population did not meet the EAR. Based on the previous nutrient adequacy data that showed a similar trend as shown in this analysis, the USDA Dietary Guideline (DG) Committee has classified vitamin E as a shortfall nutrient low in the American diet (USDA 2004). None of population consumes vitamin E above UL levels; in the 2005-2006 NHANES, the 90th percentile intakes were estimated to be 5.1 and 12.5 mg/d in young children aged 1-3 yr and the entire population, respectively (Cho et al. 2009). These values represent less than 1.6-3% of the ULs established for these age groups.

Table 8. Tocotrienol and vitamin E (α-T) intakes before the intended use (NHANES 2005-2006)

Age, yr	Intake Type	N	Tocotrienol				Alpha Tocopherol			
			Median	SE	90th Pctl	SE	Median	SE	90th Pctl	SE
1+	Daily intake, mg/d	8792	3.1	0.03	8.1	0.14	5.8	0.04	12.5	0.18
1+	Daily intake, mg/kg BW/d	8720	0.05	0.00	0.15	0.00	0.09	0.00	0.22	0.01
1-3	Daily intake, mg/d	789	2.2	0.06	3.5	0.17	3.6	0.07	5.1	0.22
1-3	Daily intake, mg/kg BW/d	787	0.17	0.00	0.26	0.01	0.28	0.01	0.39	0.02

Pctl = Percentile; kg BW=kg body weight

Table 9. Nutrient adequacy of vitamin E (α-T) before the intended use (NHANES 2005-2006)

Age, yr	N	Usual Intake, mg/d		EAR		RDA		UL	
		Mean	SE	% Above	SE	% Above	SE	% Above	SE
1+	8792	6.4	0.05	6.4	1.8	1.6	0.9	0	0
1-3	789	3.9	0.05	10.5	8.4	2.5	4.3	0	0

D. Estimated Daily Intake of T3 and α-T from GRAS Uses

Using food intake data reported in the 2005-2006 NHANES, exposure levels to MPOB T3 and α-T that will result from the intended uses were estimated. Results of the exposure estimates after fortification with palm TRF in foods for the U.S. population ages 1 yr and older are presented in Table 10. The intended use models are reported at two different levels: The first set of estimates is based on the assumption that 20% of the products in each food category will be under the intended use (assuming 18% of users will consume the recommended levels and additional 2% of users will consume the maximum levels of intended use; total 20%). The second set of estimates is based on the assumption that 100% of the products are used at the maximum levels of T3 specified in the intended use (assuming 95% of users will consume the recommended levels and additional 5% of users will consume the maximum levels of intended use: total 100%). This is a highly unlikely scenario since it is not possible to use all the food groups under the intended use. Also wastage and other losses should be considered.

Assuming that 20% of the product will be used under the intended use, the median intakes including palm T3 from all GRAS proposed use categories by users of one or more foods is 6.4 mg/d for children aged 1-3 yr and 8.6 mg/d for the entire population. The 90th percentile intakes including MPOB T3 from all GRAS proposed use categories by users of one or more foods are 9.4 mg/d for children aged 1-3 yr and 17.4 mg/d for the entire population. These levels correspond to 0.68 mg/kg BW/d for children aged 1-3 yr and 0.35 mg/kg BW/d for the entire population (Table 11). These levels are more than 100x below the no-observed-adverse-effect level (NOAEL) values that have been found from sub-chronic and chronic toxicity studies in rats (Nakamura et al. 2001; Tasaki et al. 2008).

Even if 100% of the product will be used under the intended use, the median intakes including T3 from all GRAS proposed use categories by users of one or more foods is 19.1 mg/d for male children aged 1-3 yr and 23.8 mg/d for the entire population. The 90th percentile intakes including MPOB T3 from all GRAS-proposed use categories by users of one or more foods are 28.6 mg/d for children aged 1-3 yr and 49.2 mg/d for the entire population. These levels correspond to 2.07 mg/kg BW/d for children aged 1-3 yr and 0.99 mg/kg BW/d for the entire population (Table 10).

Estimated exposure to vitamin E (α-T) is presented in Tables 10 and 11. Intended use would significantly improve the vitamin E intake status of Americans. An assumption that 20 % of that population are consumers of MPOB α-T leads to estimates of dietary exposure of approximately 7.1 mg/d (or 0.54 mg/kg BW/d) for children aged 1-3 yr and 17.9 mg/d (or 0.35 mg/kg BW/d) for the entire population. Even if 100% of the product will be used under the intended use, the 95th percentile intakes including α-T from all GRAS-proposed use categories are 13.3 mg/d (or 1.0 mg/kg BW/d) for children aged 1-3 yr and 29.0 mg/d (or 0.63 mg/kg BW/d) for the entire population. These levels are far below the ULs established by IOM; 200 mg/d for children aged 1-3 yr and 1,000 mg/d for adults. While no population consumes vitamin E above the UL, intended use would increase the percentages of people meeting EAR and RDA (Table 11; Cho et al. 2009). Also, these exposure levels are within the Acceptable Daily Intake (ADI) of 0.15-2 mg/kg BW/d as defined by the Joint FAO/WHO Expert Committee on Food Additives (JECFA; EFSA 2008; WHO 1986).

Table 10. Estimated exposure under the intended use

Age	Intake Type	N	Tocotrienols				Vitamin E (α-T)			
			Median	SE	90th Pctl	SE	Median	SE	90th Pctl	SE
Assuming 20% of products are under the intended use										
1+	Daily Intake, mg/d	8792	8.6	0.06	17.4	0.23	7.30	0.04	14.6	0.19
1+	Daily Intake, mg/kg BW/d	8720	0.13	0.00	0.35	0.01	0.11	0.00	0.27	0.01
1-3	Daily Intake, mg/d	789	6.4	0.13	9.4	0.36	4.7	0.08	6.4	0.33
1-3	Daily Intake, mg/kg BW/d	787	0.48	0.01	0.68	0.03	0.35	0.01	0.49	0.02
Assuming 100% of products are under the intended use										
1+	Daily Intake, mg/d	8792	23.8	0.16	49.2	0.63	11.9	0.07	22.3	0.25
1+	Daily Intake, mg/kg BW/d	8720	0.36	0.00	0.99	0.02	0.18	0.00	0.43	0.01
1-3	Daily Intake, mg/d	789	19.1	0.41	28.6	1.18	6.23	0.10	11.2	0.38
1-3	Daily Intake, mg/kg BW/d	787	1.42	0.01	2.07	0.10	0.60	0.01	0.83	0.03

Table 11. Nutrient adequacy of Vitamin E (α-T) under the intended use

Age, yr	N	Usual intake,			EAR		RDA		UL	
		Mean, mg/d	SE	Mean, % RDA	% Above	SE	% above	SE	% Above	SE
Assuming 20% of products are under the intended use										
1+	8792	7.90	0.06	62.2	17.1	2.1	5.8	1.5	0	0
1-3	789	4.93	0.06	82.0	39.0	5.9	14.4	8.3	0	0
Assuming 100% of products are under the intended use										
1+	8792	12.70	0.08	98.2	66.1	1.9	40.7	2.1	0	0
1-3	789	8.41	0.10	139.7	97.9	3.2	90.1	7.2	0	0

% Above = percentage of people consuming more than EAR, RDA, or UL.

III. Basis for GRAS determination

A. Current regulatory status.

It should be noted that α-T (as a chemical preservative and as the nutrient, vitamin E), tocopherols (as a chemical preservative and as the nutrient, vitamin E), and α-T acetate (as the nutrient, vitamin E) are already listed as GRAS ingredients by the US FDA (21 CFR, Part 184.1890, 182.8890, and 182.8892) when used in accordance with good manufacturing practice. Also, T and α-T acetate are included in the *"Everything" Added to Food in the United States* (EAFUS) list. The EAFUS list of substances contains ingredients added directly to food that FDA has either approved as food additives or listed or affirmed as GRAS. The CAS number for mixed tocopherols is 1406-18-4, which is a mixture of the following T with their own CAS numbers: d-α-T, CAS number 59-02-9; d-β-T, CAS number 148-03-8; d-γ-T, CAS number 7616-22-0; d-δ–T, CAS Number 119-13-1. As mentioned earlier, IOM (2000) and the USDA Dietary Guidelines Committee (2004) have recommended the increased consumption of vitamin E for Americans of all ages.

Currently, T3 is not listed as an approved food additive or a GRAS-affirmed substance in the U.S. However, dietary supplements containing T3 have been on the market in the U.S. as dietary supplements since 1999 under the *Dietary Supplements Health and Education Act of 1994* (DSHEA).

Due to the current GRAS approval status of T, this review for GRAS self-affirmation has focused on T3. This GRAS determination for TRF is based upon scientific procedures in accordance with section 201(s) (21 U.S.C. §321(s)) of the Federal Food, Drug, and Cosmetic Act (21U.S.C.§301 et. seq.) ("the Act"), is set forth at 21 CFR 170.30. The following is provided to briefly set the stage regarding the GRAS process (US FDA 2008):

1) Any substance of natural biological origin that has been widely consumed for its nutrient properties prior to January 1, 1958, without known detrimental effect, for which no health hazard is known, and which has been modified by processes first introduced into commercial use after January 1, 1958, which may reasonably be expected significantly to alter the composition of the substance.
2) Any substance of natural biological origin that has been widely consumed for its nutrient properties prior to January 1, 1958, without known detrimental effect, for which no health hazard is known, that has had a significant alteration of composition by breeding or selection after January 1, 1958, where the change may reasonably be expected to alter the nutritive value or concentration of its constituents.
3) Distillates, isolates, extracts and concentration of extracts of GRAS substances.
4) Reaction products of GRAS substances.
5) Substances not of a natural biological origin, including those for which evidence is offered that they are identical to a GRAS counterpart of natural biological origin.
6) Substances of natural biological origin intended for consumption for other than their nutrient properties.

In general, materials of natural biological origin that have no significant detrimental effect and no known health hazard are eligible for GRAS status. Considering that TRF is of biological origin, that TRF exists naturally in many foods that have long histories of human consumption, and that TRF does not represent a known health hazard, TRF is considered as a GRAS substance.

Documentation qualifying a substance as GRAS has been compiled, where such documentation includes technical evidence and common knowledge of safety under the

conditions of intended use, as recognized by qualified experts (the Expert Panel). The safety requirement for GRAS substances can be supported by this historical consumption (prior to 1958) as well as by scientific studies. Technical evidence of safety includes the chemical identity of the substance, the method of manufacture, analytical data on composition and specifications, estimates of dietary exposure, safety data from animal and human clinical studies, and nutritional benefits from animal and human clinical studies.

The European Scientific Committee on Food (SCF) evaluated the safety of TRF for use as a food ingredient and concluded that the use of mixed T and T3 as a source of vitamin E in food supplements for the general population at the proposed levels of use is not a safety concern (EFSA 2008). Proposed levels include up to 1000 mg of T3 per daily dose, which would result in a daily intake of 16.7 mg T3/kg BW/d for a 60 kg person. At the international level, the ADI value was estimated to be 0.15 – 2 mg/kg BW, calculated as α-T (WHO 1986). Unlike the U.S. IOM and WHO, two European expert panels set UL based on TEV. The European SCF set a UL of vitamin E (as d-α-T) for adults of 300 mg TEV/d (SCF 2003). The U.K. EVM established a NOAEL and UL of 540-970 mg TEV (EVM 2003). In this GRAS self-affirmation, the U.S. IOM standard (α-T) is adopted in estimating dietary exposure levels of vitamin E since USDA Dietary Guidelines and other dietary recommendations in the U.S. are based on the IOM standards.

B. Intended technical effects

1. Natural vitamin E

Natural vitamin E includes eight chemically distinct molecules: T; and α-, β-, γ- and δ-T3 (IOM 2000; Traber and Sies 1996). They cannot be synthesized in the body; thus, vitamin E must be obtained from the diet (IOM 2000). α-Tocopherol is known to have the most powerful

vitamin E activity among these 8 isomers. Table 12 presents the relative biological activities of the various tocopherols and tocotrienols in the rat resorption-gestation test (Kamal-Eldin and Appelqvist 1996). The biological activities of vitamin E are usually compared based on their efficacy, when administered orally, that prevents resorption-gestation in 50 % of female rats deprived of vitamin E. Symptoms caused by α-T deficiency can be alleviated by T3 (Khanna et al. 2005a; Traber et al. 2004). For example, α-T3 restored fertility in TTP-deficient mice (Jishage et al. 2001). Thus, T3 may be viewed as being members of the natural vitamin E family, not only structurally but also functionally.

Table 12. Relative biological activities (in %) of vitamin E active compounds in the rat resorption-gestation test (Kamal-Eldin and Appelqvist 1996).

Form of vitamin E	Relative activity in prevention of fetal resorption (%)
d-α-tocopherol (RRR)	100
d-β-tocopherol (RRR)	25-50
d-γ-tocopherol (RRR)	1-11
d-δ-tocopherol	1-3
d-α-tocotrienol	29-30
d-β-tocotrienol	5
d-γ-tocotrienol	Not known
d-δ-tocotrienol	Not known

Adopted from EFSA 2008

2. Antioxidant effects

It is well known that vitamin E compounds have antioxidant properties (Sen 2007; Nesaretnam et al. 2008) and have been used as a food additive to prevent oxidation (Theriault et al. 2002; Romero et al. 2004; Rossi et al. 2007). Tocotrienols, like T, are capable of scavenging and quenching free radicals. Their antioxidative activity resides mainly with its chain breaking property, which neutralizes peroxyl and alkoxyl radicals generated during lipid peroxidation

(Nesaretnam et al. 2008) and T3 is more a potent antioxidant against lipid peroxidation than T (Serbinova et al. 1991, 1994; Qureshi et al. 2000; Ahmad et al. 2005). Peroxidation of membrane lipids is known to modify and inactivate cellular components, which can have damaging effects on crucial cellular factors, leading to disease.

In addition, antioxidant properties of T3 may have a role in protection against cancer and other ailments (Watson and Preedy 2008; Ahn et al. 2007; Cuddihy et al. 2004; Khanna et al. 2003, 2005a).

C. Review of safety data

1. Bioavailability and metabolic fate of T3

Once ingested, the T3 and α-T form chylomicrons in the intestine when they are released and mixed with human gastrointestinal fluid to enter the circulation from the intestine. The conversion of chylomicrons to remnant particles results in the distribution of newly absorbed T3 and α-T to all of the circulating lipoproteins and ultimately to tissues (Traber et al. 2004). In the liver, newly absorbed dietary lipids are incorporated into nascent very low-density-lipoproteins (VLDL). The liver is responsible for the control and release of α-T into blood plasma. α-Tocopherol transfer protein (TTP) is required for the release of α-T back into the plasma. α-Tocopherol transfer protein has the ability to bind to both α-T3 as well as α-T and TTP is known to bind to α-T3 with 8.5-fold lower affinity than that for α-T (Hosomi et al. 1997; Khanna et al. 2005a). The data indicate that tissue delivery of α-T3 is likely to be compromised when α-T and α-T3 are co-supplemented (Khanna et al. 2005a). However, a study of Khanna et al. (2005a) with the TTP-deficient mice indicates the existence of a TTP-independent mechanism for the tissue delivery of oral α-T3.

Recently it has been demonstrated that γ-T is more rapidly metabolized in women than in men (Leonard et al. 2005). In rat studies, delivery of oral α-T3 to the heart of females was more efficient compared to that of males (Khanna et al. 2005b; Patel et al. 2006). The accumulation of α-T3 in various organs over time was eliminated in 7 wk when the sub-group of rats was subjected to a vitamin E-deficient diet.

1) Human clinical trials

In a human study investigating the pharmacokinetics and bioavailability of T3 (Yap et al. 2001), eight volunteers were administered a single oral dose of mixed T3 (a 300 mg dose of α-, γ-, and δ-T3) under fed or fasted conditions. The bioavailability of α-T3 under the two conditions was compared using the outcomes, peak plasma concentration (Cmax), time to reach peak plasma concentration (Tmax), and total area under the plasma concentration-time curve (AUC). The absorption of each T3 was 100% greater in the fed state and Cmax was achieved between 3 and 5 h. A statistically significant difference was observed between the fed and fasted logarithmic transformed values of Cmax ($P < 0.01$) and AUC (0-infinity) ($P < 0.01$) for all three T3. However, no statistically significant difference was observed between the fed and fasted Tmax values of the three homologues. The mean apparent elimination half-life (t(1/2)) of α-, γ-, and δ-T3 was estimated to be 4.4, 4.3 and 2.3 h, respectively, being between 4.5- to 8.7-fold shorter than that reported for α-T. No statistically significant difference was observed between the fed and fasted t(1/2) values (Hayes et al. 1993).

Some TRF preparations, such as Tocomin® Suprabio™, contain emulsifiers, self-emulsifying drug delivery systems (Sen 2007). Emulsifiers are known to increase absorption of fat-soluble

compounds. A recent study using Tocomin® Suprabio™ examined the post-absorptive fate of tocotrienol isomers (Khosla et al. 2006). The peak plasma concentration of α-T3 (~3 uM) was 2–3 times more than the peak concentration reported in previous studies using TRF supplements not containing emulsifiers (O'Byrne et al. 2000; Yap et al. 2001).

2) Animal Studies

Orally supplemented α-T3 was successfully delivered to several vital organs among which the skin, adipose, ovaries, and the heart seemed to be preferred destinations within the body of rats (Khanna et al 2005b; Patel et al. 2006). α-Tocotrienol that accumulated in organs for over 2 yr was almost completely lost from the tissues in less than 2 mo after switching to a α-T3-deficient diet (Patel et al. 2006). The shorter elimination half life of T3 than that of T seems to be dependent on its higher metabolic rate and antioxidant property (Traber and Sies 1996; Birringer et al. 2002; Cuddihy et al. 2004). The ability of long-term oral supplementation to maintain and even increase α-T3 levels in vital organs, together with the rapid elimination from all organs studied, underscores the need for continuous oral supplementation of T3. The transport efficiency of α-T3 to tissues may be maximized by eliminating the co-presence of α-T in the oral supplement (Khanna et al 2005b).

a) Nine-generation rat study (Patel et al. 2006)

Oral supplementation of T3 (5 mg/kg BW; 5 d/wk) for 3 yr in 9 generations of female and male rat did not show any adverse effects. In this study, 10 vital organs (including skin, adipose, heart, lung, brain, spinal cord, ovary, and testes) were examined. To gain insight into the turnover of α-T3 in tissues, a subset of supplemented rats was moved to a vitamin E-deficient

diet for 7 wk. Orally supplemented α-T3 was delivered to all vital organs including the brain and spinal cord in significant amounts. In organs such as the skin, adipose, and gonads the maximum level of α-T3 achieved in response to supplementation was folds higher than baseline values of α-T3 in rats maintained on laboratory chow. The metabolic fate of T3 showed a gender-dependency. The level of α-T3 was approximately one magnitude higher in ovaries than in testes. Elimination of α-T3 from the enriched ovaries, under conditions of the vitamin E-free diet, was observed to be much sharper compared to that from the testes. Females had higher levels of α-T3 compared to matched tissues (including skin, heart, and lung) of corresponding males. To gain insight into how quickly α-T3 is metabolized in the tissues, washout of α-T3 from vital organs was examined. Unlike that for the transport of α-T, the liver does not seem to serve as the hub for α-T3 distribution in the body. Levels of α-T3 in the liver were much lower than the levels noted in most organs studied. α-Tocotrienol that accumulated in vital organs over more than 2 yr was almost completely lost in less than 2 mo when the supplementation was stopped. This is in sharp contrast with findings related to α-T retention.

b) Five-generation rat study (Khanna et al. 2005b)

A long-term study was conducted to examine the effects of α-T3 or α-T supplementation (5 mg/kg BW/d) over 60 wk, either alone or in combination, on tissue levels. Orally supplemented α-T3 was successfully delivered to several vital organs. In this study, rats were maintained on a vitamin E-deficient diet and gavaged with α-T3 or α-T alone or in combination. Five generations of rats were studied over 60 wk. α-Tocopherol transfer protein-deficient mice were supplemented with α-T3 and bred to examine tissue delivery of oral α-T3. Orally supplemented α-T3 was effectively delivered to most tissues over time. When co-supplemented,

α-T outcompeted α-T3 for transport systems delivering vitamin E to tissues. To evaluate the significance of TTP in α-T3 delivery to tissues, tissue levels of α-T3 in supplemented TTP-deficient mice were studied. α-T3 was transported to several vital organs in TTP-deficient mice. α-Tocotrienol restored fertility in TTP-deficient mice. The transport efficiency of α-T3 to tissues may be maximized by eliminating the co-presence of α-T in the oral supplement.

The gender-specific effect (better transport of T3 in females than in males) was noted as a general trend across all organs studied. The level of α-T3 in the ovary was over 5-fold higher than that in the testes from the corresponding males rats. No adverse effects were found.

2. Safety/Toxicity of Palm TRF—Preclinical studies

The results of various animal studies are summarized in Table 13. All the experiments were conducted under good laboratory practice (GLP). As shown in Table 13, a 52-wk chronic toxicity study (Tasaki et al. 2008) showed the no-observed-adverse-effect level (NOAEL) as 0.4% palm TRF in the diet (or 303 mg/kg BW/d for males, and 472 mg/kg BW/d for female rats). A subchronic toxicity study of Nakamura et al. (2001) showed no abnormality at the 0.19% TRF in diet (or 120 mg/kg BW/d for males, and 130 mg/kg BW/d for female rats).

A 5 week chick study (Yu et al. 2006) indicated that safe intake was estimated to be up to 1000 mg/day. Thus, the NOAEL of TRF appears to be 0.4% in diet, Also a subacute toxicity study (14 d) on the TRF from rice bran oil in rats showed that up to 0.5% TRF in the diet was safe (Sheppard et al. 1996). In addition, various studies reported cancer-preventive effects of palm TRF at lower levels.

Table 13. Studies on subacute, subchronic and chronic toxicity of palm TRF.

Species	Route	Dose of TRF	Duration	Result	Reference
Male rat	Feed	120 mg/kg/d or 0.19% in diet (T3-78.6%; α-T-21.4%)	13 wk	No abnormalities	Nakamura et al. 2001
Female rat	Feed	130 mg/kg/d or 0.19% in diet (T3-78.6%; α-T-21.4%)	13 wk	No abnormalities	Nakamura et al. 2001
Male rat	Feed	303 mg/kg/d or 0.4% in diet (T3-78.7%; α-T-21.3%)	52 wk	No abnormalities	Tasaki et al. 2008
Female rat	Feed	472 mg/kg/d or 0.4% in diet (T3-78.7%; α-T-21.3%)	52 wk	No abnormalities	Tasaki et al. 2008
Rat	Feed	0.75% and 3% in diet (T3-78.6%; α-T-21.4%)	13 wk	Abnormal pathological changes in male liver and hematological changes in females	Nakamura et al. 2001
Rat	Feed	1-2 % in diet (T3-78.7%; α-T-21.3%)	52 wk	Abnormal pathological changes in liver	Tasaki et al. 2008
Chick	Feed	1000 mg/d	5 wk	No abnormalities	Yu et al. 2006
Rat	Gavage	5000 mg/kg (T3-80%; α-T-20%)	14 d	No abnormalities	Sheppard et al. 1996*

*All the studies used palm TRF except the study of Sheppard et al. (1996). This study employed TRF from rice bran oil.

Table 14 summarizes the toxicity studies on palm oil and rice bran oil, which share similar TRF composition profiles. This review does not include other oils whose TRF compositions are different from that of palm TRF. For example, annatto oil TRF contains 85-92% δ-T3 and palm TRF has approximately10% δ-T3.

A subacute toxicity of palm oil extract (containing 80% T3) in young rats and mice demonstrated that up to 2500 mg/kg BW was safe (Oo et al. 1992). Also, repeatedly heated refined and crude palm oils showed no mutagenicity (Manorama et al. 1989). All the experiments were conducted under GLP.

In addition, various studies showed no teratogenic effects and genotoxicity of rice bran oil. A diet containing 10% rice bran oil, as compared to peanut oil, had no teratogenic effect

(Raghuram and Rukmini 1995). Rice bran oil concentrate representing the T3-T preparation up to 5000 ug/plate had no mutagenic activity in an Ames test (Polasa and Rukmini 1987).

Table 14. Toxicity studies of palm oil and rice bran oil

Species	Route	Test material	Duration	Result	Reference
Rat	Oral	Palm oil extract, 2.5 mg/kg BW/d	30 d	No abnormalities	Oo et al. 1992
Rat	Feed	5% red palm oil in diet	42 d	No abnormalities	Watkins et al. 2001
S. typhimurium TA100 and TA98	W/ & w/o metabolic activation	Heated (8-24 h) refined and crude palm oils	48 h	No mutagenic activity	Manorama et al. 1989
S. typhimurium TA100 and TA98	W/ & w/o metabolic activation	5000 ug/plate rice bran oil concentrate	48 h	No mutagenic activity	Polasa and Rukmini 1987
Rat	Feed	10% rice bran oil	Over 3 generations	Normal reproductive performance and no teratogenicity	Raghuram and Rukmini 1995

Details

1) A 13-week sub-chronic toxicity study (Nakamura et al. 2001)

Nakamura et al. (2001) performed a 13-wk oral toxicity study in 344 5-wk-old Fischer rats of both sexes (10 rats/sex/group) at dose levels of 0 (group 1), 0.19 (group 2), 0.75 (group 3) and 3% (group 4) of palm TRF (Eisai Co., Japan) in the diet. The high dose level of 3% was determined based on a pilot study in which 5%, but not 3%, TRF diet was refused by rats. The mixture used in this study had the following composition: α-T3 21.4%, β-T3 3.5%, γ-T3 36.5%, δ-T3 8.6%, α-T 20.5%, β-T 0.7%, γ-T 1.0% and δ-T 0.5%. The composition of palm TRF used in this study was similar to those of MPOB palm TRF: α-T3 20%, β-T3 3.3%, γ-T3 25%, δ-T3 8.3%, and α-T 23.3%. Because the chemical used in this study contained T, another group (group 5)

was fed a diet containing 0.69% α-T (Tama Biochemistry Co. Ltd), which was identical to the concentration of T in the high-dose (3%) group to assess the influence of T itself.

Suppression of body weight gain was observed in group 4 males. On hematological examination, a significant decrease in mean corpuscular volume (MCV) was observed in all treated males. Platelets were significantly reduced in group 3 and 4 males. Hemoglobin concentration, MCV, and mean corpuscular hemoglobin (MCH) concentration were significantly decreased in group 3 and 4 females (0.75-3% TRF in the diet) and hematocrit (Ht) in group 4 females. Increases in the albumin/globulin ratio (A/G) and alkaline phosphatase (ALP) in all treated males, elevated alanine transaminase in group 4 of both sexes, and increases in asparagine transaminase and g-glutamyl transaminase in group 4 females were observed. With regard to relative organ weights, liver weights in group 4 of both sexes and adrenal weights in all treated males demonstrated an increase, and ovary and uterus weights in group 4 females were reduced.

As a decrease in MCV, an increase in the A/G, elevation of ALP, and increase in adrenal weight were observed in all treated males, a NOAEL could not be determined in this examination. Histopathologically, slight hepatocellular hypertrophy in group 3 and 4 males and reduction of cytoplasmic vacuolation in the adrenal cortical region in group 4 males (3% TRF in the diet) were observed. Because of pathological changes in male liver and hematological changes in females, the authors concluded that the NOAEL was 0.19% in the diet (120 mg/kg BW/d for male rats and 130 mg/kg BW/d for female rats).

2) 52 week chronic toxicity study (Tasaki et al. 2008)

Tasaki et al. 2008 performed a 52-wk chronic study using Wistar Hannover rats of both sexes (10 rats/sex/group) given the compound at doses of 0, 0.08, 0.4, or 2% palm TRF (Eisai Co., Ltd.,Tokyo, Japan) in a powdered basal diet. Complete dissociation from T was impossible and the mixture used in this study had the following composition: α-T3 21.4%, β-T3 3.5%, γ-T3 36.5%, δ-T3 8.6%, α-T 20.5%, β-T 0.7%, γ-T 1.0%, and δ-T 0.5%. Since 6 animals in the 2% male group died of hemorrhage of several organs by week 50, the maximum dose level was changed to 1% in both sexes for the last 2 wk. A decrease in body weight gain was observed in the 2% males from week 5 and females from week 10, persisting to the end of the study. With the high dose, prolongation of prothrombin time (PT) and increase of serum ALT in males, as well as an increase of serum ALP in both sexes, were observed. In male and female rats receiving 0.4% or less, there were no toxicological changes in any of the criteria examined. At necropsy, multiple cyst-like nodules on the liver surface were macroscopically pronounced in both sexes receiving 2%. On histopathological examination, hepatocellular nodules were evident with distortion of hepatic cords and compression of the surrounding tissue, almost all including areas of spongiosis hepatis. The constituent hepatocytes were immunohistochemically stained with proliferation cell nuclear antigen at high rates. Nevertheless, they did not exhibit overt atypia and the basic lobular architecture remained intact. Additionally, they were consistently negative for glutathione *S*-transferase placental form (GST-P). Quantitative GST-P analysis of the liver sections overall showed numbers of GST-P foci in the high dose females to be significantly elevated as compared to the control value. Based on the data demonstrating nodular liver lesions only at the high dose of both sexes, the authors concluded that the NOAEL was 0.4% (303 mg/kg BW/d for males and 472 mg/kg BW/d for females).

3) 4 week chick study (Yu et al. 2006)

Diets supplemented with 50 (115 umol/kg diet), 100 (230 umol/kg), 250 (575 umol/kg), 500 (1150 umol/kg), 1000(2300umol/kg), or 2000 (4600 umol/kg) ppm of palmTRF, α-T, α-T3, γ-T3, or δ-T3 were fed to 204 White Leghorn female chickens (divided into 34 groups) weighing 40-50 g for 4 wk. Supplemental palm TRF produced a dose-response (50-2000 ppm) lowering of serum total and LDL cholesterol levels of 22% and 52% ($P < 0.05$), respectively, compared with the control group. Supplemental α-T3 within the 50-500 ppm range produced a dose-response lowering of total (17%) and LDL (33%) cholesterol levels. The more potent γ- and δ-isomers yielded dose-response (50-2,000 ppm) reductions of serum total (32%) and LDL (66%) cholesterol levels. A lowering of LDL cholesterol was achieved with higher levels of α-T; 2,000 ppm showed a modest response compared with responses to palm TRF and the individual T3, yielding a 6% reduction. HDL-cholesterol levels were minimally impacted by the T3; thus HDL/LDL cholesterol ratios were markedly improved (123-150%) by the supplements. Serum triglyceride levels were significantly lower in subjects receiving the higher supplements.

There were no supplement-induced changes in weight gain, weight of organs, or feed consumption. The study suggest that a T3 and/or α-T concentration up to 1000-2000 ppm (or 1000-2000 mg/kg diet) in the diet can show beneficial effects without having side effects.

4) Subacute toxicity studies of palm oil extract in mice and rats (Oo et al. 1992)

Palm oil extract (containing 80% T3 and 20% α-T) administered to young Sprague-Dawley rats (weighing 150-180 g) and albino mice (weighing 15-20 g) at a dose of up to 25,000 mg/kg BW for 30 d showed no appreciable adverse effects in animals with respect to physical

manifestations or behavioral changes (Oo et al. 1992). In this study, the animals were fed daily doses (250, 500, 1000, or 2500 mg/kg) of palm oil extract in refined, bleached and deodorized olein for 30 d. Results indicated that treated rodents did not exhibit any physical abnormalities or unusual behavior. However, some sluggish motor activity was manifested. Hematology, serum biochemistry, and histopathological observations were not made in this study. Interestingly, treated mice gained weight significantly whereas treated rats grew normally over the 30 d period. The authors concluded that palm oil extract, in extremely high doses, did not appear to produce appreciable adverse effects in the animals used.

Watkins et al. (2001) studied the effect of red palm olein on bone metabolism in growing rats. Rat growth was not affected by the dietary lipid treatments after 42 d of feeding (up to 5% red palm oil in diet). As compared to the soybean oil group (3.5% in diet), the concentrations of saturated fatty acids and monounsaturated fatty acids were higher but polyunsaturated fatty acids (PUFA) lower in tibia marrow of rats given the palm oil treatments compared with those given soybean oil. The reduction in total n-6 PUFA concentration in bone of rats given palm oil was associated with higher bone specific alkaline phosphatase activity and endosteal mineral apposition rate and bone formation rate in tibia. No difference was found in *ex vivo* prostaglandin E2 (PGE_2) production in femur from rats. The histomorphometric analyses indicated that red palm olein might support bone formation and bone modeling in growing rats. Palm oil or palm antioxidants may play a role in bone formation.

5) Carcinogenicity

At high concentration (2% in diet), T3 may be a hepatocarcinogen in female rats (Tasaki et al. 2008). However, long-term administration of T3 at a dose of 30 ppm was shown to exert a

preventive effect on liver tumor development in the Solt and Farber hepatocarcinogenesis model (Rahmat et al. 1993). This study investigated the effects of long-term administration of T3 on hepatocarcinogenesis in rats (28 male 7- to 8-wk-old *Rattus norwegicus* rats, weighing 120 to 160 g) induced by diethylnitrosamine (DEN) and 2-acetylaminofluorene (AAF). The T3 was composed of 80% γ-T3, 20% α- and β-T3 supplied by PORIM, Malaysia. The rats were killed after 9 mo and the livers were examined morphologically. Grayish white nodules (2/liver) were found in all the DEN/AAF-treated rats (n = 10), but only one of the rats treated with DEN/AAF and supplemented with T3 (n = 6) had liver nodules. The authors concluded that T3 supplementation attenuated the impact of the carcinogenesis in the rats.

Tocotrienols inhibit the growth of hepatoma cells but not that of hepatocytes from healthy rat liver (Sakai et al. 2004). Tocotrienols killed murine liver cancer cells but not normal cells (Har and Keong, 2005). Research indicates that T3 has an inhibitory effect on angiogenic factor secretion from cancer cells and mediates inhibition of growth factor-induced survival, migration, and angiogenesis signals (Miyazawa et al. 2008, 2009). Tocotrienols act on cell proliferation in a dose-dependent manner and can induce programmed cell death (Nesaretnam et al. 2008). At concentrations of 0.5-5.0 ug/mL, palm TRF dose-dependently protected against LPS-induced cell death (Ahn et al. 2007).

Consistent with observations made in other in vitro systems, T3 inhibits the growth of breast cancer cells in vitro and in vivo (Guthrie et al. 1997; Yu et al. 1999; Takahashi and Loo 2004; Nesaretnam et al. 1998, 2000, 2004; Elangovan 2008) as well as prostate cancer cells (Kumar et al. 2006; Srivastava and Gupta, 2006), gastric cancer cells (Sun 2008, 2009), and colon cancer cells (Shibata et al. 2008).

Tocotrienol is effective in suppressing mevalonate synthesis. By doing so, T3 can deplete tumor tissues of two intermediate products, farnesyl pyrophosphate and geranylgeranyl Pyrophosphate (Pearce et al. 1994).

6) Genotoxicity

The mutagenic potential of repeatedly heated (8- 24 h) refined and crude palm oils has been examined using the Ames test with *Salmonella typhimurium* strains TA100 and TA98 with and without metabolic activation (rat liver S9 fraction; Manorama et al. 1989). Benzo (a) pyrene (5 ug/plate) and sodium azide (1 ug/plate) were used as positive controls for TA 98 and TA 100 respectively. Dimethylsulfoxide (DMSO)-based extracts of the unheated crude and heated refined palm oils did not show mutagenic activity in these tests.

Oliveira et al. (1994) investigated the genotoxicity of red palm oil on bone marrow cells from 8-10 wk old Balb/C female mice. Experimental groups received daily doses of 4.5 g/kg supernatant and sediment of red palm oil (no information on TRF content). Corn oil served as the negative control and cyclophosphamide (20 mg/kg intraperitoneally) was used as the positive control. There were no differences in the frequency of chromosomal aberrations between the two groups of mice.

7) Related studies

a) Subacute toxicity study on the TRF from rice bran oil in rats

Exposure of rats up to 0.5% TRF in diet (rice bran oil source, representing the T3 and T preparation) by gavage resulted in no deaths and no abnormal clinical observations during the 14 d observation period (Sheppard et al. 1996).

b) The mutagenicity of rice bran oil concentrate.

A dimethylsulfoxide (DMSO)-based extract of rice bran oil (up to 5000 ug per plate) was reported to reveal no mutagenic activity in an Ames test using *Salmonella typhimurium* strains TA98 and TA100 with and without metabolic activation (Polasa and Rukmini 1987). Also snack foods fried in rice bran oil did not have any mutagenic effects in the bacterial screening system (Polasa et al. 1987; Raghuram and Rukmini 1995).

c) Reproductive and developmental toxicity

A review of Raghuram and Rukmini (1995) documented normal reproductive performance and lack of teratogenicity of rice bran oil in albino rats of NIN Wistar strain (15 males and 15 females in a three generation study). A diet containing 10% rice bran oil, as compared to peanut oil containing relatively lower amounts of T3, did not result in differences in conception, birth weights, litter size, weaning weights, and pre-weaning mortality, and had no teratogenic effect. There were no differences in body weight, feed efficiency ratio, and nitrogen, phosphorus, and calcium balances in the animals over three generations.

3. Safety/Toxicity of T3 and/or Palm TRF - Clinical studies

A number of clinical trials have been conducted with T3 and T and few side effects have been reported (EFSA 2008). Palm oil is a rich source of T3 that has been a traditional staple food in several Asian countries and also in some African countries. Table 15 summarizes human clinical studies on palm TRFs which had primary endpoints of DNA damage, blood lipid profiles, oxidative stress, and/or bioavailability. As shown in Table 15, daily consumption of up to 330-360 mg palm TRF was not associated with any adverse effects in healthy persons as well as in

subjects with hypercholesterolemia or non-insulin-dependent diabetes mellitus (NIDDM). In these studies, the percentages of T3 in palm TRF varied from 70% to 100%, and the proportion of α-T ranged from 0 to 30%.

Table 15. Human clinical studies reporting no adverse effects of palm TRF

Number and status of subjects	Dose of TRF	Ratio of T3: α-T	Duration	Reference
22 healthy	60 mg/d	70:30	6 mo.	Tan et al. 1991
36 healthy	80-320 mg/d	Mixed (15% δ-)	8 wk	Rasool et al. 2006
64 healthy	160 mg/d	74:26	6 mo	Chin et al. 2008
52 healthy	250 mg/d	Individual doses of α-, γ- or δ-tocotrienyl acetate (81%)	8 wk	O'Byrne et al. 2003*
8 healthy males	300 mg	84.1:2.3	Single dose	Yap et al. 2001
25 hypercholesterolemic	200 mg/d	70:30	6 wk	Qureshi et al. 1991
25 hypercholesterolemic	200 mg γ-T3/d	γ-T3 only	4 wk	Qureshi et al. 1991
36 hypercholesterolemic	260 mg/d	85:15	4 wk	Qureshi et al. 1995
36 hypercholesterolemic	200 mg γ-T3	γ-T3 only	4 wk	Qureshi et al. 1995
44 hypercholesterolemic	240 mg/d	70:30	20 wk	Walqvist et al. 1992
41 mildly hypercholesterolemic	240 mg/d	2:1	4 wk	Mensink et al. 1999
28 mildly hypercholesterolemic	73 mg/d, every other day	50:22.9	4 wk	Ajuluchukwu et al. 2007
50 carotid atherosclerosis	330 mg/d	72.7:27.3	18 mo	Tomeo et al. 1995
50 carotid atherosclerosis	56 mg/d	40:16	2-3 yr	Kooyenga et al. 1997, 2001
32 NIDDM	360 mg/d	80:20	60 d	Wan Nazaimoon et al. 1996

*Individual doses of tocotrienyl acetate

In addition, studies on rice bran TRF did not report any adverse effects (Qureshi et al. 1997, 2001, 2002; Kooyenga et al. 2001; Baliarshingh et al. 2005; Rubin et al. 2008).

Table 16. Human clinical studies reporting no adverse effects of rice bran TRF

Number and status of subjects	Dose of T3	Ratio of T3: α-T	Duration	Reference
28 hypercholesterolemic	3 mg/day T3; 0.04 mg/kg BW/day;	T3: α-T: δ-T= 86.9:8.7: 4.4	35-70 d	Qureshi et al. 2001
36 hypercholesterolemic	0.33mg/kg BW/d from a processed rice bran oil	25 mg α-T, 42 mg γ-T, 20 mg δ-T	4 wk	Qureshi et al. 1997
90 hypercholesterolemic	25, 50, 100, and 200 mg/d	T3: α-T: δ-T= 86.9:8.7: 4.4	5 wk	Qureshi et al. 2002
25 carotid atherosclerosis	112 mg/d	T3:α-T=52:60	3 yr	Kooyenga et al. 2001
19 NIDDM	3mg/kg BW/d	T3:α-T=92.5: 7.5	60 d	Baliarshingh et al. 2005
23 familial dysautonomia	100 mg/d	NA	3–4 mo	Rubin et al. 2008

Details

1) DNA damage study—at 160 mg/d for 6 mo

Chin et al. (2008) evaluated the effect of T3 on DNA damage. In this study, a daily dose of 160 mg of commercially available palm TRF (a mixture of α -, β-, γ-, and δ-T3 and α-T) was given for 6 mo to 64 healthy subjects aged 37-78 yr. The total T3 isomer ratio compared with T is approximately 74:26. No adverse effects were reported. Instead, results showed a significant reduction in DNA damage as measured by the comet assay after 3 mo ($P < 0.01$) and remained low at 6 mo ($P < 0.01$).

2) Lipid profile studies- 140-360 mg/d for up to 36 mo.

In general, the palm TRF lowered blood cholesterol concentrations without showing any adverse effects in healthy volunteers and subjects with hypercholesterolemia or NIDDM. For example, administration of 60 mg palm TRF in 22 healthy Malaysian subjects (with no age limitation) for 30 d lowered both serum total cholesterol (TC) and low-density-lipoprotein

cholesterol (LDL-C) concentrations. The magnitude of reduction of serum TC ranged from 5 to 35.9% whereas the reduction of LDL-C values ranged from 0.9 to 37.0% when compared with their respective starting values. No adverse effects were reported (Tan et al. 1991).

Rasool et al. (2006) assessed the effects of TRF supplementation (up to 320 mg/d of TRF composed of 34.6% α-T3, 24.6% γ-T3, 15% δ-T3, and 26.2% α-T: Golden Hope Plantations, Malaysia) for 2 mo on blood lipid profiles and arterial compliance in 36 healthy Malaysian men. The authors stated that the palm TRF supplements at doses up to 320 mg/d were well-tolerated with no serious adverse events.

The palm TRF was tested in 16-25 hypercholesterolemic Malaysian subjects aged 30-60 yr at a dose of 200 -300 mg/d over a 4-8 wk period (Qureshi et al. 1991, 1995). Significant reductions in serum total cholesterol, LDL cholesterol, apolipoprotein B, thromboxane B2, and platelet factor were observed. No adverse effects were reported.

In a study with 44 Australian subjects with hypercholesterolemia (aged 25-75 yr; Wahlqvist et al. 1992), administration of palm oil for 20 wk (up to 240 mg T3/d) did not show any changes in serum lipid profiles and plasma prostaglandins and platelet functions. No adverse effects were reported.

Mensink et al. (1999) reported that the TRF supplements (240 mg palm TRF/d for 4 wk) used had no adverse effects in 41 Netherlands men with slightly elevated lipid concentrations (aged 21-61 yr). Also, the study of Wan Nazaimoon et al. (1996) showed that administration of 360 mg palm TRF/d (a mixture of 288 mg T3 and 72 mg α-T) for 60 d did not cause adverse changes in 32 Malaysian subjects with NIDDM (aged 31-70 yr).

Ajuluchukwu et al. (2007) studied the effect of palm oil TRF on the serum lipid profiles of 28 individuals aged 18-80 yr with mild hypercholesterolemia and another cardiovascular risk

factor. Subjects were randomly assigned to consume tocotrienols as TOCOVID™ Suprabio™ capsules (consisting of 15.38 mg α-T3, 28.20 mg γ-T3, 6.42 mg δ-T3 and 22.90 mg α-T) or 500 mg or 1000 mg vitamin E (α-T) capsules per day or every other day (n=16) for 4 weeks. Compared to the T supplement, the T3 supplement significantly decreased total cholesterol and LDL-cholesterol, but not HDL-cholesterol or triglyceride concentrations. No information about adverse effects was reported.

Kooyenga et al. (1997, 2001) investigated the effect of supplementation with an extract from palm oil (52 mg of T3, 60 mg of α-T) for 2-3 yr on carotid atherosclerosis. The authors stated that there were no reports of headache, intestinal upset, muscle weakness, or persistent liver weakness abnormalities in the 50 Malaysian subjects studied.

In another placebo controlled study, α-, γ-, and δ–T3 acetates were administered individually to 52 American healthy subjects at a daily dose of 250 mg for 8 wk (O'Byrne *et al*. 2000). No adverse effects of T3 ester ingestion were observed in this study.

As shown in Table 16, several studies reported no adverse effects with the supplementation of TRF or a processed oil extracted from rice bran oil. Qureshi *et al*. (1997) performed a randomized double blind placebo controlled trial in which 41 hypercholesterolemic Malaysian subjects were first placed on the low fat diet for 4 wk and then given 200 mg/d of TRF from rice bran oil or corn oil as placebo. No adverse effects due to T3 ingestion were observed.

Qureshi et al. (2001) investigated the efficacy of TRF of rice bran alone and in combination with the cholesterol lowering drug, lovastatin. The TRF consisted of 8.7% α-T, 4.4% δ-T, and 86.9% T3. The combination of TRF and lovastatin significantly reduced lipid metabolites by 20 - 25% ($P < 0.001$) in these subjects. The authors stated that subjects reported no side effects during the 25-wk study.

Qureshi et al. (2002) investigated the effect of 4 doses (25, 50, 100, and 200 mg/d) of a TRF (8.7 % α-T, 15.5 % α-T3, 1.6% β-T3, 39.4 % γ-T3, 5.2 % δ-T3, and 4.4 % δ-T, 20.9% D-desmethyl (D-P_{21}-T3), plus D-didesmethyl (D-P_{25}-T3) T3, 4.3 % unidentified tocotrienols) extracted and purified from rice bran oil on the suppression of serum cholesterol in Malaysian adults. No information on adverse effects was reported in the study.

Baliarsingh et al. (2005) reported the therapeutic impacts of palm TRF (3 mg palm TRF/kg BW/d, 60 d) on serum lipoprotein lipid levels in 19 Indian subjects with NIDDM. The composition of tocols in palm TRF was 7.5% α-T, 14.6% α-T3, 2.2% β–T3, 38.8% γ-T3, 4.6% γ-T, and 29.9% δ-T3. No adverse effects were reported.

Kooyenga et al. (2001) investigated the effect of supplementation with an extract from rice bran for 1 yr on carotid atherosclerosis. Each rice bran oil capsule (produced by Rangsit Biotech, Bangkok) provided 52 mg of T3 and 60 mg of α-T. The authors stated that there were no reports of headache, intestinal upset, muscle weakness, or persistent liver weakness abnormalities in the 50 Malaysian subjects studied.

3) Oxidative stress and biomarker test- 240 mg/d T3 and 90 mg/d α-T for 18 mo

In a randomized, placebo-controlled study, 50 subjects (aged 49-83 yr) with carotid atherosclerosis received palm TRF (240 mg T3 and 90 mg T/d) or placebo in addition to standard medical therapy over 18 mo (Tomeo et al. 1995). Serum measures of oxidative stress (TBARS) showed a significant decrease with no appreciable change in serum lipids or other biochemical markers. Both T3 and placebo groups displayed attenuated collagen-induced platelet aggregation responses ($P < 0.05$) as compared with entry values. Serum total cholesterol, low density

lipoprotein cholesterol, and triglyceride values remained unchanged in both groups, as did the plasma high density lipoprotein cholesterol values. No adverse effects were reported.

4) Bioavailability study - 300 mg, a single dose

In a study reported by Yap et al. (2001), healthy volunteers were administered a single oral dose of 300 mg TRF (841 mg T3 and 23.3 mg α-T, Tocovid, Hovid Pte.Ltd, Malaysia) under fed or fasted conditions to study their bioavailability. No adverse effects were reported.

4. Safety of vitamin E

Three international expert groups have recently evaluated the safety of vitamin E: the Antioxidant Panel of the Food and Nutrition Board at the IOM of the U.S. National Academy of Sciences (IOM 2000), the EU Scientific Committee on Food (SCF 2003), and the UK Expert group on Vitamins and Minerals (EVM 2003). The IOM set the tolerable upper intake level (UL) for adults at 1000 mg/d of any supplemental form of α-T (IOM 2000). Unlike the IOM's recommendations that are based on α-T only, two European expert groups set the ULs of vitamin E based on TEV. The SCF, based on a placebo controlled, dose-response supplementation study in 88 healthy humans (Meydani et al.1998), set the NOAEL at 540 mg TEV, the highest dose used in the study. Considering an uncertainty factor of 2 to cover for inter-individual differences in sensitivity, the SCF established an UL of 270 mg TEV for adults, which was rounded to 300 mg TEV (SCF 2003). The EVM established a NOAEL of 540-970 mg TEV, based on three placebo controlled human studies (Gillilan et al. 1977, Meydani et al. 1998, Stephens et al. 1996, EVM 2003). An uncertainty factor to account for inter-individual differences was not considered since the results of the larger trial by Stephens et al. (1996) support a NOAEL of 540 mg TEV, so that the UL (defined by the EVM as a

safe UL for consumption over a lifetime) was established as 540 mg TEV for supplemental vitamin E (EVM 2003).

Most of the safety studies on vitamin E were carried out in the past few decades. Table 17 summarizes subacute and subchronic toxicity studies that have been carried out in animals (Demole 1939; Weissberger and Harris 1943; Levander et al. 1973; Dysma and Park 1975; Krasavage and Terhaar 1977; Abdo et al. 1986; Diplock 1995). There is no evidence that there is any detrimental effect attributable to vitamin E up to 500 mg/kg diet.

Table 17. Studies on subacute and subchronic toxicity of vitamin E.

Species	Route	Dose	Duration	Result	Reference
Rat	Oral	130 mg/kg/d	2 mo	No abnormalities	Demole 1939
Rat	Oral	100 mg/d	4 mo	Small increase in phosphorus turnover in the femur. Otherwise, no abnormalities	Weissberger and Harris 1943
Rat	Oral	10 mg/d	4 mo	No abnormalities	Weissberger and Harris 1943
Rat	Feed	500 mg/kg diet	1 mo	Small effect on liver TG	Levander et al. 1973.
Rat	Feed	875 and 1750 mg/kg	3 mo	No abnormalities	Dysma and Park 1975
Rat	Feed	0.0002 and 0.2% in diet	3 mo	No abnormalities	Krasavage and Terhaar 1977
Rat	Gavage	125-500 mg/kg 2000 mg/kg	3 mo 3 mo	No abnormalities Extended prothrombin time	Abdo et al. 1986

Adopted from Diplock 1995

Table 18 shows that there is no adverse effect of vitamin E (up to 2% in diet) with respect to the teratogenicity and reproductive toxicity (Hook et al. 1974; Krasavage and Terhaar 1977; Diplock 1995).

Table18. Studies on teratogenesis and reproductive toxicity of vitamin E.

Species	Route	Dose	Duration	Result	Reference
Rat	Feed	0.00, 0.2, and 2.0%	Days 6-16 of pregnancy	No difference between treatment and placebo groups	Krasavage and Terhaar 1997
Rat	Feed	0.002, 0.2, and 2.0%	F_1 generation: mated on days 112 and 175 of dosage: F_0 generation: 268 d	No effects on reproductive toxicity, teratogenicity, clinical and hematologic indexes	Krasavage and Terhaar 1997
Mouse	Gavage	591 IU/mouse	Days 7-11 of pregnancy	One malformation in 91 mice	Hook et al. 1974

Adopted from Diplock 1995

As shown in Table 19, vitamin E has no carcinogenic activity (up to 0.2% in diet for 24 mo; Weldon et al. 1983; Yang and Desai 1977; Diplock 1995). Table 20 shows that α-T intake of up to 600 mg/d for 12 wk did not show adverse effects as evaluated by clinical chemical indices, liver, kidney and thyroid functions, serum lipids, PT time, and urinary indices (Farrell and Bieri 1975; Corrigan et al. 1982; Stampfer et al. 1983; Kitagawa et al. 1989; Diplock 1995). These data support the IOM's assessment that up to a vitamin E (as α-T) intake of 1000 mg/d is safe for human consumption.

Table19. Studies on chronic toxicity and carcinogenicity of vitamin E

Species	Route	Dose	Duration	Result	Reference
Rat	Feed	500, 1000, and 2000 mg/kg/d	24 mo	No significant effects on a range of clinical chemical indexes; slight increase in liver weight and in liver enzymes in serum; hemorrhage (anti-vitamin K effect) at 2000 mg/kg/d; decrease in mammary tumors	Weldon et al. 1983
Rat	Feed	25, 250, 2500, 10,000, and 25,000 IU/kg	8-16 mo	No abnormalities at low doses; increase in serum alkaline phosphatase and relative heart and spleen weight at high doses (>10,000 IU/kg); no change in prothrombin time; no carcinogenicity	Yang and Desai 1977

Adopted from Diplock 1995

Table 20. Human clinical studies of vitamin E.

Subjects	Dose	Duration	Result	Reference
Adults, *n* = 28	100-800 IU/day: T	4 mo to 21 yr (mean 2.9 yr)	No evidence of toxicity judged by clinical chemical blood indexes (blood coagulation, liver enzymes, and thyroid hormones)	Farrell and Bieri 1975
Volunteers, n = 30 (20)	800 IU/d vitamin E	16 wk	No differences between groups	Stampfer et al. 1983
Volunteers, n = 19	600 mg/d α-T	12 wk	No adverse effects (clinical chemical indices, liver, kidney, and thyroid function, serum lipids, and prothrombin time, and urinary indices all normal)	Kitagawa and Mino 1989

Adopted from Diplock 1995

IV. Conclusions

The information/data provided by NutraSource/MPOB in this report and supplemented by the publicly available literature/toxicity data on T3 and T provide a sufficient basis for an assessment of the safety of T3 for the proposed use as an ingredient in food, when prepared according to appropriate specifications and used according to GMP. Key findings are summarized here:

1. T3 and T are well characterized and of consistent quality across lots and free from chemical and microbial contamination.
2. T3 and T have a long history of use in foods and beverages in the U.S.
3. T3 and T extraction processes have been safely used for many yrs in the dietary supplement industry.
4. The safety and nutritional benefits of T3 and T are well established by human clinical trials and animal studies of T3. There are no indications of significant adverse effects related to T3 consumption in the publicly available literature at the proposed use levels.
5. Increased consumption of vitamin E has been recommended by the USDA Dietary Guidelines Committee and IOM.
6. Intended use of T3 as part of the proposed food use results in levels of exposure significantly below those associated with any adverse effects and provides a reasonable certainty of safety.

Therefore, not only is the proposed use of TRF safe within the terms of the Federal Food, Drug, and Cosmetic Act (meeting the standard of reasonable certainty of no harm), but because of this consensus among experts, it is also *Generally Recognized as Safe* (GRAS).

V. Discussion of Information Inconsistent with GRAS Determination

We are not aware of information that would be inconsistent with a finding that the proposed use of T3 preparations in foods and beverages, meeting appropriate specifications and used according to GMP, is GRAS.

References

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Our Reference	. Doc / GRAS / 1
COA No.	: CT_307000_1A/1
Sampling Date	: 18th March 2009
Released Date	25th March 2009
Manufacturing Date	. 18th March 2009
Best Before Date	· 17th March 2012
Batch No.	B7000_1_180309
Country Of Origin	. Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Reddish Oil Suspension	
2	Tocotrienol/Tocopherol Complex, %	In House Method – CTM_0011/04	30.0 Min	31.3
	d-Gamma Tocotrienol, %		12.0 – 14.0	12 0
	d-Beta Tocotrienol, %		1.0 Min	1.3
	d-Delta Tocotrienol, %		3.3 – 5.0	3.5
	d-Alpha Tocotrienol, %		6.5 – 9.0	7 0
	d-Alpha Tocopherol, %		6.0 – 9.0	7.5
3	Moisture (WT, %) (Karl Fisher)	AOCS 5th Edition Ca 2e-84	1.00 Max	0.09
4	*Peroxide Value, meq/kg	AOCS 5th Edition Cd 8-53	10.0 Max	1.8
5	Plant Squalene, %	In House Method – CTM_0017/00	4.00 Min	13 16
6	Phytosterols Content, %	In House Method – CTM_0018/02	1.00 Min	7.14
7	*Mixed Carotene, %	In House Method – CTM_0013/02	0.03 Min	0.13
8	*Lead (Pb), ppm	AOCS 5th Edition Ca 15-75	3.0 Max	< 3 0
9	*Heavy Metals, ppm	AOCS 5th Edition Ca 15-75	10.0 Max	< 10.0
10	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	< 10
11	**Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	< 10

Certified By

Ms Chung Yoke Lying
Senior Quality Control Chemist
Issued Date . 16th June 2009

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TOCOMIN® 30% (NON-GMO)

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NATURAL FULL SPECTRUM TOCOTRIENOL/TOCOPHEROL COMPLEX 30% OIL SUSPENSION

Our Reference	Doc / GRAS / 2
COA No	: CT_307000_1/1
Sampling Date	: 27th August 2008
Released Date	: 6th September 2008
Manufacturing Date	: 27th August 2008
Best Before Date	: 26th August 2011
Batch No.	: B7000_1_270808
Country Of Origin	: Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Reddish Oil Suspension	
2	Tocotrienol/Tocopherol Complex, %	In House Method – CTM_0011/04	30.0 Min	30.5
	d-Gamma Tocotrienol, %		12.0 – 14.0	12.0
	d-Beta Tocotrienol, %		1.0 Min	1.4
	d-Delta Tocotrienol, %		3.3 – 5.0	3.6
	d-Alpha Tocotrienol, %		6.5 – 9.0	7.0
	d-Alpha Tocopherol, %		6.0 – 9.0	6.5
3	Moisture (WT. %) (Karl Fisher)	AOCS 5th Edition Ca 2e-84	1.00 Max	0.07
4	*Peroxide Value, meq/kg	AOCS 5th Edition Cd 8-53	10.0 Max	1.6
5	Plant Squalene, %	In House Method – CTM_0017/00	4 00 Min	9.62
6	Phytosterols Content, %	In House Method – CTM_0018/02	1.00 Min	5 93
7	*Mixed Carotene, %	In House Method – CTM_0013/02	0.03 Min	0.19
8	*Lead (Pb), ppm	AOCS 5th Edition Ca 15-75	3.0 Max	< 3.0
9	*Heavy Metals, ppm	AOCS 5th Edition Ca 15-75	10.0 Max	< 10.0
10	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	< 10
11	**Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	< 10

Certified By

Ms. Chung Yoke Lying
Senior Quality Control Chemist
Issued Date 16th June 2009

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Carotech

CERTIFICATE OF ANALYSIS

TOCOMIN® 30% (NON-GMO)

KOSHER MS 1500 2004

NATURAL FULL SPECTRUM TOCOTRIENOL/TOCOPHEROL COMPLEX 30% OIL SUSPENSION

Our Reference	: Doc / GRAS / 3
COA No.	. CT_306517_1/3
Sampling Date	: 2nd September 2008
Released Date	: 10th September 2008
Manufacturing Date	: 2nd September 2008
Best Before Date	: 1st September 2011
Batch No.	: B6517_1_020908
Country Of Origin	: Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Reddish Oil Suspension	
2	Tocotrienol/Tocopherol Complex, %	In House Method – CTM_0011/04	30.0 Min	31.7
	d-Gamma Tocotrienol, %		12.0 – 14.0	12.0
	d-Beta Tocotrienol, %		1 0 Min	1.4
	d-Delta Tocotrienol, %		3.3 – 5.0	4.3
	d-Alpha Tocotrienol, %		6.5 – 9.0	7.4
	d-Alpha Tocopherol, %		6.0 – 9.0	6.6
3	Moisture (WT, %) (Karl Fisher)	AOCS 5th Edition Ca 2e-84	1.00 Max	0.07
4	*Peroxide Value, meq/kg	AOCS 5th Edition Cd 8-53	10.0 Max	1.5
5	Plant Squalene, %	In House Method – CTM_0017/00	4 00 Min	6.5
6	Phytosterols Content, %	In House Method – CTM_0018/02	1.00 Min	3.9
7	*Mixed Carotene, %	In House Method – CTM_0013/02	0 03 Min	0.09
8	*Lead (Pb), ppm	AOCS 5th Edition Ca 15-75	3.0 Max	< 3.0
9	*Heavy Metals, ppm	AOCS 5th Edition Ca 15-75	10.0 Max	< 10.0
10	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	< 10
11	**Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	< 10

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CERTIFICATE OF ANALYSIS

TOCOMIN® 50% (NON-GMO)

KOSHER MS 1500 2004

NATURAL FULL SPECTRUM TOCOTRIENOL/TOCOPHEROL COMPLEX 50% OIL SUSPENSION

Our Reference	: Doc / GRAS / 1
COA No.	: CT_507073_1/2
Sampling Date	: 24th April 2009
Released Date	: 30th April 2009
Manufacturing Date	: 24th April 2009
Best Before Date	: 23rd April 2012
Batch No.	: B7073_1_230409
Country Of Origin	: Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Reddish Oil Suspension	
2	Tocotrienol/Tocopherol Complex, %	In House Method – CTM_0001/05	50.0 Min	51.1
	d-Gamma Tocotrienol, %		20.0 – 24.0	20.0
	d-Beta Tocotrienol, %		1.5 Min	2.3
	d-Delta Tocotrienol, %		5.5 – 7.0	5.7
	d-Alpha Tocotrienol, %		11.0 –14.0	12.6
	d-Alpha Tocopherol, %		10.0 –14.0	10.5
3	Moisture (WT, %) (Karl Fisher)	AOCS 5th Edition Ca 2e-84	1.00 Max	0.07
4	*Peroxide Value, meq/kg	AOCS 5th Edition Cd 8-53	10.0 Max	1.4
5	Plant Squalene, %	In House Method – CTM_0007/04	8.00 Min	8.02
6	Phytosterols Content, %	In House Method – CTM_0008/04	3.00 Min	4.14
7	*Mixed Carotene, %	In House Method – CTM_0003/04	0.05 Min	0.09
8	*Lead (Pb), ppm	AOCS 5th Edition Ca 15-75	3.0 Max	< 3.0
9	*Heavy Metals, ppm	AOCS 5th Edition Ca 15-75	10.0 Max	< 10.0
10	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	< 10
11	**Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	< 10

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TOCOMIN® 50% (NON-GMO)

KOSHER MS 500:2004

NATURAL FULL SPECTRUM TOCOTRIENOL/TOCOPHEROL COMPLEX 50% OIL SUSPENSION

Our Reference	: Doc / GRAS / 2
COA No.	: CT_507062_1/6
Sampling Date	: 13th January 2009
Released Date	: 22nd January 2009
Manufacturing Date	: 13th January 2009
Best Before Date	: 12th January 2012
Batch No.	: B7062_1_130109
Country Of Origin	: Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Reddish Oil Suspension	
2	Tocotrienol/Tocopherol Complex, %	In House Method – CTM_0001/05	50.0 Min	51.7
	d-Gamma Tocotrienol, %		20.0 – 24.0	20.0
	d-Beta Tocotrienol, %		1.5 Min	2.1
	d-Delta Tocotrienol, %		5.5 – 7.0	6.5
	d-Alpha Tocotrienol, %		11.0 –14.0	12.7
	d-Alpha Tocopherol, %		10.0 –14.0	10.4
3	Moisture (WT, %) (Karl Fisher)	AOCS 5th Edition Ca 2e-84	1.00 Max	0.06
4	*Peroxide Value, meq/kg	AOCS 5th Edition Cd 8-53	10.0 Max	1.2
5	Plant Squalene, %	In House Method – CTM_0007/04	8.00 Min	8.00
6	Phytosterols Content, %	In House Method – CTM_0008/04	3.00 Min	3.08
7	*Mixed Carotene, %	In House Method – CTM_0003/04	0.05 Min	0.05
8	*Lead (Pb), ppm	AOCS 5th Edition Ca 15-75	3.0 Max	< 3.0
9	*Heavy Metals, ppm	AOCS 5th Edition Ca 15-75	10.0 Max	< 10.0
10	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	< 10
11	**Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	< 10

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TOCOMIN® 50% (NON-GMO)

KOSHER

MS 1500: 2004

NATURAL FULL SPECTRUM TOCOTRIENOL/TOCOPHEROL COMPLEX 50% OIL SUSPENSION

Our Reference	: Doc / GRAS / 3
COA No.	: CT_506976_1/7
Sampling Date	: 12th March 2009
Released Date	: 18th March 2009
Manufacturing Date	: 12th March 2009
Best Before Date	: 11th March 2012
Batch No.	: B6976_1_120309
Country Of Origin	: Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Reddish Oil Suspension	
2	Tocotrienol/Tocopherol Complex, %	In House Method – CTM_0001/05	50.0 Min	52.3
	d-Gamma Tocotrienol, %		20.0 – 24.0	20.0
	d-Beta Tocotrienol, %		1.5 Min	2.5
	d-Delta Tocotrienol, %		5.5 – 7.0	6.1
	d-Alpha Tocotrienol, %		11.0 –14.0	12.8
	d-Alpha Tocopherol, %		10.0 –14.0	10.9
3	Moisture (WT, %) (Karl Fisher)	AOCS 5th Edition Ca 2e-84	1.00 Max	0.08
4	*Peroxide Value, meq/kg	AOCS 5th Edition Cd 8-53	10.0 Max	1.7
5	Plant Squalene, %	In House Method – CTM_0007/04	8.00 Min	8.01
6	Phytosterols Content, %	In House Method – CTM_0008/04	3.00 Min	3.00
7	*Mixed Carotene, %	In House Method – CTM_0003/04	0.05 Min	0.07
9	*Lead (Pb), ppm	AOCS 5th Edition Ca 15-75	3.0 Max	< 3.0
10	*Heavy Metals, ppm	AOCS 5th Edition Ca 15-75	10.0 Max	< 10.0
11	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	< 10
12	**Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	< 10

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CERTIFICATE OF ANALYSIS

TOCOMAX™ 20% (GMO FREE)

KOSHER MS 500 2004

WATER DISPERSIBLE NATURAL FULL SPECTRUM TOCOTRIENOL/TOCOPHEROL COMPLEX 20% POWDER

Our Reference	: Doc / GRAS / 1
COA No.	: CT_Tocomax_310309_1/13
Sampling Date	: 4th April 2009
Released Date	: 8th April 2009
Manufacturing Date	: 31st March 2009
Best Before Date	: 30th March 2010
Batch No.	: Tocomax_1_310309
Country Of Origin	: Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Dry Stabilized Orange Powder	
2	Tocotrienol/Tocopherol Complex, %	In House Method – CTM_0030/04	20.0% Min	21.3
	d-Gamma Tocotrienol, %		8.0 – 10.0	8.1
	d-Beta Tocotrienol, %		0.5 Min	1.0
	d-Delta Tocotrienol, %		2.0 – 3.0	2.6
	d-Alpha Tocotrienol, %		4.5 – 6.0	5.3
	d-Alpha Tocopherol, %		4.0 – 6.0	4.3
3	Moisture (WT, %) (Karl Fisher)	AOCS 5th Edition Ca 2e-84	8.00 Max	0 54
4	Plant Squalene, %	In House Method – CTM_0035/00	3.0 Min	3.1
5	Phytosterols Content, %	In House Method – CTM_0036/02	1.0 Min	3.7
6	*Lead (Pb), ppm	AOCS 5th Edition Ca 15-75	3.0 Max	< 3.0
7	*Heavy Metals, ppm	AOCS 5th Edition Ca 15-75	10.0 Max	< 10.0
8	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	< 10
9	** Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	< 10

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TOCOMAX™ 20% (GMO FREE)

KOSHER MS 1500 2004

WATER DISPERSIBLE NATURAL FULL SPECTRUM TOCOTRIENOL/TOCOPHEROL COMPLEX 20% POWDER

Our Reference	: Doc / GRAS / 2
COA No.	. CT_Tocomax_020309_1/8
Sampling Date	: 7th March 2009
Released Date	: 12th March 2009
Manufacturing Date	: 2nd March 2009
Best Before Date	: 1st March 2010
Batch No.	: Tocomax_1_020309
Country Of Origin	: Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Dry Stabilized Orange Powder	
2	Tocotrienol/Tocopherol Complex, %	In House Method – CTM_0030/04	20.0% Min	20.2
	d-Gamma Tocotrienol, %		8.0 – 10.0	8.0
	d-Beta Tocotrienol, %		0.5 Min	0.9
	d-Delta Tocotrienol, %		2.0 – 3.0	2.4
	d-Alpha Tocotnenol, %		4.5 – 6.0	4.5
	d-Alpha Tocopherol, %		4.0 – 6.0	4.4
3	Moisture (WT, %) (Karl Fisher)	AOCS 5th Edition Ca 2e-84	8.00 Max	0.76
4	Plant Squalene, %	In House Method – CTM_0035/00	3.0 Min	4.5
5	Phytosterols Content, %	In House Method – CTM_0036/02	1.0 Min	1.9
6	*Lead (Pb), ppm	AOCS 5th Edition Ca 15-75	3.0 Max	< 3.0
7	*Heavy Metals, ppm	AOCS 5th Edition Ca 15-75	10.0 Max	< 10.0
8	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	< 10
9	** Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	< 10

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KOSHER MS 1500 2004

WATER DISPERSIBLE NATURAL FULL SPECTRUM TOCOTRIENOL/TOCOPHEROL COMPLEX 20% POWDER

Our Reference	: Doc / GRAS / 3
COA No.	: CT_Tocomax_180509_1/3
Sampling Date	: 25th May 2009
Released Date	: 30th May 2009
Manufacturing Date	: 18th May 2009
Best Before Date	: 17th May 2010
Batch No.	: Tocomax_1_180509
Country Of Origin	: Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Dry Stabilized Orange Powder	
2	Tocotrienol/Tocopherol Complex, %	In House Method – CTM_0030/04	20.0% Min	20.6
	d-Gamma Tocotrienol, %		8.0 – 10.0	8.1
	d-Beta Tocotrienol, %		0.5 Min	1.0
	d-Delta Tocotrienol, %		2.0 – 3.0	2.4
	d-Alpha Tocotrienol, %		4.5 – 6.0	5.0
	d-Alpha Tocopherol, %		4.0 – 6.0	4.1
3	Moisture (WT, %) (Karl Fisher)	AOCS 5th Edition Ca 2e-84	8.00 Max	0.46
4	Plant Squalene, %	In House Method – CTM_0035/00	3.0 Min	3.9
5	Phytosterols Content, %	In House Method – CTM_0036/02	1.0 Min	1.1
6	*Lead (Pb), ppm	AOCS 5th Edition Ca 15-75	3.0 Max	< 3.0
7	*Heavy Metals, ppm	AOCS 5th Edition Ca 15-75	10.0 Max	< 10.0
8	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	< 10
9	**Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	< 10

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TOCOMIN® SUPRABIO™ 20% (NON-GMO)

KOSHER MS 1500 2004

BIO-ENHANCED NATURAL FULL SPECTRUM TOCOTRIENOL/TOCOPHEROL COMPLEX 20% SUSPENSION

Our Reference	: Doc / GRAS / 1
COA No.	: CSB_20030609_B1/2
Sampling Date	: 3rd June 2009
Released Date	: 8th June 2009
Manufacturing Date	: 3rd June 2009
Best Before Date	: 2nd June 2011
Batch No.	: SupraBio/J_B1_030609
Country Of Origin	: Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Light Amber Suspension	
2	Tocotrienol/Tocopherol Complex, %	In House Method – CTM_0048/01	20.0 Min	21.5
	d-Gamma Tocotrienol, %		8.0 – 9.6	8.1
	d-Beta Tocotrienol, %		0.6 Min	0.9
	d-Delta Tocotrienol, %		2.2 – 2.8	2.5
	d-Alpha Tocotrienol, %		4.4 – 6.0	5.2
	d-Alpha Tocopherol, %		4.0 – 5.6	4.8
3	Moisture (WT, %) (Karl Fisher)	AOCS 5th Edition Ca 2e-84	1.00 Max	0.8
4	Plant Squalene, %	In House Method – CTM_0053/00	3.0 Min	3.6
5	Phytosterols Content, %	In House Method – CTM_0054/00	1.5 Min	1.6
6	*Mixed Carotene, %	In House Method – CTM_0050/00	0.02 Min	0.03
7	*Lead (Pb), ppm	AOCS 5th Edition Ca 15-75	3.0 Max	< 3.0
8	*Heavy Metals, ppm	AOCS 5th Edition Ca 15-75	10.0 Max	< 10.0
9	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	< 10
10	**Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	< 10

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Ms. Chung Yoke Tying
Senior Quality Control Chemist
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KOSHER MS 1500 2004

BIO-ENHANCED NATURAL FULL SPECTRUM TOCOTRIENOL/TOCOPHEROL COMPLEX 20% SUSPENSION

Our Reference	: Doc / GRAS / 2
COA No.	: CSB_20210509_B1/3
Sampling Date	: 21st May 2009
Released Date	: 28th May 2009
Manufacturing Date	: 21st May 2009
Best Before Date	. 20th May 2011
Batch No.	: SupraBio/J_B1_210509
Country Of Origin	· Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Light Amber Suspension	
2	Tocotrienol/Tocopherol Complex, %	In House Method – CTM_0048/01	20.0 Min	20.3
	d-Gamma Tocotrienol, %		8.0 – 9.6	8.0
	d-Beta Tocotrienol, %		0.6 Min	0.9
	d-Delta Tocotrienol, %		2.2 – 2.8	2.2
	d-Alpha Tocotrienol, %		4.4 – 6.0	4.9
	d-Alpha Tocopherol, %		4.0 – 5.6	4.3
3	Moisture (WT, %) (Karl Fisher)	AOCS 5th Edition Ca 2e-84	1.00 Max	0.8
4	Plant Squalene, %	In House Method – CTM_0053/00	3.0 Min	3.8
5	Phytosterols Content, %	In House Method – CTM_0054/00	1.5 Min	1.5
6	*Mixed Carotene, %	In House Method – CTM_0050/00	0.02 Min	0.02
7	*Lead (Pb), ppm	AOCS 5th Edition Ca 15-75	3.0 Max	< 3.0
8	*Heavy Metals, ppm	AOCS 5th Edition Ca 15-75	10.0 Max	< 10.0
9	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	< 10
10	**Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	< 10

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TOCOMIN® SUPRABIO™ 20% (NON-GMO)

KOSHER MS 1500 2004

BIO-ENHANCED NATURAL FULL SPECTRUM TOCOTRIENOL/TOCOPHEROL COMPLEX 20% SUSPENSION

Our Reference	: Doc / GRAS / 3
COA No.	: CSB_20100209_B1/3
Sampling Date	: 12th February 2009
Released Date	: 20th February 2009
Manufacturing Date	: 10th February 2009
Best Before Date	: 9th February 2011
Batch No.	: SupraBio/J_B1_100209
Country Of Origin	: Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Light Amber Suspension	
2	Tocotrienol/Tocopherol Complex, %	In House Method – CTM_0048/01	20.0 Min	20.2
	d-Gamma Tocotrienol, %		8.0 – 9.6	8.0
	d-Beta Tocotrienol, %		0.6 Min	1.0
	d-Delta Tocotrienol, %		2.2 – 2.8	2.5
	d-Alpha Tocotrienol, %		4.4 – 6.0	4.7
	d-Alpha Tocopherol, %		4.0 – 5.6	4.0
3	Moisture (WT, %) (Karl Fisher)	AOCS 5th Edition Ca 2e-84	1.00 Max	0.65
4	Plant Squalene, %	In House Method – CTM_0053/00	3.0 Min	3.0
5	Phytosterols Content, %	In House Method – CTM_0054/00	1.5 Min	1.5
6	*Mixed Carotene, %	In House Method – CTM_0050/00	0.02 Min	0.04
7	*Lead (Pb), ppm	AOCS 5th Edition Ca 15-75	3.0 Max	< 3.0
8	*Heavy Metals, ppm	AOCS 5th Edition Ca 15-75	10.0 Max	< 10.0
9	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	< 10
10	**Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	< 10

Certified By

Ms. Chung Yoke Tying
Senior Quality Control Chemist
Issued Date : 16th June 2009

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Carotech

CERTIFICATE OF ANALYSIS

TOCOMIN® SUPRABIO™ 25% (NON-GMO)

KOSHER MS 1500 2004

BIO-ENHANCED NATURAL FULL SPECTRUM TOCOTRIENOL/TOCOPHEROL COMPLEX 25% SUSPENSION

Our Reference	: Doc / GRAS / 1
COA No.	: CSB_SupraBio_221208/4
Sampling Date	: 22nd December 2008
Released Date	: 29th December 2008
Manufacturing Date	: 22nd December 2008
Best Before Date	. 21st December 2010
Batch No.	· SupraBio/N_C1_221208
Country of Origin	. Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Light Amber Suspension	
2	Tocotrienol/Tocopherol Complex, %	In House Method – CTM_0048/01	25.0 Min	26.4
	d-Gamma Tocotrienol, %		10.0 – 12.0	10.1
	d-Beta Tocotrienol, %		0.75 Min	1.2
	d-Delta Tocotrienol, %		2.75 – 3 50	3.5
	d-Alpha Tocotrienol, %		5.5 – 7.0	6.1
	d-Alpha Tocopherol, %		5.0 – 7.0	5.5
3	Moisture (WT, %) (Karl Fisher)	AOCS 5th Edition Ca 2e-84	1.0 Max	0.2
4	*Peroxide Value, meq/kg	AOCS 5th Edition Cd 8-53	10.0 Max	1.4
5	Plant Squalene, %	In House Method – CTM_0053/00	4.0 Min	10.9
6	Phytosterols Content, %	In House Method – CTM_0054/00	1.5 Min	8.2
7	*Mixed Carotene, %	In House Method – CTM_0050/00	0.02 Min	0.31
8	*Lead (Pb), ppm	AOCS 5th Edition Ca 15-75	3.00 Max	< 3.0
9	*Heavy Metals, ppm	AOCS 5th Edition Ca 15-75	10.0 Max	< 10.0
10	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	< 10
11	**Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	< 10

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TOCOMIN® SUPRABIO™ 25% (NON-GMO)

KOSHER MS 1500 2004

BIO-ENHANCED NATURAL FULL SPECTRUM TOCOTRIENOL/TOCOPHEROL COMPLEX 25% SUSPENSION

Our Reference	: Doc / GRAS / 2
COA No.	: CSB_SupraBio_121207/8
Sampling Date	: 12th December 2007
Released Date	: 19th December 2007
Manufacturing Date	: 12th December 2007
Best Before Date	: 11th December 2009
Batch No.	: SupraBio/N_C1_121207
Country of Origin	: Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Light Amber Suspension	
2	Tocotrienol/Tocopherol Complex, %	In House Method – CTM_0048/01	25.0 Min	26.7
	d-Gamma Tocotrienol, %		10.0 – 12.0	10.1
	d-Beta Tocotrienol, %		0.75 Min	1.3
	d-Delta Tocotrienol, %		2.75 – 3.50	3.1
	d-Alpha Tocotrienol, %		5.5 – 7.0	6.5
	d-Alpha Tocopherol, %		5.0 – 7.0	5.7
3	Moisture (WT, %) (Karl Fisher)	AOCS 5th Edition Ca 2e-84	1.0 Max	0.3
4	*Peroxide Value, meq/kg	AOCS 5th Edition Cd 8-53	10.0 Max	1.8
5	Plant Squalene, %	In House Method – CTM_0053/00	4.0 Min	7.4
6	Phytosterols Content, %	In House Method – CTM_0054/00	1.5 Min	5.6
7	*Mixed Carotene, %	In House Method – CTM_0050/00	0.02 Min	0.28
8	*Lead (Pb), ppm	AOCS 5th Edition Ca 15-75	3.00 Max	< 3.0
9	*Heavy Metals, ppm	AOCS 5th Edition Ca 15-75	10.0 Max	< 10.0
10	**Total Aerobic Microbial Count	BP 2005, Plate Count Method	1000 cfu/g Max	< 10
11	**Total Combined Molds And Yeasts	BP 2005, Plate Count Method	100 cfu/g Max	< 10

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KOSHER MS 1500:2004

BIO-ENHANCED NATURAL FULL SPECTRUM TOCOTRIENOL/TOCOPHEROL COMPLEX 25% SUSPENSION

Our Reference	: Doc / GRAS / 3
COA No.	: CSB_SupraBio_041208/2
Sampling Date	: 4th December 2008
Released Date	: 9th December 2008
Manufacturing Date	: 4th December 2008
Best Before Date	: 3rd December 2010
Batch No.	: SupraBio/N_C1_041208
Country of Origin	: Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Light Amber Suspension	
2	Tocotrienol/Tocopherol Complex, %	In House Method – CTM_0048/01	25.0 Min	25.8
	d-Gamma Tocotrienol, %		10.0 – 12.0	10.1
	d-Beta Tocotrienol, %		0.75 Min	1.1
	d-Delta Tocotrienol, %		2.75 – 3.50	3.0
	d-Alpha Tocotrienol, %		5.5 – 7.0	6.1
	d-Alpha Tocopherol, %		5.0 – 7.0	5.5
3	Moisture (WT, %) (Karl Fisher)	AOCS 5th Edition Ca 2e-84	1.0 Max	0.3
4	*Peroxide Value, meq/kg	AOCS 5th Edition Cd 8-53	10.0 Max	1.8
5	Plant Squalene, %	In House Method – CTM_0053/00	4.0 Min	7.4
6	Phytosterols Content, %	In House Method – CTM_0054/00	1.5 Min	5.6
7	*Mixed Carotene, %	In House Method – CTM_0050/00	0.02 Min	0.28
8	*Lead (Pb), ppm	AOCS 5th Edition Ca 15-75	3.00 Max	< 3.0
9	*Heavy Metals, ppm	AOCS 5th Edition Ca 15-75	10.0 Max	< 10.0
10	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	< 10
11	**Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	< 10

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CERTIFICATE OF ANALYSIS

TOCOSEM™ 1.5% (NON-GMO)

KOSHER MS 1500 2004

WATER SOLUBLE NATURAL FULL SPECTRUM TOCOTRIENOL/TOCOPHEROL COMPLEX 1.5% EMULSION

Our Reference	: Doc / GRAS / 1
COA No.	: Tocosem_200808_1/2
Sampling Date	: 20th August 2008
Released Date	: 26th August 2008
Manufacturing Date	: 20th August 2008
Best Before Date	: 19th August 2009
Batch No.	: Tocosem_1_200808
Country Of Origin	: Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Clear Orange-Color Liquid	
2	Tocotrienol/Tocopherol Complex, %	In House Method – HPLC-VitE_10	1.48 Min	1.56
	d-Gamma Tocotrienol, %		0.5 Min	0.52
	d-Beta Tocotrienol, %		0.05 Min	0.06
	d-Delta Tocotrienol, %		0.3 Min	0.33
	d-Alpha Tocotrienol, %		0.3 Min	0.36
	d-Alpha Tocopherol, %		0.28 Min	0.29
3	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	< 10
4	** Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	< 10
5	***Escherichia coli*	BP 2005/Volume IV/Appendix XVI B	Absent/1g	Absent
6	***Salmonella*	BP 2005/Volume IV/Appendix XVI B	Absent/10g	Absent

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CERTIFICATE OF ANALYSIS

TOCOSEM™ 1.5% (NON-GMO)

KOSHER MS 1500 2004

Our Reference	: Doc / GRAS / 2
COA No	: Tocosem_090908_1/2
Sampling Date	: 9th September 2008
Released Date	: 15th September 2008
Manufacturing Date	: 9th September 2008
Best Before Date	: 8th September 2009
Batch No.	: Tocosem_1_090908
Country Of Origin	: Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Orange Slightly Viscous Liquid	
2	Tocotrienol/Tocopherol Complex, %	In House Method – CIN_HPLC_VitE_10	1.48 Min	1.51
	d-Gamma Tocotrienol, %		0.5 Min	0.50
	d-Beta Tocotrienol, %		0.05 Min	0.06
	d-Delta Tocotrienol, %		0.3 Min	0.32
	d-Alpha Tocotrienol, %		0.3 Min	0.35
	d-Alpha Tocopherol, %		0.28 Min	0.28
3	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	< 10
4	** Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	< 10
5	***Escherichia coli*	BP 2005/Volume IV/Appendix XVI B	Absent/1g	Absent
6	***Salmonella*	BP 2005/Volume IV/Appendix XVI B	Absent/10g	Absent

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KOSHER MS 1500 2004

WATER SOLUBLE NATURAL FULL SPECTRUM TOCOTRIENOL/TOCOPHEROL COMPLEX 1.5% EMULSION

Our Reference	. Doc / GRAS / 3
COA No.	: Tocosem_050109_1/1
Sampling Date	: 5th January 2009
Released Date	: 10th January 2009
Manufacturing Date	: 5th January 2009
Best Before Date	: 4th January 2010
Batch No.	: Tocosem_1_050109
Country Of Origin	: Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Clear Orange-Color Liquid	
2	Tocotrienol/Tocopherol Complex, %	In House Method – HPLC-VitE_10	1.48 Min	1.52
	d-Gamma Tocotrienol, %		0.5 Min	0.51
	d-Beta Tocotrienol, %		0.05 Min	0.06
	d-Delta Tocotrienol, %		0.3 Min	0.32
	d-Alpha Tocotrienol, %		0.3 Min	0.35
	d-Alpha Tocopherol, %		0.28 Min	0.28
3	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	< 10
4	** Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	< 10
5	***Escherichia coli*	BP 2005/Volume IV/Appendix XVI B	Absent/1g	Absent
6	***Salmonella*	BP 2005/Volume IV/Appendix XVI B	Absent/10g	Absent

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CERTIFICATE OF ANALYSIS

TOCOBEADS™ 7.5% (NON-GMO)

KOSHER MS 1500 2004

NATURAL FULL SPECTRUM TOCOTRIENOL/TOCOPHEROL COMPLEX 7.5% BEADLETS

Our Reference	: Doc / GRAS / 1
COA No.	: Tocobeads_15-311208_1/2
Sampling Date	: 31st December 2008
Released Date	: 5th January 2009
Manufacturing Date	: 31st December 2008
Best Before Date	: 30th December 2010
Batch No.	: Tocobeads_1_311208
Country Of Origin	: Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Slight Orange Beadlets	
2	Tocotrienol/Tocopherol Complex, %	In House Method – CTM_0011/04	7.5 Min	7.52
	d-Gamma Tocotrienol, %		3.0-3.6	3.05
	d-Beta Tocotrienol, %		0.15 Min	0.34
	d-Delta Tocotrienol, %		0.8 – 1.05	0 89
	d-Alpha Tocotrienol, %		1.65 – 2.10	1.66
	d-Alpha Tocopherol, %		1.5 – 2.1	1.58
3	Moisture Content, %	Karl Fischer Method	8.0 Max	4.8
4	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	< 10
5	** Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	< 10
6	**Coliform Count	BP 2005/Volume IV/Appendix XVI B	1 cfu/g Max	< 1
7	***Escherichia coli*	BP 2005/Volume IV/Appendix XVI B	Absent/1g	Absent
8	***Salmonella*	BP 2005/Volume IV/Appendix XVI B	Absent/10g	Absent

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TOCOBEADS™ 7.5% (NON-GMO)

KOSHER MS 1500 2004

NATURAL FULL SPECTRUM TOCOTRIENOL/TOCOPHEROL COMPLEX 7.5% BEADLETS

Our Reference	: Doc / GRAS / 2
COA No.	: CT_T75-07807PB /2
Sampling Date	: 12th August 2008
Released Date	: 17th August 2008
Manufacturing Date	: 12th August 2008
Best Before Date	: 11th August 2010
Batch No.	: Tocobeads_1_120808
Country Of Origin	: Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Slight Orange Beadlets	
2	Tocotrienol/Tocopherol Complex, %	In House Method – CTM_0011/04	7.5 Min	7.62
	d-Gamma Tocotrienol, %		3.0-3.6	3.10
	d-Beta Tocotrienol, %		0.15 Min	0.34
	d-Delta Tocotrienol, %		0.8 – 1.05	0.90
	d-Alpha Tocotrienol, %		1.65 – 2.10	1.68
	d-Alpha Tocopherol, %		1.5 – 2.1	1.60
3	Moisture Content, %	Karl Fischer Method	8.0 Max	5.0
4	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	< 10
5	** Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	< 10
6	**Coliform Count	BP 2005/Volume IV/Appendix XVI B	1 cfu/g Max	< 1
7	***Escherichia coli*	BP 2005/Volume IV/Appendix XVI B	Absent/1g	Absent
8	***Salmonella*	BP 2005/Volume IV/Appendix XVI B	Absent/10g	Absent

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Ms Chung Yoke Tying
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KOSHER MS 1500 2004

NATURAL FULL SPECTRUM TOCOTRIENOL/TOCOPHEROL COMPLEX 7.5% BEADLETS

Our Reference	: Doc / GRAS / 3
COA No.	: Tocobeads_15-170209_1/1
Sampling Date	: 17th February 2009
Released Date	: 22nd February 2009
Manufacturing Date	: 17th February 2009
Best Before Date	: 16th February 2011
Batch No.	: Tocobeads_1_170209
Country Of Origin	: Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Slight Orange Beadlets	
2	Tocotrienol/Tocopherol Complex, %	In House Method – CTM_0011/04	7.5 Min	7.61
	d-Gamma Tocotrienol, %		3.0-3.6	3.09
	d-Beta Tocotrienol, %		0.15 Min	0.34
	d-Delta Tocotrienol, %		0.8 – 1.05	0.90
	d-Alpha Tocotrienol, %		1.65 – 2.10	1.68
	d-Alpha Tocopherol, %		1.5 – 2.1	1.60
3	Moisture Content, %	Karl Fischer Method	8.0 Max	4.5
4	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	< 10
5	** Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	< 10
6	**Coliform Count	BP 2005/Volume IV/Appendix XVI B	1 cfu/g Max	< 1
7	***Escherichia coli*	BP 2005/Volume IV/Appendix XVI B	Absent/1g	Absent
8	***Salmonella*	BP 2005/Volume IV/Appendix XVI B	Absent/10g	Absent

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c/o Golden Jomalina Food Industries Sdn Bhd
9th Mile, Jalan Klang-Banting,
42500 Telok Panglima Garang Industrial Estate,
Kuala Langat, Selangor Darul Ehsan, Malaysia
Tel 603-31226301& 603-31226211
Fax 603-31220782
Email bioganic@simedarby.com

Product Specifications
Bio Tri.E 20

I Definition

Bio Tri.E 20 is an emulsion of tocotrienols and tocopherols of palm fruit *(Elaeis Guineensis)* origin

II Description

Bio Tri.E 20 is an amber viscous liquid Vitamin E containing minimum 20% of tocotrienols and tocopherols.

III Product Use

Dietary supplements, nutraceuticals, functional food ingredients and cosmeceuticals.

IV Physical Properties

1.	Appearance and Color	:	amber liquid
2.	Odor and Taste	:	bland/Neutral
3.	Solubility	:	soluble in oil
4.	Moisture content (%)	:	not more than 1.0
5.	Acid Value, AV (mgKOH/g)	:	not more than 5
6.	Peroxide Value, (meq/kg)	:	not more than 10.0

V Microbial Properties

1.	Total Plate Count	:	not more than 3000 cfu/g
2.	Coliforms	:	negative/g

VI Composition

Total Tocols (Tocotrienols and Tocopherol): **200 mg/g min**

***Typical ratio in tocols**

Total tocotrienols	:	**75%**
Total tocopherols	:	**25%**

Actual composition as per CoA

VII Packaging and Storage

1, 5, 10, 20 kg in Aluminium canister
Store in cool and dry place; Recommended at 20 °C. Protect from light and moisture.

VIII Shelf life

18 months in original unopened container
12 – 15 months in opened container.

Sime Darby Bioganic Sdn Bhd,

c/o Golden Jomalina Food Industries Sdn Bhd
9th Mile, Jalan Klang-Banting,
42500 Telok Panglima Garang Industrial Estate,
Kuala Langat, Selangor Darul Ehsan, Malaysia
Tel . 603-31226301& 603-31226211
Fax 603-31220782
Email : bioganic@simedarby.com

Product Specifications
Bio Tri.E 30

I Definition

Bio Tri.E 30 is an emulsion of tocotrienols and tocopherols of palm fruit *(Elaeis Guineensis)* origin.

II Description

Bio Tri.E 30 is an amber viscous liquid Vitamin E containing minimum 30% of tocotrienols and tocopherols.

III Product Use

Dietary supplements, nutraceuticals, functional food ingredients and cosmeceuticals.

IV Physical Properties

1.	Appearance and Color	:	amber viscous liquid
2.	Odor and Taste	:	bland/Neutral
3.	Solubility	:	soluble in oil
4.	Moisture content (%)	:	not more than 1.0
5.	Acid Value, AV (mgKOH/g)	:	not more than 5
6.	Peroxide Value, (meq/kg)	:	not more than 10.0

V Microbial Properties

1.	Total Plate Count	:	not more than 3000 cfu/g
2.	Coliforms	:	negative/g

VI Composition

Total Tocols (Tocotrienols and Tocopherol): **300 mg/g min**

***Typical ratio in tocols**

Total tocotrienols	:	**75%**
Total tocopherols	:	**25%**

Actual composition as per CoA

VII Packaging and Storage

1, 5, 10, 20 kg in Aluminium canister
Store in cool and dry place; Recommended at 20 °C. Protect from light and moisture.

VIII Shelf life

18 months in original unopened container
12 – 15 months in opened container

Sime Darby Bioganic Sdn Bhd,

c/o Golden Jomalina Food Industries Sdn Bhd
9th Mile, Jalan Klang-Banting,
42500 Telok Panglima Garang Industrial Estate,
Kuala Langat, Selangor Darul Ehsan, Malaysia.
Tel 603-31226301& 603-31226211
Fax . 603-31220782
Email . bioganic@simedarby com

Product Specifications
Gold Tri.E 30

I Definition

Gold Tri.E 30 contains a mixture of tocotrienols and tocopherols liquid extracted from palm fruit *(Elaeis Guineensis)*

II Description

Gold Tri.E 30 is an amber viscous liquid Vitamin E containing minimum 30% of tocotrienols and tocopherols.

III Product Use

Dietary supplements, nutraceuticals, functional food ingredients and cosmeceuticals.

IV Physical Properties

1.	Appearance and Color	:	amber liquid
2.	Odor and Taste	:	bland/Neutral
3.	Solubility	:	soluble in oil
4.	Moisture content (%)	:	not more than 1.0
5.	Acid Value, AV (mgKOH/g)	:	not more than 5
6.	Peroxide Value, (meq/kg)	:	not more than 10.0

V Microbial Properties

1.	Total Plate Count	:	not more than 3000 cfu/g
2.	Coliforms	:	negative/g

VI Composition

Total Tocols (Tocotrienols and Tocopherol): **300 mg/g min**

***Typical ratio in tocols**

Total tocotrienols	:	**75%**
Total tocopherols	:	**25%**

Actual composition as per CoA

VII Packaging and Storage

1, 5, 10, 20 kg in Aluminium canister
Store in cool and dry place; Recommended at 20 °C. Protect from light and moisture.

VIII Shelf life

3 years in original packing at recommended storage conditions

Sime Darby Bioganic Sdn Bhd,

c/o Golden Jomalina Food Industries Sdn Bhd
9th Mile, Jalan Klang-Banting,
42500 Telok Panglima Garang Industrial Estate,
Kuala Langat, Selangor Darul Ehsan, Malaysia.
Tel : 603-31226301& 603-31226211
Fax : 603-31220782
Email : bioganic@simedarby.com

Product Specifications
Gold Tri.E 50

I Definition

Gold Tri.E 50 contains a mixture of tocotrienols and tocopherols liquid extracted from palm fruit *(Elaeis Guineensis)*

II Description

Gold Tri.E 50 is an amber viscous liquid Vitamin E containing minimum 50% of tocotrienols and tocopherols.

III Product Use

Dietary supplements, nutraceuticals, functional food ingredients and cosmeceuticals.

IV Physical Properties

1.	Appearance and Color	:	amber liquid
2.	Odor and Taste	:	bland/Neutral
3.	Solubility	:	soluble in oil
4.	Moisture content (%)	:	not more than 1.0
5.	Acid Value, AV (mgKOH/g)	:	not more than 5
6.	Peroxide Value, (meq/kg)	:	not more than 10.0

V Microbial Properties

1.	Total Plate Count	:	not more than 3000 cfu/g
2.	Coliforms	:	negative/g

VI Composition

Total Tocols (Tocotrienols and Tocopherol): **500 mg/g min**

***Typical ratio in tocols**

Total tocotrienols	:	**75%**
Total tocopherols	:	**25%**

Actual composition as per CoA

VII Packaging and Storage

1, 5, 10, 20 kg in Aluminium canister
Store in cool and dry place; Recommended at 20 °C. Protect from light and moisture.

VIII Shelf life

3 years in original packing at recommended storage conditions

Sime Darby Bioganic Sdn Bhd,

c/o Golden Jomalina Food Industries Sdn Bhd
9th Mile, Jalan Klang-Banting,
42500 Telok Panglima Garang Industrial Estate,
Kuala Langat, Selangor Darul Ehsan, Malaysia.
Tel : 603-31226301& 603-31226211
Fax : 603-31220782
Email bioganic@simedarby.com

Product Specifications
Gold Tri.E 70

I Definition

Gold Tri.E 70 contains a mixture of tocotrienols and tocopherols liquid extracted from palm fruit *(Elaeis Guineensis)*

II Description

Gold Tri.E 70 is an amber viscous liquid Vitamin E containing minimum 65% of tocotrienols and tocopherols.

III Product Use

Dietary supplements, nutraceuticals, functional food ingredients and cosmeceuticals.

IV Physical and Chemical Properties

1.	Appearance and Color	:	amber liquid
2.	Odor and Taste	:	bland/Neutral
3.	Solubility	:	soluble in oil
4.	Moisture content (%)	:	not more than 1.0
5.	Acid Value, AV (mgKOH/g)	:	not more than 5
6.	Peroxide Value, (meq/kg)	:	not more than 10.0

V Microbial Properties

1.	Total Plate Count	:	not more than 3000 cfu/g
2.	Coliforms	:	negative/g

VI Composition

Total Tocols (Tocotrienols and Tocopherol): **700 mg/g min**

***Typical ratio in tocols**

Total tocotrienols	:	**75%**
Total tocopherols	:	**25%**

Actual composition as per CoA

VII Packaging and Storage

1, 5, 10, 20 kg in Aluminium canister
Store in cool and dry place; Recommended at 20 °C. Protect from light and moisture.

VIII Shelf life

3 years in original packing at recommended storage conditions

Sime Darby Bioganic Sdn Bhd,

c/o Golden Jomalina Food Industries Sdn Bhd
9th Mile, Jalan Klang-Banting,
42500 Telok Panglima Garang Industrial Estate,
Kuala Langat, Selangor Darul Ehsan, Malaysia
Tel · 603-31226301& 603-31226211
Fax : 603-31220782
Email . bioganic@simedarby com

Product Specifications
Gold Tri.E 20 Powder

I Definition

Gold Tri.E 20 Powder contains a mixture of tocotrienols and tocopherols powder extracted from palm fruit *(Elaeis Guineensis)*

II Description

Gold Tri.E 20 Powder is a yellowish powdered Vitamin E Derivatives containing minimum 20% of tocotrienol and tocopherol.

III Product Use

Dietary supplements, nutraceuticals and functional food ingredients.

IV Physical and Chemical Properties

1.	Appearance and Color	:	Yellowish powder
2.	Odor and Taste	:	Bland/Neutral
3.	Solubility	:	Disperses in water
4.	Loss on Drying	:	Not more than 5.0

V Microbial Properties

1.	Total Plate Count	:	Not more than 3000 cfu/g
2.	Coliforms	:	Negative/g

VI Composition

Total Tocols (Tocotrienols and Tocopherol)	:	200 mg/g min

Typical ratio in tocols

Total tocotrienols	:	**75%**
Total tocopherols	:	**25%**

Actual composition as per CoA

VII Packaging and Storage

0.5 and 1.0 kg in Aluminium Foil
Store in cool and dry place. Recommended at 20 °C. Protect from light and moisture.

VIII Shelf life

2 years in original packing at recommended storage conditions.

Sime Darby Bioganic Sdn Bhd,

c/o Golden Jomalina Food Industries Sdn Bhd
9th Mile, Jalan Klang-Banting,
42500 Telok Panglima Garang Industrial Estate,
Kuala Langat, Selangor Darul Ehsan, Malaysia.
Tel : 603-31226301& 603-31226211
Fax . 603-31220782
Email . bioganic@simedarby com

Product Specifications
Gold Tri.E 30 Powder

I Definition

Gold Tri.E 30 Powder contains a mixture of tocotrienols and tocopherols powder extracted from palm fruit *(Elaeis Guineensis)*

II Description

Gold Tri.E 30 Powder is a yellowish powdered Vitamin E Derivatives containing minimum 30% of tocotrienol and tocopherol.

III Product Use

Dietary supplements, nutraceuticals and functional food ingredients.

IV Physical and Chemical Properties

1.	Appearance and Color	:	Yellowish powder
2.	Odor and Taste	:	Bland/Neutral
3.	Solubility	:	Disperses in water
4.	Loss on Drying	:	Not more than 5.0

V Microbial Properties

1.	Total Plate Count	:	Not more than 3000 cfu/g
2.	Coliforms	:	Negative/g

VI Composition

Total Tocols (Tocotrienols and Tocopherol)	:	300 mg/g min

Typical ratio in tocols

Total tocotrienols	**:**	**75%**
Total tocopherols	**:**	**25%**

Actual composition as per CoA

VII Packaging and Storage

0.5 and 1.0 kg in Aluminium Foil
Store in cool and dry place. Recommended at 20 °C. Protect from light and moisture.

VIII Shelf life

2 years in original packing at recommended storage conditions.

Golden Hope Bioganic Sdn. Bhd.

Golden Hope Bioganic

CERTIFICATE OF ANALYSIS OF Gold-Leaf™

PALM FOG OLEIN OIL RICH FRACTION

Name: Leaf 50
Batch No: GHBG F0709180150NE

[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
TOTAL	244.2mg/g

[illegible]

Golden Hope Bioganic Sdn. Bhd.
(Co No. 189028-K)

c/o Golden Jomalina Food Industries Sdn Bhd
9th Mile, Jalan Klang-Banting,
42500 Telok Panglima Garang Industrial Estate
Kuala Langat, Selangor Darul Ehsan, Malaysia
Tel [illegible]
Fax [illegible]
Website [illegible]
E-mail [illegible]

Golden Hope Bioganic

CERTIFICATE OF ANALYSIS OF Gold-Tri E ™

PALM TOCOTRIENOLS RICH FRACTION

Gold-Tri E 20 Nt
Batch No : GHB-T0806050N - 02

α-Tocopherol	26 2 mg/g
α-Tocotrienol	63 2 mg/g
β-Tocotrienol	6 0 mg/g
γ-Tocotrienol	69 1 mg/g
δ-Tocotrienol	43 4 mg/g
TOTAL	**207.9 mg/g**

Ref Standard 0954/090300

Mfg date 06/08

Best Before 06/11

(b) (6)

KANANTHAN
Chemist
E-mail ananthan krishnan@simedarby com

DATE : 20/06/2008

In GHB-T0806050N-02 sada folder

Golden Hope Bioganic Sdn. Bhd. (Co No 189028 K
c/o Golden Jomalina Food Industries Sdn Bhd
9th Mile Jalan Banting-Kiang, 42500 Telok Panglima Garang Industrial Estate, Selangor Darul Ehsan, Malaysia
Tel (603) 3122 6302 9676 Fax (603) 3122 0762 6389
Web Page www.goldenhope.com E-mail bioganic@simedarby.com

Golden Hope Bioganic

CERTIFICATE OF ANALYSIS OF Gold-Tri E ™

PALM TOCOTRIENOLS RICH FRACTION

Gold-Tri E 20 N1
Batch No : GHB-T0710050N - 01

α-Tocopherol	26 7 mg/g
α-Tocotrienol	64 5 mg/g
β-Tocotrienol	6.2 mg/g
γ Tocotrienol	68.9 mg/g
δ-Tocotrienol	45 1 mg/g
TOTAL	211.4 mg/g

Ref Standard . 0954/090300

Mfg date : 10/07

Best Before . 10/10

(b) (6)

KANANTHAN
Chemist
E-mail ananthan krishnan@simedarby com

DATE : 20/06/2008

Ref GHB-T0710050 - 01 Gold-tri folder

A member of the Sime Darby Group

Golden Hope Bioganic

Golden Hope Bioganic Sdn. Bhd.
(Co. No. 189028 K)

c/o Golden Jomalina Food Industries Sdn. Bhd
9th Mile, Jalan Klang-Banting,
42500 Telok Panglima Garang Industrial Estate
Kuala Langat, Selangor Darul Ehsan, Malaysia
Tel : 603-3122 6301 & 03-3122 6211
Fax : 603-3122 6688 & 03-3122 9539
Web page www.goldtrie.com
Email bioganic@goldenhope.com

CERTIFICATE OF ANALYSIS OF Gold Tri.E ™

PALM TOCOTRIENOLS RICH FRACTION

Nano E70
Batch No : GHB CF070918070NE

α-Tocopherol	45.3 mg/g
α-Tocotrienol	102.8 mg/g
β-Tocotrienol	25.2 mg/g
γ-Tocotrienol	104.3 mg/g
δ-Tocotrienol	65.2 mg/g
TOTAL	342.8 mg/g

Ref. Standard : 0669 / 14042005
Mfg Date : 09/07
Best Before : 09/10

K.ANANTHAN
Chemist
Email : ananthan.krishnan@simedarby.com
Date : 18/09/2007

Golden Hope Bioganic Sdn. Bhd. (Co No 189028-K)
c/o Golden Jomalina Food Industries Sdn Bhd
9th Mile, Jalan Banting-Klang, 42500 Telok Panglima Garang Industrial Estate, Selangor Darul Ensan Malaysia
Tel (603) 31226301 & (603) 31226211 Fax. (603) 31226688 & (603) 31229539
Web Page www.goldtrie.com E-mail bioganic@goldenhope.com

Golden Hope Bioganic

CERTIFICATE OF ANALYSIS OF Gold-Tri E ™

PALM TOCOTRIENOLS RICH FRACTION

Nano E70
Batch No : GHB-CF070918070NE

α-Tocopherol	90.5 mg/g
α-Tocotrienol	86.4 mg/g
β-Tocotrienol	15.0 mg/g
γ-Tocotrienol	67.1 mg/g
δ-Tocotrienol	43.0 mg/g
TOTAL	**302.0mg/g**

Ref. Standard : 0669/14042005
Mfg date : 09/07
Best Before : 09/10

(b) (6)

K.ANANTHAN
Chemist
E-mail : ananthan.krishnan@simedarby.com

DATE : 04/03/2008

F:\ GHB-CF070918070NE D16(d)

Golden Hope Bioganic Sdn. Bhd.

Golden Hope Bioganic

CERTIFICATE OF ANALYSIS OF Gold-Tri E ™

PALM TOCOTRIENOLS RICH FRACTION

Gold-Tri E 50
Batch No : GHB071113196298R

α-Tocopherol	73.9 mg/g
α-Tocotrienol	167.1 mg/g
β-Tocotrienol	41.[illegible] mg/g
γ-Tocotrienol	165.2 mg/g
δ-Tocotrienol	98.5 mg/g
TOTAL	**545.8mg/g**

Ref. Standard 0869/140420/5

Mfg. Date 14/[illegible]/07

Best Before 14/[illegible]/10

K. ANANTHAN
Research Officer
[illegible]@goldenhope.com

DATE : 16/11/2007

Golden Hope Bioganic

CERTIFICATE OF ANALYSIS OF Gold-Tri E™

PALM DOCOTRIENOLS RICH EXTRACTION

Seed Lot No.: 50
Batch No.: GHB8508200050R

[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
TOTAL	50 [illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

Golden Hope Bioganic Sdn. Bhd.

Golden Hope Bioganic

CERTIFICATE OF ANALYSIS OF Gold-Tri E™

PALM TOCOTRIENOL RICH FRACTION

Gold-Tri E 50
Batch No. GHB07111419620998

[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
TOTAL	576.0mg/g

[illegible]

Golden Hope Bioganic

CERTIFICATE OF ANALYSIS OF Gold-Tri E 70

PALM TOCOTRIENOLS RICH FRACTION

Gold Tri E 70
Batch No : GHB 080200070R-02

[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
TOTAL	650.3mg/g

[illegible]

K. ANANTHAN

[illegible]

Golden Hope Bioganic

CERTIFICATE OF ANALYSIS OF GoldeTri E™

PALM TOCOTRIENOLS RICH FRACTION

GoldeTri E 70
Batch No. GHBio80512171289

[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
TOTAL	711.3mg/g

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

Golden Hope Bioganic Sdn. Bhd.

Golden Hope Bioganic

CERTIFICATE OF ANALYSIS OF Gold-Tri E™

PALM TOCOTRIENOLS RICH FRACTION

Gold-Tri E 70
Batch No. GHB0809220070

[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
TOTAL	783.1 mg/g

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

Golden Hope Bioganic Sdn. Bhd.
(Co. No. 189028-K)

c/o Golden Jomalina Food Industries Sdn Bhd.
9th Mile, Jalan Klang-Banting,
42500 Telok Panglima Garang Industrial Estate,
Kuala Langat, Selangor Darul Ehsan, Malaysia
Tel (603) 3122 [illegible]
Fax (603) 3122 0782 [illegible]
Website www.gold tri [illegible] com
E-mail bioganic@simedarby.com

Golden Hope Bioganic

CERTIFICATE OF ANALYSIS OF Gold-Tri E ™

PALM TOCOTRIENOLS RICH FRACTION

Gold-Tri E 20 Nt
Batch No : GHB-T0806050N - 02

α-Tocopherol	26.2 mg/g
α-Tocotrienol	63.2 mg/g
β-Tocotrienol	6.0 mg/g
γ-Tocotrienol	69.1 mg/g
δ-Tocotrienol	43.4 mg/g
TOTAL	**207.9 mg/g**

Ref Standard : 0954/090300

Mfg date : 06/08

Best Before : 06/11

(b) (6)

K.ANANTHAN
Chemist
E-mail. ananthan.krishnan@simedarby.com

DATE : 20/06/2008

In *GHB-T0806050N-02/santa folder*

Golden Hope Bioganic

Golden Hope Bioganic Sdn. Bhd. (Co No 189026-K)
c/o Golden Jomalina Food Industries Sdn. Bhd
9th Mile, Jalan Banting-Klang, 42500 Telok Panglima Garang Industrial Estate, Selangor Darul Ehsan Malaysia
Tel (603) 3122 6302/9676 Fax (603) 3122 0782/6688
Web Page www.gold-trie.com E-mail bioganic@simedarby.com

CERTIFICATE OF ANALYSIS OF Gold-Tri E ™

PALM TOCOTRIENOLS RICH FRACTION

Gold-Tri E 20 Nt
Batch No : GHB-T0710050N - 01

α-Tocopherol	26.7 mg/g
α-Tocotrienol	64.5 mg/g
β-Tocotrienol	6.2 mg/g
γ-Tocotrienol	68.9 mg/g
δ-Tocotrienol	45.1 mg/g
TOTAL	**211.4 mg/g**

Ref Standard . 0954/090300

Mfg date . 10/07

Best Before : 10/10

(b) (6)

K.ANANTHAN
Chemist
E-mail: ananthan.krishnan@simedarby.com

DATE : 20/06/2008

In *GHB-T0710050N-01/santo folder*

Golden Hope Bioganic

Golden Hope Bioganic Sdn. Bhd.

CERTIFICATE OF ANALYSIS OF Gold-Tri E ™

PALM TOCOTRIENOLS RICH FRACTION

Gold-Tri E 50
Batch No : GHB071113196298R

α-Tocopherol	73.9 mg/g
α-Tocotrienol	167.1 mg/g
β-Tocotrienol	41.1 mg/g
γ-Tocotrienol	165.2 mg/g
δ-Tocotrienol	98.5 mg/g
TOTAL	**545.8mg/g**

Ref Standard 0669/14042005

Mfg date 11/07

Best Before 11/10

(b) (6)

K.ANANTHAN
Research Officer
E-mail ananthan@goldenhope.com

DATE : 16/11/2007

Golden Hope Bioganic Sdn. Bhd.

Golden Hope Bioganic

CERTIFICATE OF ANALYSIS OF Gold-Tri E™

PALM TOCOTRIENOLS RICH FRACTION

Gold-Tri E 50
Batch No : GHB08082000050R

α-Tocopherol	[illegible] mg/g
α-Tocotrienol	[illegible] mg/g
γ-Tocotrienol	[illegible] mg/g
γ-Tocopherol	[illegible] mg/g
δ-Tocotrienol	[illegible] ppm
TOTAL	**562.7 mg/g**

[illegible]

[illegible]

[illegible]

K. ANANTHAN

[illegible]

DATE : 21.08.2008

[illegible]

Golden Hope Bioganic

Golden Hope Bioganic Sdn. Bhd.
[illegible]

CERTIFICATE OF ANALYSIS OF Gold-Tri E™

PALM TOCOTRIENOLS RICH FRACTION

Gold-Tri E 50
Batch No : GHB071114196299R

α-Tocopherol	72.5 mg/g
α-Tocotrienol	175.0 mg/g
β-Tocotrienol	15.0 mg/g
Tocotrienol	100.7 mg/g
γ-Tocotrienol	112.5 mg/g
TOTAL	**576.0mg/g**

Ref. Standard [illegible]

Methane [illegible]

[illegible] [illegible]

R. ANANTHAN
[illegible]
[illegible]

DATE : 15.11.2007

Golden Hope Bioganic

Golden Hope Bioganic Sdn. Bhd.

CERTIFICATE OF ANALYSIS OF Gold-Tri E ™

PALM TOCOTRIENOLS RICH FRACTION

Gold-Tri E 70
Batch No : GHB0805121 74289

α-Tocopherol	[illegible] mg/g
α-Tocotrienol	176.7 mg/g
β-Tocotrienol	[illegible] mg/g
γ-Tocotrienol	[illegible] mg/g
δ-Tocotrienol	149.0 mg/g
TOTAL	**711.3mg/g**

Ref. Standard: [illegible]

[illegible] date: 05.08

Best before: 05.[illegible]

K. ANANTHAN
Manager
[illegible]

DATE : 22/05/2008

[illegible]

Golden Hope Bioganic

Golden Hope Bioganic Sdn. Bhd.
[illegible]
[illegible] Sungai [illegible]
9½ Mile Jalan Klang Banting
42500 Telok Panglima Garang [illegible]
Kuala Langat Selangor Darul Ehsan Malaysia
[illegible]

CERTIFICATE OF ANALYSIS OF Gold-Tri E ™

PALM TOCOTRIENOLS RICH FRACTION

Gold-Tri E 70
Batch No : GHB080922070

α-Tocopherol	153.6 mg/g
α-Tocotrienol	205.8 mg/g
β-Tocotrienol	26.5 mg/g
γ-Tocotrienol	243.2 mg/g
δ-Tocotrienol	154.0 mg/g
TOTAL	**783.1 mg/g**

Ref Standard [illegible]

Mfg date [illegible]

Best Before [illegible]

KANANTHAN
[illegible]
[illegible] sanedarby.com

DATE : 25/09/2008

[illegible]

Golden Hope Bioganic Sdn. Bhd.

Golden Hope Bioganic

CERTIFICATE OF ANALYSIS OF Gold-Tri E™

PALM TOCOTRIENOLS RICH FRACTION

Nano E50
Batch No : GHB-CT070918050NE

α-Tocopherol	57.8 mg/g
α-Tocotrienol	25.0 mg/g
β-Tocotrienol	12.2 mg/g
γ-Tocotrienol	56.4 mg/g
δ-Tocotrienol	42.2 mg/g
TOTAL	**244.2mg/g**

Ref. Standard: USP 1204200X

Mfg Date: [illegible]

Best Before: [illegible]

(b) (6)

K. ANANTHAN

[illegible]

DATE : 01.03.2008

Golden Hope Bioganic

Golden Hope Bioganic Sdn. Bhd.

CERTIFICATE OF ANALYSIS OF Gold-Tri E ™

PALM TOCOTRIENOLS RICH FRACTION

Gold-Tri E 70
Batch No : GHB 08020070R-02

α-Tocopherol	148.3 mg/g
α-Tocotrienol	202.4 mg/g
β-Tocotrienol	40.2 mg/g
γ-Tocotrienol	156.2 mg/g
δ-Tocotrienol	80.2 mg/g
TOTAL	**650.3mg/g**

Ref Standard [illegible] 14942-02S

Mfg date 02/08

Best Before 02/11

(b) (6)

K. ANANTHAN
Chemist
[illegible]@smedarby.com

DATE : 13/03/2008

Golden Hope Bioganic

Golden Hope Bioganic Sdn. Bhd.
(Co. No. 189028 K)

c/o Golden Jomalina Food Industries Sdn. Bhd
9th Mile, Jalan Klang-Banting,
42500 Telok Panglima Garang Industrial Estate
Kuala Langat, Selangor Darul Ehsan, Malaysia
Tel : 603-3122 6301 & 03-3122 6211
Fax : 603-3122 6688 & 03-3122 9539
Web page: www.goldtrie.com
Email bioganic@goldenhope.com

CERTIFICATE OF ANALYSIS OF Gold Tri.E ™

PALM TOCOTRIENOLS RICH FRACTION

Nano E70
Batch No : GHB CF070918070NE

α-Tocopherol	45.3 mg/g
α-Tocotrienol	102.8 mg/g
β-Tocotrienol	25.2 mg/g
γ-Tocotrienol	104.3 mg/g
δ-Tocotrienol	65.2 mg/g
TOTAL	342.8 mg/g

Ref. Standard : 0669 / 14042005
Mfg Date : 09/07
Best Before : 09/10

(b) (6)

K.ANANTHAN
Chemist
Email : ananthan.krishnan@simedarby.com
Date : 18/09/2007

Golden Hope Bioganic

Golden Hope Bioganic Sdn. Bhd. (Co. No. 189028-K)
c/o Golden Jomalina Food Industries Sdn Bhd.
9th Mile, Jalan Banting-Klang, 42500 Telok Panglima Garang Industrial Estate, Selangor Darul Ehsan Malaysia
Tel (603) 31226301 & (603) 31226211 Fax: (603) 31226688 & (603) 31229539
Web Page: www.goldtrie com E-mail. bioganic@goldenhope com

CERTIFICATE OF ANALYSIS OF Gold-Tri E ™

PALM TOCOTRIENOLS RICH FRACTION

Nano E70
Batch No : GHB-CF070918070NE

α-Tocopherol	90.5 mg/g
α-Tocotrienol	86.4 mg/g
β-Tocotrienol	15.0 mg/g
γ-Tocotrienol	67.1 mg/g
δ-Tocotrienol	43.0 mg/g
TOTAL	**302.0mg/g**

Ref. Standard : 0669/14042005
Mfg date : 09/07
Best Before : 09/10

(b) (6)

K.ANANTHAN
Chemist
E-mail : ananthan.krishnan@simedarby.com

DATE : 04/03/2008

FN: GHB-CF070918070NE/D16(d)

RCC - CCR STUDY NUMBER 1152200

SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI REVERSE MUTATION ASSAY

WITH

Tocotrienol

REPORT

STUDY COMPLETION DATE:
May 20, 2008

RCC

1 COPY OF GLP CERTIFICATE

HESSEN

Gute Laborpraxis/Good Laboratory Practice

GLP-Bescheinigung/Statement of GLP Compliance
(gemäß/according to § 19b Abs. 1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 88/320/EG wurde durchgeführt in

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 88/320/EEC at:

☒ Prüfeinrichtung/Test facility ☐ Prüfstandort/Test site

RCC – Cytotest Cell Research GmbH
RCC – Cytotest Cell Research GmbH
In den Leppsteinswiesen 19
64380 Rossdorf

(Unverwechselbare Bezeichnung und Adresse/Unequivocal name and adress)

Prüfungen nach Kategorien/Areas of Expertise
(gemäß/according chemVwV-GLP Nr. 5.3/OECD guidance)

2 Prüfungen zur Bestimmung der toxikologischen Eigenschaften	2 Toxicity studies
3 Prüfungen zur Bestimmung der erbgutverändernden Eigenschaften (in vitro und in vivo)	3 Mutagenicity studies
6 Prüfungen zur Bestimmung von Rückständen	6 Residues
8 Analytische Prüfungen an biologischen Materialien	8 Analytical studies on biological materials
9 Virussicherheitsprüfungen	9 Virus validation studies

02.09.2006
Datum der Inspektion/Date of Inspection
(Tag Monat Jahr/day month year)

Die genannte Prüfeinrichtung befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

The above mentioned test facility is included in the national GLP Compliance Programme and is inspected on a regular basis.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung die oben genannten Prüfungen unter Einhaltung der GLP- Grundsätze durchgeführt werden können.

Based on the inspection report it can be confirmed, that this test facility is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Im Auftrag
(b) (6)

Th. Zimmermann, Referent, Wiesbaden, den **19. Januar 2007**
(Name und Funktion der verantwortlichen Person/
Name and function of responsible person)

Hess. Ministerium für Umwelt, ländlichen Raum und Verbraucherschutz,
Mainzer Straße 80 D65189 Wiesbaden
(Name und Adresse der GLP-Überwachungsbehörde/Name and address of the GLP Monitoring Authority

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3 PREFACE

3.1 General

Title:	Salmonella typhimurium and Escherichia coli Reverse Mutation Assay with Tocotrienol
Sponsor:	Davos Life Science Pte. Ltd. Mr. CY Ho (C.O.O) 11 Biopolis Way Helios #07-03/04 138667 Singapore Singapore
Study Monitor:	Davos Life Science Pte. Ltd. Ms. Hui Heng Foo c/o Miguel Servet 16 nave 17 Elche Parque Industrial 03203 Elche (Alicante) Spain
Test Facility:	R C C Cytotest Cell Research GmbH (RCC-CCR) In den Leppsteinswiesen 19 64380 Rossdorf Germany
Contracting Institute:	R C C Ltd 4452 Itingen Switzerland
Reference Number:	B76443

3.2 Responsibilities

Study Director:	Dipl. Biol. Andrea Sokolowski
Deputy Study Director:	Dr. Hans-Eric Wollny
Management:	Dr. Wolfgang Völkner
Head of Quality Assurance Unit:	Frauke Hermann

3.3 Schedule

Experimental Starting Date:	March 27, 2008
Experimental Completion Date:	April 04, 2008

3.4 Project Staff Signatures

Study Director Dipl. Biol. Andrea Sokolowski

(b) (6)

.....

Date: May 20, 2008

Management Dr. Wolfgang Völkner

(b) (6)

.....

Date: May 20, 2008

3.5 Good Laboratory Practice

The study was performed in compliance with:

"Chemikaliengesetz" (Chemicals Act) of the Federal Republic of Germany, "Anhang 1" (Annex 1) dated July 25, 1994 ("BGBl. I 1994", pp. 1703), last revision dated June 27, 2002

"OECD Principles of Good Laboratory Practice", as revised in 1997 [C(97)186/Final]

3.6 Guidelines

This study followed the procedures indicated by the following internationally accepted guidelines and recommendations:

"Ninth Addendum to OECD Guidelines for Testing of Chemicals", Section 4, No. 471: "Bacterial Reverse Mutation Test", adopted July 21, 1997

"Commission Directive 2000/32/EC, L1362000, Annex 4D", dated May 19, 2000

3.7 Archiving

RCC Cytotest Cell Research GmbH will archive the following data for 15 years:

Raw data, study plan, report, and a sample of the test item.

No data will be discarded without the sponsor's consent.

3.8 Deviations to Study Plan

There were no deviations to study plan.

4 STATEMENT OF COMPLIANCE

Study Number:	1152200
Test Item:	Tocotrienol
Study Director:	Dipl. Biol. Andrea Sokolowski
Title:	Salmonella typhimurium and Escherichia coli Reverse Mutation Assay with Tocotrienol

This study performed in the test facility of RCC Cytotest Cell Research GmbH was conducted in compliance with Good Laboratory Practice Regulations:

"Chemikaliengesetz" (Chemicals Act) of the Federal Republic of Germany, "Anhang 1" (Annex 1) dated July 25, 1994 ("BGBl. I 1994", pp. 1703), last revision dated June 27, 2002.

"OECD Principles of Good Laboratory Practice", as revised in 1997 [C(97)186/Final]

There were no circumstances that may have affected the quality or integrity of the study.

Study Director

R C C - C C R
Dipl. Biol. Andrea Sokolowski

(b) (6)

.....

Date: May 20, 2008

5 STATEMENT OF QUALITY ASSURANCE UNIT

Study Number: 1152200

Test Item: Tocotrienol

Study Director: Dipl. Biol. Andrea Sokolowski

Title: Salmonella typhimurium and Escherichia coli Reverse Mutation Assay with Tocotrienol

The general facilities and activities of RCC Cytotest Cell Research GmbH are inspected periodically and the results are reported to the responsible person and the management.

Study procedures were inspected periodically. The study plan and this report were audited by the Quality Assurance Unit. The dates are given below.

Phases and Dates of QAU Inspections/ Audits		Dates of Reports to the Study Director and to Management
Study Plan:	March 06, 2008	March 06, 2008
Process Inspection (application):	March 31, 2008	March 31, 2008
Report:	May 14, 2008	May 14, 2008

This statement is to confirm that the present report reflects the raw data.

Head of Quality Assurance Unit for Frauke Hermann
Karlheinz Werner

(b) (6)

.....

Date: May 20, 2008

6 SUMMARY OF RESULTS

This study was performed to investigate the potential of Tocotrienol to induce gene mutations in the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using the Salmonella typhimurium strains TA 1535, TA 1537, TA 98, and TA 100, and the Escherichia coli strain WP2 uvrA.

The assay was performed in two independent experiments both with and without liver microsomal activation. Each concentration, including the controls, was tested in triplicate. The test item was tested at the following concentrations:

Pre-Experiment/Experiment I:	3, 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate
Experiment II:	33; 100; 333; 1000; 2500; and 5000 µg/plate

The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without metabolic activation in both independent experiments.

No toxic effects, evident as a reduction in the number of revertants, occurred in the test groups with and without metabolic activation. The slight reduction in the number of revertants in strain TA 1537 at 333 µg/plate without S9 mix in experiment II, is judged to be based on biologically irrelevant fluctuations in the number of colonies and does not represent a true toxic effect.

No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with Tocotrienol at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.

Appropriate reference mutagens were used as positive controls and showed a distinct increase of induced revertant colonies.

6.1 Conclusion

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

Therefore, Tocotrienol is considered to be non-mutagenic in this Salmonella typhimurium and Escherichia coli reverse mutation assay.

7 OBJECTIVE

7.1 Aims of the Study

The experiments were performed to assess the potential of the test item to induce gene mutations by means of two independent Salmonella typhimurium and Escherichia coli reverse mutation assays. Experiment I was performed as a plate incorporation assay. Since a negative result was obtained in this experiment, experiment II was performed as a pre-incubation assay.

7.2 Reasons for the Study

The most widely used assays for detecting gene mutations are those using bacteria (3). They are relatively simple and rapid to perform, and give reliable data on the ability of an agent to interact with DNA and produce mutations.

Reverse mutation assays determine the frequency with which an agent reverses or suppresses the effect of the forward mutation. The genetic target presented to an agent is therefore small, specific and selective. Several bacterial strains, or a single strain with multiple markers are necessary to overcome the effects of mutagen specificity. The reversion of bacteria from growth-dependence on a particular amino acid to growth in the absence of that amino acid (reversion from auxotrophy to prototrophy) is the most widely used marker.

The Salmonella typhimurium histidine (his) and the E. coli tryptophan (trp) reversion system measures $his^- \rightarrow his^+$ and $trp^- \rightarrow trp^+$ reversions, respectively. The S. typhimurium and Escherichia coli strains are constructed to differentiate between base pair (TA 1535, TA 100, and WP2 uvrA) and frameshift (TA 1537, TA 98) mutations.

According to the direct plate incorporation or the pre-incubation method the bacteria are exposed to the test item with and without metabolic activation and plated on selective medium. After a suitable period of incubation, revertant colonies are counted.

To establish a dose response effect at least 6 dose levels with adequately spaced intervals were tested. The maximum dose level was 5000 µg/plate.

To validate the test, reference mutagens were tested in parallel to the test item.

8 MATERIALS AND METHODS

8.1 Test Item

Internal RCC-CCR Test Item Number: S8318 11

The test item and the information concerning the test item were provided by the sponsor.

Identity:	Tocotrienol
Batch No.:	A0601560
Purity:	Total Tocotrienols and Tocopherols 59.4
Stability in Solvent:	Not indicated by the sponsor
Storage:	At room temperature, light and heat protected,
Expiration Date:	Not indicated by the sponsor

On the day of the experiment, the test item Tocotrienol was dissolved in ethanol (MERCK, D-64293 Darmstadt; purity > 99 %). The solvent was chosen because of its solubility properties and its relative non-toxicity to the bacteria (5).

The test item precipitated in the test tubes from 1000 up to 5000 in experiment I and from 2500 up to 5000 µg/plate in experiment II. Precipitation was also observed on the agar plates from 2500 up to 5000 µg/plate in both experiments.

8.2 Controls

8.2.1 Negative Controls

Concurrent untreated and solvent controls were performed.

8.2.2 Positive Control Substances

Without metabolic activation

Strains:	TA 1535, TA 100
Name:	sodium azide, NaN_3
Supplier:	SERVA, D-69042 Heidelberg
Catalogue No.:	30175
Purity:	at least 99 %
Dissolved in:	water deionised
Concentration:	10 µg/plate

Strains:	TA 1537, TA 98
Name:	4-nitro-o-phenylene-diamine, 4-NOPD
Supplier:	SIGMA, D-82041 Deisenhofen
Catalogue No.:	N 9504
Purity:	> 99.9 %
Dissolved in:	DMSO (MERCK, D-64293 Darmstadt; purity > 99 %)
Concentration:	10 µg/plate in TA 98, 50 µg/plate in TA 1537

Strain:	WP2 uvrA
Name:	methyl methane sulfonate, MMS
Supplier:	Merck-Schuchardt, D-85662 Hohenbrunn
Catalogue No.:	820775
Purity:	> 99.0 %
Dissolved in:	water deionised
Concentration:	3 µL/plate

With metabolic activation

Strains:	TA 1535, TA 1537, TA 98, TA 100, WP2 uvrA
Name:	2-aminoanthracene, 2-AA
Supplier:	SIGMA, D-82041 Deisenhofen
Catalogue No.:	A 1381
Purity:	97.5 %
Dissolved in:	DMSO (MERCK, D-64293 Darmstadt; purity > 99 %)
Concentration:	2.5 µg/plate (TA 1535, TA 1537, TA 98, TA 100), 10 µg/plate (WP2 uvrA)

The stability of the positive control substances in solution is unknown but a mutagenic response in the expected range is sufficient evidence of biological stability.

8.3 Test System

8.3.1 Characterisation of the Salmonella typhimurium Strains and E. coli Strain

The histidine dependent strains are derived from S. typhimurium strain LT2 through a mutation in the histidine locus. Additionally due to the "deep rough" (rfa-minus) mutation they possess a faulty lipopolysaccharide envelope which enables substances to penetrate the cell wall more easily. A further mutation causes a reduction in the activity of an excision repair system. The latter alteration includes mutational processes in the nitrate reductase and biotin genes produced in a UV-sensitive area of the gene named "uvrB-minus". In the strains TA 98 and TA 100 the R-factor plasmid pKM 101 carries the ampicillin resistance marker (6).

Strain WP2 (4) and its derivatives all carry the same defect in one of the genes for tryptophan biosynthesis. Tryptophan-independent (Trp^+) mutants (revertants) can arise either by a base change at the site of the original alteration or by a base change elsewhere in the chromosome so that the original defect is suppressed. This second possibility can occur in several different ways so that the system seems capable of detecting all types of mutagen which substitute one base for another. Additionally, the uvrA derivative is deficient in the DNA repair process (excision repair damage). Such a repair-deficient strain may be more readily mutated by agents.

When summarised the mutations of the TA strains and the E. coli strain, used in this study can be described as follows:

Salmonella typhimurium		
Strains	**Genotype**	**Type of mutations indicated**
TA 1537	his C 3076; rfa^-; $uvrB^-$:	frame shift mutations
TA 98	his D 3052; rfa^-; $uvrB^-$;R-factor	" "
TA 1535	his G 46; rfa^-; $uvrB^-$:	base-pair substitutions
TA 100	his G 46; rfa^-; $uvrB^-$;R-factor	" "
Escherichia coli		
WP2 uvrA	trp^-; $uvrA^-$:	base-pair substitutions and others

Regular checking of the properties of the strains regarding the membrane permeability and ampicillin resistance as well as spontaneous mutation rates is performed in RCC Cytotest Cell Research GmbH according to B. Ames et al. (1) and D. Maron and B. Ames (6). In this way it was ensured that the experimental conditions set down by Ames were fulfilled.

The bacterial strains TA 1535, TA 1537, TA 98, TA 100, and WP2 uvrA were obtained from Trinova Biochem GmbH (35394 Gießen, Germany).

8.3.2 Storage

The strain cultures were stored as stock cultures in ampoules with nutrient broth + 5 % DMSO (MERCK, D-64293 Darmstadt) in liquid nitrogen.

8.3.3 Precultures

From the thawed ampoules of the strains 0.5 mL suspension was transferred into 250 mL Erlenmeyer flasks containing 20 mL nutrient medium. A solution of 20 µL ampicillin (25 µg/mL) was added to the strains TA 98 and TA 100. This nutrient medium contains per litre:

8 g Merck Nutrient Broth (MERCK, D-64293 Darmstadt)
5 g NaCl (MERCK, D-64293 Darmstadt)

The bacterial cultures were incubated in a shaking water bath for 4 hours at 37° C.

8.3.4 Selective Agar

The plates with the selective agar were obtained from E. Merck, D-64293 Darmstadt.

8.3.5 Overlay Agar

The overlay agar contains per litre:

for Salmonella strains:	for Escherichia coli:
6.0 g MERCK Agar Agar*	6.0 g MERCK Agar Agar*
6.0 g NaCl*	6.0 g NaCl*
10.5 mg L-Histidine×HCl×H_2O*	2.5 mg Tryptophan*
12.2 mg Biotin*	

* (MERCK, D-64293 Darmstadt)

Sterilisations were performed at 121° C in an autoclave.

8.4 Mammalian Microsomal Fraction S9 Mix

The bacteria used in this assay do not possess the enzyme systems which, in mammals, are known to convert promutagens into active DNA damaging metabolites. In order to overcome this major drawback an exogenous metabolic system is added in form of mammalian microsome enzyme activation mixture.

8.4.1 S9 (Preparation by R C C - C C R)

Phenobarbital/β-Naphthoflavone induced rat liver S9 is used as the metabolic activation system. The S9 is prepared from 8 - 12 weeks old male Wistar Hanlbm rats, weight approx. 220 - 320 g induced by applications of 80 mg/kg b.w. Phenobarbital i.p. (Desitin; D-22335 Hamburg) and β-Naphthoflavone p.o. (Aldrich, D-89555 Steinheim) each on three consecutive days. The livers are prepared 24 hours after the last treatment. The S9 fractions are produced by dilution of the liver homogenate with a KCl solution (1+3) followed by centrifugation at 9000 g. Aliquots of the supernatant are frozen and stored in ampoules at -80° C. Small numbers of the ampoules can be kept at -20°C for up to one week. Each batch of S9 mix is routinely tested with 2-aminoanthracene as well as benzo(a)pyrene.

The protein concentration in the S9 preparation was 32.5 mg/mL (lot no. R 010208).

8.4.2 S9 Mix

Before the experiment an appropriate quantity of S9 supernatant was thawed and mixed with S9 co-factor solution. The amount of S9 supernatant was 10% v/v in the S9 mix. Cofactors are added to the S9 mix to reach the following concentrations in the S9 mix:

8 mM $MgCl_2$
33 mM KCl
5 mM Glucose-6-phosphate
5 mM NADP

in 100 mM sodium-ortho-phosphate-buffer, pH 7.4.

During the experiment the S9 mix was stored in an ice bath. The S9 mix preparation was performed according to Ames et al.(1).

8.5 Pre-Experiment for Toxicity

To evaluate the toxicity of the test item a pre-experiment was performed with strains TA 1535, TA 1537, TA 98, TA 100, and WP2 uvrA. Eight concentrations were tested for toxicity and mutation induction with three plates each. The experimental conditions in this pre-experiment were the same as described below for the experiment I (plate incorporation test).

Toxicity of the test item results in a reduction in the number of spontaneous revertants or a clearing of the bacterial background lawn.

The pre-experiment is reported as main experiment I, if the following criteria are met:

Evaluable plates (>0 colonies) at five concentrations or more in all strains used.

8.6 Dose Selection

In the pre-experiment the concentration range of the test item was 3 – 5000 µg/plate. The pre-experiment is reported as experiment I. Since no toxic effects were observed 5000 µg/plate were chosen as maximal concentration.

The concentration range included two logarithmic decades. The following concentrations were tested in experiment II:

33; 100; 333; 1000; 2500; and 5000 µg/plate

8.7 Experimental Performance

For each strain and dose level including the controls, three plates were used.

The following materials were mixed in a test tube and poured onto the selective agar plates:

- 100 µL Test solution at each dose level, solvent (negative control) or reference mutagen solution (positive control),
- 500 µL S9 mix (for test with metabolic activation) or S9 mix substitution buffer (for test without metabolic activation),
- 100 µL Bacteria suspension (cf. test system, pre-culture of the strains),
- 2000 µL Overlay agar

In the pre-incubation assay 50 µL test solution, 100 µL reference mutagen solution (positive control), 500 µL S9 mix / S9 mix substitution buffer and 100 µL bacterial suspension were mixed in a test tube and shaken at 37° C for 60 minutes. After pre-incubation 2.0 mL overlay agar (45° C) was added to each tube. The mixture was poured on selective agar plates.

After solidification the plates were incubated upside down for at least 48 hours at 37° C in the dark (2).

8.8 Data Recording

The colonies were counted using the Petri Viewer Mk2 (Perceptive Instruments Ltd, Suffolk CB 7BN, UK) with the software program Ames Study Manager. The counter was connected to an IBM AT compatible PC with printer to print out the individual values and the means from the plates for each concentration together with standard deviations and enhancement factors as compared to the spontaneous reversion rates (see tables of results). Due to precipitation of the test item the revertant colonies were partly counted manually.

8.9 Acceptability of the Assay

The Salmonella typhimurium and Escherichia coli reverse mutation assay is considered acceptable if it meets the following criteria:

- regular background growth in the negative and solvent control
- the spontaneous reversion rates in the negative and solvent control are in the range of our historical data
- the positive control substances should produce a significant increase in mutant colony frequencies

8.10 Evaluation of Results

A test item is considered as a mutagen if a biologically relevant increase in the number of revertants exceeding the threshold of twice (strains TA 98, TA 100, and WP2 uvrA) or thrice (strains TA 1535 and TA 1537) the colony count of the corresponding solvent control is observed (3).

A dose dependent increase is considered biologically relevant if the threshold is exceeded at more than one concentration (2).

An increase exceeding the threshold at only one concentration is judged as biologically relevant if reproduced in an independent second experiment.

A dose dependent increase in the number of revertant colonies below the threshold is regarded as an indication of a mutagenic potential if reproduced in an independent second experiment. However, whenever the colony counts remain within the historical range of negative and solvent controls such an increase is not considered biologically relevant.

8.11 Biometry

According to the OECD guideline 471, a statistical analysis of the data is not mandatory.

9 DISCUSSION OF RESULTS

The test item Tocotrienol was assessed for its potential to induce gene mutations in the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using Salmonella typhimurium strains TA 1535, TA 1537, TA 98, and TA 100, and the Escherichia coli strain WP2 uvrA.

The assay was performed in two independent experiments both with and without liver microsomal activation. Each concentration and the controls were tested in triplicate. The test item was tested at the following concentrations:

Pre-Experiment/Experiment I:	3, 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate
Experiment II:	33; 100; 333; 1000; 2500; and 5000 µg/plate

The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without S9 mix in both experiments.

No toxic effects, evident as a reduction in the number of revertants, occurred in the test groups with and without metabolic activation. The slight reduction in the number of revertants in strain TA 1537 at 333 µg/plate without S9 mix in experiment II, is judged to be based on biologically irrelevant fluctuations in the number of colonies and does not represent a true toxic effect.

No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with Tocotrienol at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.

Appropriate reference mutagens were used as positive controls. They showed a distinct increase of induced revertant colonies.

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

10 REFERENCES

1. Ames, B.N., J. McCann, and E. Yamasaki (1977)
   Methods for detecting carcinogens and mutagens with the Salmonella/mammalian microsome mutagenicity test
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2. de Serres F.J. and M.D. Shelby (1979)
   Recommendations on data production and analysis using the Salmonella/microsome mutagenicity assay
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3. Hollstein,M., J. McCann, F.A. Angelosanto and W.W. Nichols (1979)
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   Mutagen Testing Using TRP^+ Reversion in Escherichia Coli
   Mutation. Res. 38, 3- 32

5. Maron D.M., J. Katzenellenbogen and B.N. Ames, (1981)
   Compatibility of organic solvents with the Salmonella/Microsome Test
   Mutation Res. 88, 343-350

6. Maron D.M., Ames, B.N. (1983)
   Revised methods for the Salmonella mutagenicity test
   Mutation Res. 113, 173-215

11 DISTRIBUTION OF THE REPORT

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Study Director	1 × (original)

12 SUMMARY OF RESULTS

12.1 Summary of Results Pre-Experiment and Experiment I

Study Name: 1152200
Experiment: 1152200 VV Plate
Assay Conditions:

Study Code: RCC-CCR 1152200
Date Plated: 27/03/2008
Date Counted: 31/03/2008

Metabolic Activation	Test Group	Dose Level (µg/plate)	Revertant Colony Counts (Mean ±SD)				
			TA 1535	TA 1537	TA 98	TA 100	WP2 uvrA
Without Activation	Ethanol		17 ± 2	15 ± 7	33 ± 12	107 ± 14	47 ± 8
	Untreated		18 ± 4	21 ± 6	29 ± 2	124 ± 3	47 ± 5
	Tocotrienol	3 µg	13 ± 3	17 ± 3	40 ± 5	105 ± 11	43 ± 5
		10 µg	15 ± 3	16 ± 4	38 ± 6	106 ± 16	45 ± 4
		33 µg	16 ± 6	17 ± 4	31 ± 3	116 ± 5	40 ± 2
		100 µg	17 ± 4	14 ± 2	35 ± 2	110 ± 11	50 ± 7
		333 µg	16 ± 3	16 ± 3	34 ± 8	113 ± 4	44 ± 8
		1000 µg	15 ± 2	13 ± 2	37 ± 10	113 ± 2	44 ± 3
		2500 µg	14 ± 3 P M	14 ± 2 P M	29 ± 6 P M	106 ± 6 P M	45 ± 5 P M
		5000 µg	12 ± 3 P M	12 ± 3 P M	28 ± 4 P M	103 ± 11 P M	42 ± 3 P M
	NaN3	10 µg	2135 ± 78			2494 ± 85	
	4-NOPD	10 µg			377 ± 51		
	4-NOPD	50 µg		112 ± 2			
	MMS	3.0 µL					1203 ± 131
With Activation	Ethanol		22 ± 5	22 ± 8	45 ± 10	122 ± 10	67 ± 10
	Untreated		23 ± 4	22 ± 2	51 ± 10	157 ± 22	66 ± 5
	Tocotrienol	3 µg	23 ± 5	26 ± 5	47 ± 11	128 ± 7	66 ± 11
		10 µg	17 ± 4	22 ± 7	39 ± 11	123 ± 5	57 ± 3
		33 µg	21 ± 3	18 ± 7	46 ± 7	126 ± 6	56 ± 6
		100 µg	24 ± 7	19 ± 3	44 ± 6	117 ± 11	55 ± 5
		333 µg	23 ± 7	23 ± 6	48 ± 8	119 ± 14	61 ± 4
		1000 µg	28 ± 5	22 ± 4	46 ± 5	122 ± 26	56 ± 1
		2500 µg	15 ± 3 P M	14 ± 2 P M	39 ± 5 P M	120 ± 22 P M	49 ± 11 P M
		5000 µg	15 ± 1 P M	16 ± 4 P M	34 ± 7 P M	117 ± 12 P M	48 ± 2 P M
	2-AA	2.5 µg	298 ± 25	218 ± 58	1375 ± 278	1618 ± 67	
	2-AA	10.0 µg					248 ± 33

Key to Positive Controls

NaN3	sodium azide
2-AA	2-aminoanthracene
4-NOPD	4-nitro-o-phenylene-diamine
MMS	methyl methane sulfonate

Key to Plate Postfix Codes

P	Precipitate
M	Manual count

12.2 Summary of Results Experiment II

Study Name: 1152200
Experiment: 1152200 HV2 Pre
Assay Conditions:

Study Code: RCC-CCR 1152200
Date Plated: 01/04/2008
Date Counted: 04/04/2008

Metabolic Activation	Test Group	Dose Level (µg/plate)	Revertant Colony Counts (Mean ±SD)				
			TA 1535	TA 1537	TA 98	TA 100	WP2 uvrA
Without Activation	Ethanol		20 ± 4	14 ± 4	31 ± 3	120 ± 8	48 ± 1
	Untreated		17 ± 4	14 ± 5	35 ± 13	133 ± 17	55 ± 1
	Tocotrienol	33 µg	23 ± 2	8 ± 2	33 ± 3	122 ± 9	47 ± 4
		100 µg	16 ± 4	10 ± 4	34 ± 4	127 ± 7	49 ± 8
		333 µg	18 ± 5	6 ± 1	34 ± 6	118 ± 9	53 ± 9
		1000 µg	16 ± 5	10 ± 5	33 ± 4	131 ± 9	54 ± 3
		2500 µg	17 ± 2 P	10 ± 3 P	27 ± 3 P	126 ± 11 P	49 ± 5 P
		5000 µg	13 ± 4 PM	7 ± 3 PM	33 ± 3 PM	119 ± 11 PM	52 ± 4 PM
	NaN3	10 µg	2131 ± 39			2396 ± 29	
	4-NOPD	10 µg			392 ± 7		
	4-NOPD	50 µg		91 ± 14			
	MMS	3.0 µL					417 ± 13
With Activation	Ethanol		25 ± 4	12 ± 3	44 ± 3	155 ± 14	63 ± 7
	Untreated		23 ± 2	17 ± 2	45 ± 12	169 ± 14	62 ± 3
	Tocotrienol	33 µg	31 ± 4	12 ± 5	45 ± 10	138 ± 11	46 ± 5
		100 µg	17 ± 1	13 ± 1	45 ± 11	136 ± 6	64 ± 7
		333 µg	18 ± 11	13 ± 1	62 ± 14	136 ± 7	70 ± 12
		1000 µg	25 ± 8	16 ± 6	51 ± 6	132 ± 9	62 ± 14
		2500 µg	28 ± 5 PM	12 ± 1 PM	56 ± 8 PM	146 ± 14 PM	69 ± 15 PM
		5000 µg	23 ± 2 PM	9 ± 1 PM	37 ± 4 PM	130 ± 7 PM	63 ± 5 PM
	2-AA	2.5 µg	275 ± 49	147 ± 16	821 ± 73	1605 ± 79	
	2-AA	10.0 µg					316 ± 8

Key to Positive Controls

NaN3	sodium azide
2-AA	2-aminoanthracene
4-NOPD	4-nitro-o-phenylene-diamine
MMS	methyl methane sulfonate

Key to Plate Postfix Codes

P	Precipitate
M	Manual count

13 HISTORICAL CONTROL DATA

These data represent the laboratory's historical control data from January 2007 until July 2007 representing approx. 200 experiments (WP2 uvrA the historical data are based on approx. 100 experiments).

Strain		without S9 mix				with S9 mix			
		Mean	SD	Min	Max	Mean	SD	Min	Max
TA 1535	Solvent control	17	4.51	7	40	21	4.95	8	41
	Negative control	17	4.57	9	34	21	5.55	9	42
	Positive control	1878	203.83	852	2347	270	83.95	98	636
TA1537	Solvent control	11	2.91	6	23	16	4.19	6	35
	Negative control	11	2.97	6	24	17	4.97	7	37
	Positive control	125	41.01	75	424	180	64.61	73	475
TA 98	Solvent control	32	6.78	18	66	40	6.24	24	64
	Negative control	35	6.67	17	62	41	6.56	23	67
	Positive control	534	163.95	172	1916	1193	491.42	184	2759
TA 100	Solvent control	138	25.59	84	213	157	27.89	94	254
	Negative control	145	21.66	97	210	161	25.76	94	217
	Positive control	1953	492.35	572	2943	1763	713.99	542	3886
WP2uvrA	Solvent control	50	8.32	32	82	63	8.86	39	90
	Negative control	50	7.89	34	76	63	8.53	44	89
	Positive control	913	460.35	191	2023	327	128.21	161	1345

Mean = mean value of revertants/plate
SD = standard deviation Min = minimal value/Max = maximal value

14 ANNEX: TABLES OF RESULTS (8 PAGES)

Pre-Experiment and Experiment I: 1152200 VV Plate Incorporation (4 pages)

Experiment II: 1152200 Pre-Incubation (4 pages)

Study Name: 1152200
Experiment: 1152200 VV Plate
Assay Conditions:

Study Code: RCC-CCR 1152200
Date Plated: 27/03/2008
Date Counted: 31/03/2008

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**Tocotrienol**	3 µg	*13.0*	*3.0*	*0.8*	16, 10, 13
		10 µg	*15.3*	*3 2*	*0.9*	14, 13, 19
		33 µg	*16.3*	*6 4*	*1.0*	19, 21, 9
		100 µg	*17.0*	*4.4*	*1.0*	22, 14, 15
		333 µg	*16.3*	*3 2*	*1.0*	15, 14, 20
		1000 µg	*15.0*	*2.0*	*0.9*	17, 15, 13
		2500 µg	*13.7*	*3.2*	*0.8*	15 P M, 16 P M, 10 P M
		5000 µg	*12.0*	*2.6*	*0 7*	11 P M, 10 P M, 15 P M
	Ethanol		*17.0*	*2.0*		17, 19, 15
	Untreated Control		*18.3*	*3.8*		14, 20, 21
TA 1537	**Tocotrienol**	3 µg	*16.7*	*2.5*	*1 1*	19, 17, 14
		10 µg	*16.0*	*4.0*	*1.0*	12, 20, 16
		33 µg	*16.7*	*4 0*	*1.1*	16, 21, 13
		100 µg	*13.7*	*2.1*	*0.9*	16, 13, 12
		333 µg	*16.0*	*3.0*	*1.0*	13, 19, 16
		1000 µg	*13.3*	*1.5*	*0.9*	15, 12, 13
		2500 µg	*14.0*	*2.0*	*0.9*	12 P M, 14 P M, 16 P M
		5000 µg	*12.3*	*2.5*	*0.8*	10 P M, 15 P M, 12 P M
	Ethanol		*15.3*	*6.5*		22, 15, 9
	Untreated Control		*21.0*	*5.6*		15, 26, 22
TA 98	**Tocotrienol**	3 µg	*39.7*	*4.7*	*1.2*	45, 38, 36
		10 µg	*38.3*	*6.4*	*1.1*	41, 43, 31
		33 µg	*31.0*	*3.0*	*0.9*	34, 28, 31
		100 µg	*35.3*	*1.5*	*1.1*	34, 37, 35
		333 µg	*33.7*	*8.1*	*1.0*	28, 30, 43
		1000 µg	*37.3*	*9.8*	*1.1*	43, 43, 26
		2500 µg	*28.7*	*5.5*	*0.9*	35 P M, 26 P M, 25 P M
		5000 µg	*28.0*	*3.6*	*0.8*	31 P M, 29 P M, 24 P M
	Ethanol		*33.3*	*12.1*		24, 47, 29
	Untreated Control		*28.7*	*1.5*		29, 30, 27
TA 100	**Tocotrienol**	3 µg	*105 3*	*11.0*	*1.0*	118, 100, 98
		10 µg	*105.7*	*16.4*	*1.0*	118, 87, 112
		33 µg	*116.3*	*4.5*	*1.1*	116, 112, 121
		100 µg	*109.7*	*11.1*	*1 0*	111, 98, 120
		333 µg	*113.3*	*3.8*	*1.1*	109, 116, 115
		1000 µg	*112.7*	*2.1*	*1.0*	115, 111, 112
		2500 µg	*105.7*	*5.5*	*1.0*	100 P M, 106 P M, 111 P M
		5000 µg	*103.0*	*10.6*	*1 0*	99 P M, 95 P M, 115 P M
	Ethanol		*107 3*	*13.9*		111, 119, 92
	Untreated Control		*124.3*	*3 2*		123, 128, 122

Key to Plate Postfix Codes

P	Precipitate
M	Manual count

Study Name: 1152200
Experiment: 1152200 VV Plate
Assay Conditions:

Study Code: RCC-CCR 1152200
Date Plated: 27/03/2008
Date Counted: 31/03/2008

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2 uvrA	**Tocotrienol**	3 µg	*43.3*	*4.7*	*0.9*	47, 38, 45
		10 µg	*45.0*	*4.0*	*1.0*	45, 41, 49
		33 µg	*39.7*	*2.3*	*0.8*	37, 41, 41
		100 µg	*50.0*	*6.9*	*1.1*	54, 54, 42
		333 µg	*43.7*	*7.6*	*0.9*	35, 49, 47
		1000 µg	*44.3*	*2.5*	*0.9*	47, 42, 44
		2500 µg	*45.0*	*5.3*	*1.0*	47 P M, 49 P M, 39 P M
		5000 µg	*41.7*	*3.2*	*0.9*	43 P M, 38 P M, 44 P M
	Ethanol		*47.0*	*8.2*		45, 40, 56
	Untreated Control		*46.7*	*4.7*		45, 52, 43
TA 1535	**NaN3**	10 µg	*2134.7*	*77.7*	*125.6*	2141, 2209, 2054
TA 1537	**4-NOPD**	50 µg	*112.0*	*1.7*	*7.3*	111, 111, 114
TA 98	**4-NOPD**	10 µg	*376.7*	*50.6*	*11.3*	389, 420, 321
TA 100	**NaN3**	10 µg	*2493.7*	*85.1*	*23.2*	2581, 2489, 2411
WP2 uvrA	**MMS**	3.0 µL	*1202.7*	*131.2*	*25.6*	1083, 1182, 1343

Key to Positive Controls

NaN3	sodium azide
4-NOPD	4-nitro-o-phenylene-diamine
MMS	methyl methane sulfonate

Key to Plate Postfix Codes

P	Precipitate
M	Manual count

Study Name: 1152200
Experiment: 1152200 VV Plate
Assay Conditions:

Study Code: RCC-CCR 1152200
Date Plated: 27/03/2008
Date Counted: 31/03/2008

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**Tocotrienol**	3 µg	*23.3*	*4.9*	*1.1*	20, 21, 29
		10 µg	*16 7*	*4.0*	*0 8*	16, 21, 13
		33 µg	*21.0*	*3.5*	*1 0*	23, 17, 23
		100 µg	*24.3*	*7 4*	*1.1*	30, 27, 16
		333 µg	*23.0*	*6.6*	*1.0*	29, 24, 16
		1000 µg	*27.7*	*4.9*	*1.3*	30, 31, 22
		2500 µg	*15.3*	*3.2*	*0.7*	14 P M, 19 P M, 13 P M
		5000 µg	*14.7*	*0.6*	*0.7*	14 P M, 15 P M, 15 P M
	Ethanol		*22.0*	*5.3*		24, 16, 26
	Untreated Control		*23.0*	*3.6*		26, 19, 24
TA 1537	**Tocotrienol**	3 µg	*26 0*	*5.0*	*1.2*	26, 21, 31
		10 µg	*21.7*	*7.1*	*1.0*	23, 28, 14
		33 µg	*18.0*	*7.0*	*0.8*	15, 13, 26
		100 µg	*19.0*	*3.5*	*0.9*	15, 21, 21
		333 µg	*23.0*	*5.6*	*1.0*	17, 24, 28
		1000 µg	*21.7*	*4.0*	*1.0*	24, 24, 17
		2500 µg	*14 3*	*2.1*	*0 6*	16 P M, 12 P M, 15 P M
		5000 µg	*16 0*	*3.6*	*0.7*	15 P M, 13 P M, 20 P M
	Ethanol		*22.3*	*7.6*		17, 31, 19
	Untreated Control		*22.3*	*2.1*		23, 20, 24
TA 98	**Tocotrienol**	3 µg	*46.7*	*11.0*	*1.0*	59, 43, 38
		10 µg	*39.3*	*11.0*	*0.9*	43, 48, 27
		33 µg	*46.0*	*7.2*	*1.0*	54, 44, 40
		100 µg	*44.0*	*5.6*	*1.0*	49, 45, 38
		333 µg	*48.3*	*7.5*	*1.1*	44, 57, 44
		1000 µg	*45.7*	*5.1*	*1.0*	50, 47, 40
		2500 µg	*39.0*	*4.6*	*0.9*	40 P M, 34 P M, 43 P M
		5000 µg	*33.7*	*6.5*	*0.8*	27 P M, 34 P M, 40 P M
	Ethanol		*44 7*	*10.3*		36, 56, 42
	Untreated Control		*51.3*	*10.0*		59, 40, 55
TA 100	**Tocotrienol**	3 µg	*128.0*	*6.9*	*1.0*	120, 132, 132
		10 µg	*123.3*	*5.1*	*1 0*	122, 129, 119
		33 µg	*126.3*	*6.4*	*1 0*	130, 119, 130
		100 µg	*116.7*	*10.6*	*1.0*	128, 115, 107
		333 µg	*119.3*	*14.0*	*1.0*	108, 115, 135
		1000 µg	*121.7*	*26.1*	*1.0*	149, 97, 119
		2500 µg	*120.0*	*21.8*	*1.0*	110 P M, 105 P M, 145 P M
		5000 µg	*116.7*	*11.6*	*1.0*	111 P M, 109 P M, 130 P M
	Ethanol		*122.3*	*10.2*		115, 134, 118
	Untreated Control		*157.3*	*21.5*		135, 159, 178

Key to Plate Postfix Codes

P	Precipitate
M	Manual count

Study Name: 1152200
Experiment: 1152200 VV Plate
Assay Conditions:

Study Code: RCC-CCR 1152200
Date Plated: 27/03/2008
Date Counted: 31/03/2008

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2 uvrA	**Tocotrienol**	3 µg	*66.0*	*10.5*	*1.0*	55, 67, 76
		10 µg	*57.0*	*2.6*	*0.9*	59, 54, 58
		33 µg	*56.0*	*5.6*	*0.8*	55, 62, 51
		100 µg	*55.0*	*5.2*	*0.8*	49, 58, 58
		333 µg	*61.0*	*4.4*	*0.9*	64, 63, 56
		1000 µg	*56.3*	*0.6*	*0.8*	57, 56, 56
		2500 µg	*48.7*	*10.6*	*0 7*	47 P M, 39 P M, 60 P M
		5000 µg	*47.7*	*2.1*	*0.7*	47 P M, 46 P M, 50 P M
	Ethanol		*66 7*	*10 1*		55, 73, 72
	Untreated Control		*65.7*	*5.0*		61, 65, 71
TA 1535	**2-AA**	2 5 µg	*297.7*	*24.7*	*13.5*	294, 275, 324
TA 1537	**2-AA**	2.5 µg	*217.7*	*58.1*	*9.7*	202, 169, 282
TA 98	**2-AA**	2.5 µg	*1375.3*	*278.3*	*30.8*	1054, 1537, 1535
TA 100	**2-AA**	2.5 µg	*1618.3*	*67.0*	*13.2*	1621, 1550, 1684
WP2 uvrA	**2-AA**	10.0 µg	*247.7*	*33.3*	*3.7*	237, 221, 285

Key to Positive Controls

2-AA 2-aminoanthracene

Key to Plate Postfix Codes

P Precipitate
M Manual count

Study Name: 1152200
Experiment: 1152200 HV2 Pre
Assay Conditions:

Study Code: RCC-CCR 1152200
Date Plated: 01/04/2008
Date Counted: 04/04/2008

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**Tocotrienol**	33 µg	*23.0*	*1.7*	*1.2*	24, 21, 24
		100 µg	*15.7*	*3.8*	*0.8*	14, 20, 13
		333 µg	*18.0*	*5.3*	*0.9*	22, 20, 12
		1000 µg	*16.0*	*4.6*	*0.8*	21, 15, 12
		2500 µg	*17.0*	*2.0*	*0 9*	19 P, 17 P, 15 P
		5000 µg	*13.3*	*3 8*	*0.7*	16 P M, 9 P M, 15 P M
	Ethanol		*19.7*	*4.0*		19, 24, 16
	Untreated Control		*16.7*	*4.0*		21, 13, 16
TA 1537	**Tocotrienol**	33 µg	*7.7*	*1.5*	*0.5*	8, 9, 6
		100 µg	*10.3*	*3.8*	*0.7*	6, 12, 13
		333 µg	*5.7*	*1.2*	*0.4*	7, 5, 5
		1000 µg	*10.3*	*4.9*	*0.7*	16, 7, 8
		2500 µg	*10.0*	*2.6*	*0.7*	9 P, 13 P, 8 P
		5000 µg	*7.0*	*3.0*	*0.5*	4 P M, 10 P M, 7 P M
	Ethanol		*14.3*	*4.0*		12, 12, 19
	Untreated Control		*13.7*	*5.0*		19, 13, 9
TA 98	**Tocotrienol**	33 µg	*32.7*	*2.5*	*1.1*	35, 33, 30
		100 µg	*34.3*	*3.8*	*1 1*	30, 36, 37
		333 µg	*34.0*	*5.6*	*1.1*	40, 29, 33
		1000 µg	*33.0*	*4.0*	*1.1*	37, 29, 33
		2500 µg	*27.0*	*2.6*	*0.9*	29 P, 24 P, 28 P
		5000 µg	*32.7*	*2.5*	*1.1*	33 P M, 30 P M, 35 P M
	Ethanol		*31.0*	*3.0*		31, 34, 28
	Untreated Control		*35.3*	*12.9*		26, 50, 30
TA 100	**Tocotrienol**	33 µg	*121.7*	*8.5*	*1.0*	128, 125, 112
		100 µg	*126.7*	*7.2*	*1.1*	123, 135, 122
		333 µg	*118.3*	*9.3*	*1.0*	129, 112, 114
		1000 µg	*131.3*	*9.1*	*1.1*	138, 135, 121
		2500 µg	*126.0*	*11.1*	*1 0*	128 P, 136 P, 114 P
		5000 µg	*118.7*	*10.7*	*1 0*	121 P M, 128 P M, 107 P M
	Ethanol		*120.3*	*7.8*		118, 129, 114
	Untreated Control		*132.7*	*16.9*		147, 137, 114
WP2 uvrA	**Tocotrienol**	33 µg	*47.3*	*3.5*	*1 0*	51, 47, 44
		100 µg	*49.3*	*8.3*	*1 0*	52, 56, 40
		333 µg	*52.7*	*9.0*	*1 1*	44, 52, 62
		1000 µg	*54.0*	*3.0*	*1.1*	57, 51, 54
		2500 µg	*49.3*	*5 1*	*1.0*	45 P, 55 P, 48 P
		5000 µg	*52.0*	*3 6*	*1.1*	53 P M, 48 P M, 55 P M
	Ethanol		*48.0*	*1 0*		47, 49, 48
	Untreated Control		*55 3*	*1 2*		56, 54, 56

Key to Plate Postfix Codes

P	Precipitate
M	Manual count

Study Name: 1152200
Experiment: 1152200 HV2 Pre
Assay Conditions:

Study Code: RCC-CCR 1152200
Date Plated: 01/04/2008
Date Counted: 04/04/2008

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**NaN3**	10 µg	*2130.7*	*39.1*	*108.3*	2101, 2175, 2116
TA 1537	**4-NOPD**	50 µg	*90 7*	*13.9*	*6.3*	87, 79, 106
TA 98	**4-NOPD**	10 µg	*392.0*	*7.0*	*12.6*	392, 399, 385
TA 100	**NaN3**	10 µg	*2396.3*	*28.7*	*19.9*	2423, 2366, 2400
WP2 uvrA	**MMS**	3.0 µL	*416.7*	*12.5*	*8.7*	404, 417, 429

Key to Positive Controls

NaN3	sodium azide
4-NOPD	4-nitro-o-phenylene-diamine
MMS	methyl methane sulfonate

Study Name: 1152200
Experiment: 1152200 HV2 Pre
Assay Conditions:

Study Code: RCC-CCR 1152200
Date Plated: 01/04/2008
Date Counted: 04/04/2008

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**Tocotrienol**	33 µg	*31.0*	*3.6*	*1.3*	35, 30, 28
		100 µg	*16.7*	*0.6*	*0.7*	16, 17, 17
		333 µg	*17.7*	*11.0*	*0.7*	29, 17, 7
		1000 µg	*25.0*	*7.9*	*1.0*	31, 16, 28
		2500 µg	*27.7*	*4.7*	*1.1*	33 P M, 26 P M, 24 P M
		5000 µg	*23.3*	*1.5*	*0.9*	23 P M, 22 P M, 25 P M
	Ethanol		*24.7*	*4.2*		28, 26, 20
	Untreated Control		*22.7*	*1.5*		24, 23, 21
TA 1537	**Tocotrienol**	33 µg	*12.3*	*4.7*	*1.0*	16, 14, 7
		100 µg	*13.3*	*0.6*	*1.1*	13, 13, 14
		333 µg	*13.0*	*1.0*	*1.1*	13, 12, 14
		1000 µg	*16.0*	*6.0*	*1.3*	10, 22, 16
		2500 µg	*12.0*	*1.0*	*1.0*	11 P M, 13 P M, 12 P M
		5000 µg	*8.7*	*0.6*	*0.7*	9 P M, 8 P M, 9 P M
	Ethanol		*12.0*	*3.0*		15, 9, 12
	Untreated Control		*16.7*	*2.1*		19, 15, 16
TA 98	**Tocotrienol**	33 µg	*44.7*	*10.0*	*1.0*	55, 44, 35
		100 µg	*45.3*	*10.6*	*1.0*	34, 55, 47
		333 µg	*61.7*	*13.7*	*1.4*	64, 47, 74
		1000 µg	*51.0*	*5.6*	*1.2*	52, 45, 56
		2500 µg	*55.7*	*7.5*	*1 3*	63 P M, 56 P M, 48 P M
		5000 µg	*37.0*	*3.6*	*0.8*	36 P M, 41 P M, 34 P M
	Ethanol		*43.7*	*3.1*		47, 41, 43
	Untreated Control		*45.0*	*12.5*		55, 49, 31
TA 100	**Tocotrienol**	33 µg	*137.7*	*10.5*	*0.9*	148, 138, 127
		100 µg	*136.3*	*5.9*	*0.9*	143, 132, 134
		333 µg	*136.3*	*7 1*	*0 9*	135, 130, 144
		1000 µg	*131.7*	*9.0*	*0.8*	126, 127, 142
		2500 µg	*146.0*	*14.2*	*0 9*	135 P M, 162 P M, 141 P M
		5000 µg	*130.3*	*6.7*	*0.8*	127 P M, 138 P M, 126 P M
	Ethanol		*155.3*	*13.6*		171, 147, 148
	Untreated Control		*169.0*	*13.5*		170, 155, 182
WP2 uvrA	**Tocotrienol**	33 µg	*46.3*	*4.9*	*0.7*	43, 44, 52
		100 µg	*64 3*	*6.8*	*1.0*	72, 62, 59
		333 µg	*70.3*	*11.8*	*1.1*	64, 63, 84
		1000 µg	*61.7*	*14.4*	*1.0*	70, 45, 70
		2500 µg	*68.7*	*15.3*	*1.1*	86 P M, 63 P M, 57 P M
		5000 µg	*62.7*	*5.0*	*1 0*	58 P M, 62 P M, 68 P M
	Ethanol		*62.7*	*7.2*		58, 59, 71
	Untreated Control		*62.0*	*2 6*		64, 59, 63

Key to Plate Postfix Codes

P	Precipitate
M	Manual count

Study Name. 1152200
Experiment: 1152200 HV2 Pre
Assay Conditions:

Study Code. RCC-CCR 1152200
Date Plated: 01/04/2008
Date Counted: 04/04/2008

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**2-AA**	2.5 µg	*274.7*	*48.9*	*11.1*	249, 331, 244
TA 1537	**2-AA**	2.5 µg	*146.7*	*16.3*	*12 2*	129, 150, 161
TA 98	**2-AA**	2 5 µg	*821.3*	*72.9*	*18.8*	802, 760, 902
TA 100	**2-AA**	2 5 µg	*1604.7*	*79.1*	*10.3*	1593, 1532, 1689
WP2 uvrA	**2-AA**	10.0 µg	*316.3*	*8 4*	*5.0*	312, 311, 326

Key to Positive Controls

2-AA 2-aminoanthracene

TEST REPORT: 57Q0705291-02-00

Date: 19 SEP 2008 Tel: +65 68851323 Fax: +65 67784301

Client's Ref: Email: yang.li@tuv-sud-psb.sg

Note: This report is issued subject to TUV SUD PSB's "Terms and Conditions Governing Technical Services" The terms and conditions governing the issue of this report are set out as attached within this report

PSB Singapore

Choose certainty.
Add value.

1 GENERAL

1.1 STUDY TITLE

In vitro bacterial reverse mutation (Ames) study of Super T25 Tocotrienol concentrates

1.2 TEST ITEM IDENTIFICATION

Test Item Name	Super T25
Lot No	BST247/248-65-AM0808
Sterilization Condition	Non-sterile
Quantity	150g
Expiry Date	Jan 2010

1.3 REFERENCE ITEM IDENTIFICATION

No reference item was used in this study.

1.4 CHARACTERISATION OF TEST ITEM

The content (wt/wt%) of the test item is stated as follows:

Active ingredient	Content, wt/wt%
Total Tocopherol & Tocotrienols	25.1
d-alpha- Tocopherol	5.2
d-alpha- Tocotrienol	6.7
d-beta- Tocotrienol	0.5
d-gamma- Tocotrienol	8.7
d-delta- Tocotrienol	4.0

The certificate of analysis of the test item is provided by the sponsor

Purity 25% (declared)

Color/ state Reddish colored oil

2 SPONSOR

Super Vitamins Sdn Bhd
P.O. Box No. 1, 81757 Masai,
Johor, Malaysia

Attn: Ms Fang Yin Hui

3 TESTING FACILITY, TESTING SITES AND STAFF

3.1 TESTING FACILITY

Chemical and Materials
Testing Services
TÜV SÜD PSB Pte Ltd
No 1 Science Park Drive
Singapore 118221

3.2 STAFF

Study Director	Ms Li Yang
Study Personnel	Ms Seow Hwee Teng Ms Ng Yeng Ting
The above staff are located at	Chemical & Materials Testing Services TÜV SÜD PSB Pte Ltd 1 Science Park Drive Singapore 118221

4 STUDY SCHEDULE AND GUIDELINES

4.1 STUDY SCHEDULE

Experiment commencement date	30 Jul 2008
Experiment completion date	04 Aug 2008

4.2 STUDY GUIDELINES

OECD Guideline for testing of chemicals 471: Bacterial reverse mutation test. Adopted: 1997-07-21.

5 QUALITY ASSURANCE STATEMENT

This study was audited by Quality Assurance personnel of Chemical and Materials Testing Services as follows:

5.1 Study plan was audited on 29 Jul 2008 and the audit report was submitted to Study Director and Test Facility Management on the same day.

5.2 A study-based audit was conducted on 01 Aug 2008 and the audit report was submitted to Study Director and Test Facility Management on the same day.

5.3 Audit of the raw data and study report was conducted on 19 Sep 2008 and the audit report was submitted to Study Director and Test Facility Management on the same day.

The final report has been found to reflect the raw data obtained.

(b) (6)

DR LI SIHAI
QUALITY ASSURANCE
CHEMICAL AND MATERIALS
TESTING SERVICES

19 Sep 2008
DATE

6 STATEMENT OF COMPLIANCE

6.1 The procedure of the study complies with the study plan as agreed by the sponsor. The conclusion of the test report is based on the raw data obtained from the study.

6.2 This study is conducted in accordance with Organisation for Economic Co-operation and Development (OECD) Environmental Health and Safety Publications ENV/MC/CHEM(98)17, Series on Principles of Good Laboratory Practice and Compliance Monitoring No.1, OECD Principles of Good Laboratory Practice (as revised in 1997), Paris 1998.

(b) (6)

19 Sep 2008

MS LI YANG
STUDY DIRECTOR
CHEMICAL AND MATERIALS
TESTING SERVICES

DATE

7 MATERIALS AND METHODS

7.1 PREPARATION OF TEST SUBSTANCES AND CONTROLS

7.1.1 The test item was used directly as test substance without pre-treatment.

7.1.2 Negative control item was palm oil which is used as vehicle for the test substance. The palm oil used in this study was provided by the sponsor

7.1.3 Preparation of positive control
The positive control substances used in the study were sodium azide (CAS No 26628-22-8) at 5 µg/plate and ICR 191 (CAS No 17070-45-0) at 5 µg/plate in the absence of S9 mixture; Benzopyrene (CAS No 50-32-8) at 2 µg/plate was used as positive control in the presence of S9 fraction at the concentration of 10%

Metabolic activation	Mutagen	Concentration
Without S9	Sodium azide (CAS No 26628-22-8)	5 µg/ plate
Without S9	ICR 191 (CAS No 17070-45-0)	5 µg/ plate
With S9	Benzopyrene (CAS No 50-32-8)	2 µg/ plate

7.2 TEST SYSTEM AND TEST CONDITIONS

7.2.1 Test system

7.2.1.1 The test systems used were:

a) *Salmonella typhimurium* TA 1535 (ATCC 29629)
b) *Salmonella typhimurium* TA 1537 (ATCC 29630)
c) *Salmonella typhimurium* TA 100 (NBRC 14194)

The three test systems were purchased from American Type Culture Collection (ATCC) and NITE Biological Resource Centre (NBRC), Japan.

7.2.1.2 In the presence of substances that induce mutations which revert mutations present in these amino acid-requiring strains, the capability of the bacteria to synthesize the essential amino acid will be restored. The revertant or mutant bacteria can be detected by their ability to grow in the absence of the amino acid required by the parent strain.

7.2.1.3 All of the three test systems were maintained using bacteria broth containing $MgSO_4 \cdot 7H_2O$ 1g/L, yeast extract 2g/L and polypeptone 10g/L as recommended by ATCC and NBRC.

7.2.2 Bacteria exposure and counting procedure

7.2.2.1 Triplicate plates were used for each concentration of test substance, negative control and positive control respectively. The minimal glucose medium with 1 x Vogel-Bonner (VB) medium E and 2% glucose was used for each plate. The total aliquots required were calculated according to the total number of plates needed for the study.

7.2.2.2 The study was conducted both with and without the presence of metabolic activation system. A co-factor supplemented post-mitochondrial fraction (S9) from the liver of Aroclor 1254 induced Sprague-Dawley rats was used as metabolic activation system.

7.2.2.3 Based on plate incorporation method, 2 ml of molten top agar were dispensed to each culture tube held at 45 to 50°C. 0.1 ml of a fresh over-night culture of the test strain, 0.1 ml or less of the test substance / negative control / positive control, 0.2 ml Biotin/Histidine and 0.5 ml of 0.2M PBS (or 0.5 ml of cold S9 mixture) were then dispensed to each agar tube. Controls with and without S9 were prepared. The concentration of S9 fraction in S9 mixture used in each plate was 10%.

7.2.2.4 Three concentrations of the test substance were used in the study. The three concentrations used were 20.0 mg / plate, 6.1 mg / plate and 1.8 mg / plate. The selection of the concentrations of the test substance followed OECD Guideline 471. The recommended maximum concentration for non-cytotoxic substance was 5 mg / plate, the dose interval was approximately 3.3 (half log). Since the purity of the test substance was 25% as declared by the sponsor, the maximum concentration selected in this study was 20 mg / plate. The concentration of negative control used in the study was 20 mg / plate.

7.2.2.5 The mixture of top agar were vortexed and then poured on to a minimal glucose agar plate. The plates were allowed to harden and the inverted plates were incubated at 37°C in a dark and vented incubator. After 48-hr to 72-hr incubation, background lawn of the plates were checked and the revertant colonies were scored.

8 TEST RESULTS

8.1 The number of revertant colonies per plate of the extracts and controls using *Salmonella typhimurium* TA 1535 (ATCC 29629) are recorded as follows:

Metabolic activation	Test substance/ controls	No of revertant colonies in Plate 1	No of revertant colonies in Plate 2	No of revertant colonies in Plate 3	Average number of revertant colonies and standard deviation	Student's t-test
Without S9 mixture	Test substance 20mg/plate	10	5	7	7 ± 3	0.51
	Test substance 6.1 mg/plate	9	10	11	10 ± 1	0.94
	Test substance 1.8 mg/plate	7	8	11	9 ± 2	0.71
	Negative control 20 mg/plate	8	15	18	14 ± 5	-
	Positive control 5 µg/ plate	892	860	958	903 ± 50	-
With S9 mixture	Test substance 20mg/plate	16	22	18	19 ± 3	0.13
	Test substance 6.1 mg/plate	22	26	22	23 ± 2	0.73
	Test substance 1.8 mg/plate	16	18	17	17 ± 1	0.04
	Negative control 20 mg/plate	29	23	21	24 ± 4	-
	Positive control 5 µg/ plate	19	25	24	23 ± 3	-

8.1.1 Student's t-test was used to assess whether there was any significant difference between the results of the test substance and its negative control.

8.1.2 There was no significant difference between the number of revertant colonies in the test substance and its concurrent negative control without S9 metabolic activation at $p = 0.05$.

8.1.3 There was no significant difference between the number of revertant colonies in the test substance and its concurrent negative control with S9 metabolic activation at $p = 0.05$, except that the result of test on test substance at 1.8 mg/plate was significantly lower than its negative control at $p = 0.05$.

8.1.4 However, the number of revertant colonies per plate of the positive control with the presence of metabolic activation was lower than expected. This is likely to be an isolated incidence.

8.2 The number of revertant colonies per plate of each extract and control using *Salmonella typhimurium* TA 1537 (ATCC 29630) are noted as follows:

Metabolic activation	Test substance/ controls	No of revertant colonies in Plate 1	No of revertant colonies in Plate 2	No of revertant colonies in Plate 3	Average number of revertant colonies and standard deviation	Student's t-test
Without S9 mixture	Test substance 20mg/plate	8	11	8	9 ± 2	0.16
	Test substance 6.1 mg/plate	0	12	9	7 ± 6	0.87
	Test substance 1.8 mg/plate	8	12	12	11 ± 2	0.07
	Negative control 20 mg/plate	8	7	4	6 ± 2	-
	Positive control 5 µg/ plate	14	3064	2946	2008 ± 1728	-
With S9 mixture	Test substance 20mg/plate	16	15	12	14 ± 2	0.79
	Test substance 6.1 mg/plate	14	14	17	15 ± 2	0.59
	Test substance 1.8 mg/plate	21	16	18	18 ± 3	0.13
	Negative control 20 mg/plate	14	10	17	14 ± 4	-
	Positive control 5 µg/ plate	45	42	53	47 ± 6	-

8.2.1 Student's t-test was used to assess whether there was any significant difference between the results of the test substance and its negative control.

8.2.2 There was no significant difference between the number of revertant colonies in the test substance and its concurrent negative control without S9 metabolic activation at p = 0.05.

8.2.3 There was no significant difference between the number of revertant colonies in the test substance and its concurrent negative control with S9 metabolic activation at p = 0.05

8.3 The number of revertant colonies per plate of each extract and control using *Salmonella typhimurium* TA 100 (NBRC 14194) are noted as follows:

Metabolic activation	Test substance/ controls	No of revertant colonies in Plate 1	No of revertant colonies in Plate 2	No of revertant colonies in Plate 3	Average number of revertant colonies and standard deviation	Student's t-test
Without S9 mixture	Test substance 20mg/plate	134	148	111	131 ± 19	0.01
	Test substance 6.1 mg/plate	113	138	120	124 ± 13	0.00
	Test substance 1.8 mg/plate	141	134	157	144 ± 12	0.00
	Negative control 20 mg/plate	200	191	183	191 ± 9	-
	Positive control 5 µg/ plate	712	924	928	855 ± 124	-
With S9 mixture	Test substance 20mg/plate	158	183	206	182 ± 24	0.72
	Test substance 6.1 mg/plate	161	231	180	191 ± 36	0.93
	Test substance 1.8 mg/plate	230	220	247	232 ± 14	0.02
	Negative control 20 mg/plate	206	185	175	189 ± 16	-
	Positive control 5 µg/ plate	294	360	536	397 ± 125	-

8.3.1 Student's t-test was used to assess whether there was any significant difference between the results of the test substance and its negative control.

8.3.2 The number of revertant colonies in the test substance was significantly lower than its concurrent negative control without S9 metabolic activation at $p = 0.05$.

8.3.3 There was no significant difference between the number of revertant colonies in the test substance and its concurrent negative control with S9 metabolic activation at $p = 0.05$, except that the result of test on test substance at 1.8 mg/plate was not significantly lower than its negative control at $p = 0.05$.

9 DISCUSSION

9.1 In the study using 20mg/plate, 6.1 mg/plate and 1.8 mg/pate of Super T25 as test substance and *Salmonella typhimurium* TA 1535 (ATCC 29629) as test system, there was no significant increase in the number of revertant colonies in the test substance as compared to its negative control both with and without metabolic activation by S9.

9.2 In the study using 20mg/plate, 6.1 mg/plate and 1.8 mg/pate of Super T25 as test substance and *Salmonella typhimurium* TA 1537 (ATCC 29630) as test system, there was no significant increase in the number of revertant colonies in the test substance as compared to its negative control both with and without metabolic activation by S9.

9.3 In the study using 20mg/plate, 6.1 mg/plate and 1.8 mg/pate of Super T25 as test substance and *Salmonella typhimurium* TA 100 (NBRC 14194) as test system, there was no significant increase in the number of revertant colonies in the test substance as compared to its negative control both with and without metabolic activation by S9.

9.4 Spontaneous mutant colonies were observed in negative control with and without the presence of S9 mixture.

10 CONCLUSION

10.1 From the above study, using 20 mg/plate, 6.1 mg/plate and 1.8 mg/pate of Super T25, Lot No: BST247/248-65-AM0808 as test substance, it is concluded that the test item is non-mutagenic to *Salmonella typhimurium* TA 1535 (ATCC 29629).

10.2 From the above study, using 20 mg/plate, 6.1 mg/plate and 1.8 mg/pate of Super T25, Lot No: BST247/248-65-AM0808 as test substance, it is concluded that the test item is non-mutagenic to *Salmonella typhimurium* TA 1537 (ATCC 29630).

10.3 From the above study, using 20 mg/plate, 6.1 mg/plate and 1.8 mg/pate of Super T25, Lot No: BST247/248-65-AM0808 as test substance, it is concluded that the test item is non-mutagenic to *Salmonella typhimurium* TA 100 (NBRC 14194).

11 DEVIATIONS OF THE STUDY PROCEDURES FROM THE STUDY PROTOCOL

There is no deviation of the study procedures to the study protocol

12 ARCHIVAL

The study protocol, study plan, study schedule, all the raw data of experiment, audit report of quality assurance unit and other related documentations and the final test report are stored in the archive of TÜV SÜD PSB Pte Ltd.

REMARKS:

The above test results relate to the sample of test item as received.

(b) (6)

MS SEOW HWEE TENG
STUDY SCIENTIST
CHEMICAL AND MATERIALS
TESTING SERVICES

(b) (6)

MS LI YANG
STUDY DIRECTOR
CHEMICAL AND MATERIALS
TESTING SERVICES

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January 2008

Pages 000183 - 000333 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

SUBMISSION END

Reference List for Industry Submission, GRN 000307

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Sen CK, Khanna S, Roy S. Tocotrienols in health and disease: The other half of the natural vitamin E family. Mol Asp Med. 2007;28:692-728.

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Khanna S, Patel V, Rink C, Roy S, Sen CK. Delivery of orally supplemented alpha-tocotrienol to vital organs of rats and tocopherol-transport protein deficient mice. Free Radical Biol Med. 2005a;39: 1310-1319.

AM

Mosley, Sylvester

From: Susan S Cho [susanscho1@yahoo.com]
Sent: Wednesday, January 13, 2010 3:10 PM
To: Mosley, Sylvester; Carlson, Susan; rosidah@mpob.gov.my
Subject: GRN 307 palm oil-derived tocols

Dear Dr. Mosley,

It was nice to meet you in December.

1. I would appreciate it if you would treat all the information submitted for the GRAS notification as non-confidential information.
Analytical data are not confidential information.

2. Please change the notifier from NutraSource to Malaysian Palm Oil Board (MPOB) Technical Advisory Services (TAS). NutraSource is a consulting firm. The industry members of MPOB produces palm oil derived-tocols.
Ms. Rosidah Radzian (phone: 202-572-9768) at MPOB should be the contact at MPOB TAS. Please address all the correspondences to Rosidah.

3. I have changed the e mail address from to SusanSCho1@yahoo.com.

Thank you very much.

Sincerely,

Susan Cho, Ph.D.
NutraSource, Inc.

)

AM

From: Susan S Cho
To: Mosley, Sylvester;
Subject: GRN 307 palm oil-derived tocols
Date: Wednesday, February 10, 2010 9:56:00 AM

Page 10-

It should have been 'water dispersible', not "non-water dispersible'
May I send you 3 copies of corrected page?
I can not go out today. But I can hand deliver to you on Monday.
I apologize for the inconvenience.

Thank you very much.

Sincerely,

Susan Cho, Ph.D.

NutraSource, Inc.

)

AM

From: Susan S Cho
To: Mosley, Sylvester;
Subject: Revised page 26 for GRN307
Date: Thursday, March 25, 2010 6:22:29 PM
Attachments: TRF GRAS notice page 26 revised 3-25-10.doc

Dear Dr. Mosley,

I have revised page 26 to capture your points.
Please let me know if you have further questions.
Thank you.

Sincerely,

Susan Cho, Ph.D.
NutraSource
301-875-6454

From: "Mosley, Sylvester" <Sylvester.Mosley@fda.hhs.gov>
To: Susan S Cho <susanscho1@yahoo.com>
Sent: Tue, March 23, 2010 3:05:43 PM
Subject: Questions for GRN302

Good afternoon Dr. Cho,

I wanted to follow up our phone conversation with the written questions that I alluded to on the phone.

1. We note that in your certificate of analyses your products appear to be colored (red, amber, and orange). We request that the notifier present its view on whether any of the intended uses of palm-oil derived tocols would be exempt from the definition of color additive.
2. We note that on page 26 in the intended use section and in table 5 on page 27, there is confusion with the intended uses of fats and oils. We request that the notifier rewrite the intended uses on page 26 and in table 5 on page 27 to mirror one another. Furthermore, we request that you are more explicit in terms of designer fats and oils.

If you have any questions please feel free to ask. I will be out of the office through Monday, March 29th. I will be checking my email during the evenings on Wednesday and Thursday.

Thanks,

Sylvester L. Mosley, Ph.D.
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety-CFSAN
U.S. Food and Drug Administration
5100 Paint Branch Parkway (HFS-255)
College Park, MD 20740-3835
Phone: 301-436-1333
Fax: 301-436-2965
Email: Sylvester.Mosley@fda.hhs.gov

Conclusion of the expert panel:
Generally recognized as safe (GRAS) determination for the addition of palm tocotrienol-rich fraction (TRF) to foods

August 2009

CONCLUSION

We, the undersigned expert panel members, Susan Cho, Ph.D., Michael Falk, Ph.D., and George Fahey, Ph.D., have individually and collectively critically evaluated the materials summarized in the palm TRF GRAS report, and conclude that palm TRF is safe and GRAS for its intended use in foods.

There is broad-based and widely disseminated knowledge concerning the chemistry of tocotrienols (T3) and tocopherols (T). Vitamin E is a generic name for the family of eight major compounds that have been found to possess vitamin E activity. These compounds are α-, β-, γ-, and δ-T; and α -, β-, γ-, and δ-T3, and are often called tocols. They cannot be synthesized in the body; thus, vitamin E must be obtained from the diet. Vitamin E deficiency results in serious health hazards. Symptoms caused by α -T deficiency can be alleviated by T or T3. Both T3 and T possess antioxidant activities, although T3 has much stronger antioxidant activities against lipid peroxidation than T. Palm oil and rice bran oil represent two major nutritional sources of natural T3. The TRF described in this self-affirmation is prepared from palm oil.

Palm TRF (a mixture of α -, β-, γ-, and δ-T3 with an average α-T content of 20-30% of total tocols) proposed for use in this food product is well characterized and free from chemical and microbial contamination. Palm TRF will be used as an ingredient in foods and beverages as a source of natural vitamin E and antioxidants.

Intended food applications include fats or oils, such as salad dressing, mayonnaise, margarine, and spread, potato chips and salty snacks, bakery products, ready-to-eat cereals, meal replacement and other functional beverages, meatless soups, soup mix meatless gravies, sauces, pasta sauces, processed juices, processed fruits, juice drinks and punch, frozen dairy desserts, yogurt, pudding, gelatin products, chocolate milk, flavored milk, soy milk and products, chocolate, candy, confectionary, sweets, beverages, and chewing gum.

Assuming 20% of food products will be used under the intended use, the 90th percentile daily consumption under proposed new food use of T3 would result in 9.4 mg/d (or 0.68 mg/kg body weight (BW)/d) in young children aged 1-3 yr and 17.4 mg/d (or 0.35 mg/kg BW/d) for the population aged 1 yr and older. These levels represent more than 100x below the no-observed-adverse-effect level (NOAEL) values that have been found from sub-chronic and chronic toxicity studies in rats. An assumption that 20 % of the population are consumers of TRF leads to dietary α-T exposure estimates of approximately 6.4 mg/d (or 0.49 mg/kg BW/d) for children aged 1-3 yr and 14.6 mg/d (or 0.27 mg/kg BW/d) for the population aged 1 yr and older at the 90th

percentile. These levels are far below the tolerable upper intake levels of α-T (200 mg/d for children aged 1-3 yr and 1000 mg/d for adults) established by the U.S. Institute of Medicine.

Currently, α-T (as a nutrient and as a chemical preservative), tocopherols, and α-T acetate (as a nutrient) are already listed as GRAS ingredients under the 21 CFR Part 184 and the 21 CFR Part 182. Dietary supplements containing T3 have been on the market in the U.S. as dietary supplements since 1999 under the *Dietary Supplements Health and Education Act of 1994* (DSHEA). Due to the current GRAS approval status of T, this GRAS self-affirmation is more focused on the review of T3.

Palm TRF has a long history of safe use as a natural vitamin E and as an antioxidant in foods. There are no indications of significant adverse effects related to palm TRF in the publicly available literature. The proposed food use results in exposure at levels significantly below those associated with any adverse effects. Therefore, not only is the proposed use of palm TRF safe within the terms of the Federal Food, Drug, and Cosmetic Act (meeting the standard of reasonable certainty of no harm), but because of this consensus among experts, it is also *Generally Recognized as Safe* (GRAS) according to Title 21 Code of Federal Regulations (21 CFR).

Susan Cho, PhD
President, NutraSource, Inc., Clarksville, MD 21029

Signature: _____ Date: _____

Michael Falk, Ph.D.
Executive Director, Life Science Research Organization (LSRO), Bethesda, MD 20814

Signature: _____ Date: _____

George C. Fahey, Jr., Ph.D.
Professor, University of Illinois, Urbana-Champaign, IL 61801

Signature: _____ Date: _____

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Palm-oil derived tocols

I. Identity of Substance

A. Common or trade name: Palm TRF or T3 concentrates are from palm oil sources (the content of alpha T is approximately 20-25% of the tocols) that are manufactured by the industry members of Malaysian Palm Oil Board (MPOB), such as Carotech, Davos, Carotino, Sime Darby Bioganics, and SuperVitamins. Specific trade names include, but are not limited to, the following:

1) Carotech products such as
   Tocomin® 30% - Palm TRF, 30% Oil Suspension,
   Tocomin® 50% - Palm TRF, 50% Oil Suspension,
   Tocomax™ 20% - Palm TRF,
   20% Cold Water Dispersible Powder,
   Tocobeads® 7.5% - Palm TRF,
   7.5% Beadlets, Tocosem™ 1.5% - Palm TRF,
   1.5% Water Soluble Emulsion,
   Tocomin® Suprabio™ 20% - Palm TRF,
   20% Bio-enhanced Suspension, and
   Tocomin® Suprabio™ 25% - Palm TRF, 25% Bio-enhanced Suspension;

2) Davos palm TRF products, such as
   Tocotrienol Concentrate DVL 30,
   Tocotrienol Concentrate DVL 50,
   Tocotrienol Concentrate DVL 70,
   Tocotrienol Powder DVP 7.5,
   Tocotrienol Powder DVP 10,
   Tocotrienol Powder DVP 20,
   Tocotrienol Powder DVP 30, and
   Water Soluble (WS) Tocotrienol 1.5%;

3) Carotino TRF oil suspension products, such as
   Carotino TRF 10%,
   Carotino TRF 20%,
   Carotino TRF 50%, and
   Carotino TRF 80%;

4) SD Bioganic TRF products, such as
   Gold Tri E30,
   Gold Tri E50,
   Gold Tri E70,
   Gold Tri E20 powder,

Gold Tri E30 powder,
Bio E Tri E 30 N (emulsion), and
Bio E Tri E 20 N (emulsion);

5) SuperVitamin TRF oil suspension products, such as
Super T25,
Super T30,
Super T50,
Super T80,
Super T Powder 20%, and
Super T Powder 25%.

B. Standards of identity: We note that an ingredient that is lawfully added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity that is located in Title 21 of the Code of Federal Regulations.

C. Background

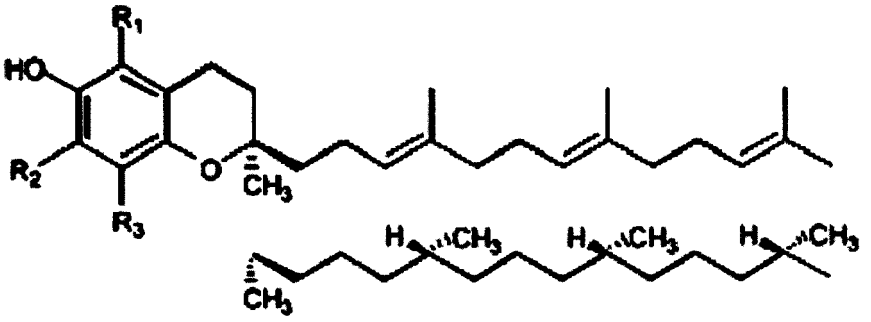
Tocotrienols are the primary form of vitamin E in the seed endosperm of most monocots, including agronomically important cereal grains such as wheat, rice, and barley. Palm oil contains significant quantities of T3 and T (Sundram et al. 2003). Crude palm oil extracted from the fruits of *Elaeis guineensis* particularly contains a high amount of T3 (up to 800 mg/kg), mainly consisting of γ-T3, α-T3, and α-T. The distribution of vitamin E in palm oil is 20-30% T and 70-80% T3 (Sundram et al. 2003). The objective of this review is to highlight the safety and potential significance of the T3.

D. Chemistry and structure of T3

Tocopherols and T3 share common structural features of a chromanol ring and a side-chain at the C-2 position (Nesaretnam et al. 2008). The difference between T and T3 is that the former contains a saturated phytyl tail, whereas the latter bears an unsaturated isoprenoid side chain, i.e., three all trans double-bonds in the hydrocarbon tail (Figure 1; Tasaki et al. 2008). Each subgroup of vitamin E contains several isoforms. Chemical formulas and molecular weights (M.W.) of 8 isomers are summarized in Table 1. Palm TRF is a mixture of T3 and α-T.

Palm-oil derived tocols

Tocotrienol isoprenoid side chain



	R_1	R_2	R_3
α:	CH_3	CH_3	CH_3
β:	CH_3	H	CH_3
γ:	H	CH_3	CH_3
δ:	H	H	CH_3

Tocopherol phytylside chain

T3 isoforms

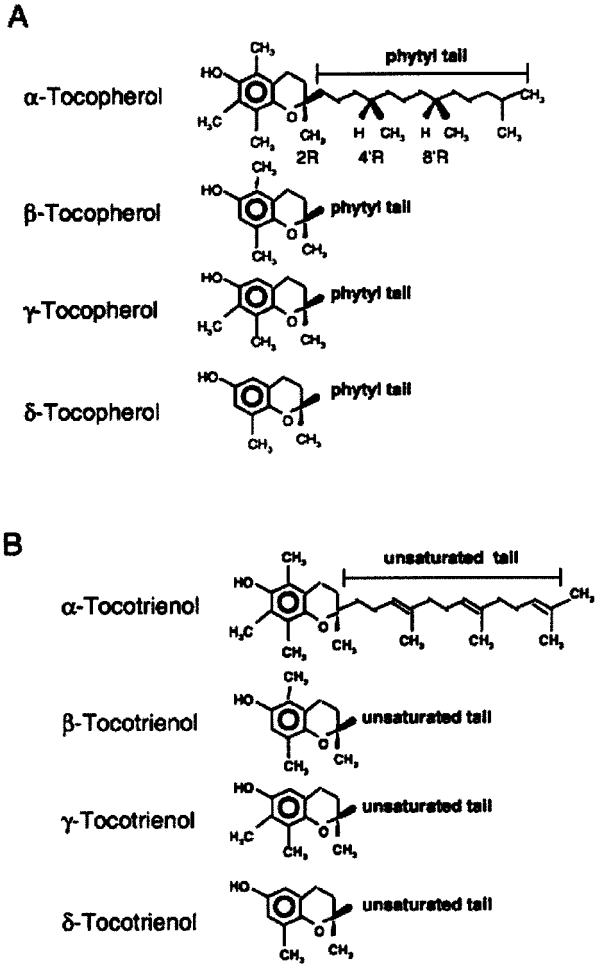


Figure 1. Structure of vitamin E stereoisomers. Tocotrienols consist of a chromanol nucleus and a lipophilic isoprenoid chain. Tocopherols have phytyl side chain. Methyl group placement in the naturally occurring isoforms α-, β-, γ-, and δ- is as indicated in the box (Adopted from Akoh and Min 2008).

Table 1. Chemical formulas, molecular weights, and CAS numbers of 8 forms of vitamin E

Compound	Chemical Formula	MW	CAS No.
α-tocotrienol	$C_{29}H_{44}O_2$	424	58864-81-6
β-tocotrienol	$C_{28}H_{42}O_2$	410	490-23-3
γ-tocotrienol	$C_{28}H_{42}O_2$	410	14101-61-2
δ-tocotrienol	$C_{27}H_{40}O_2$	396	25612-59-3
α-tocopherol	$C_{29}H_{50}O_2$	430	59-02-9
β-tocopherol	$C_{28}H_{48}O_2$	416	148-03-8
γ-tocopherol	$C_{28}H_{48}O_2$	416	7616-22-0
δ-tocopherol	$C_{27}H_{46}O_2$	402	119-13-1

Adopted from European Food Safety Authority (EFSA) 2008

E. Manufacturing Process

The industry members of MPOB describe their manufacturing process as follows:

1. TRF liquid (or TRF oil suspension):

(1) The starting raw material is crude palm oil, an edible vegetable oil extracted from the mesocarp of the oil palm. Crude palm oil is naturally reddish in color because it contains a high amount of natural mixed carotenoids. Palm TRFs (oil suspensions) are manufactured from crude palm oil using a trans-esterification reaction, followed by a multi-stage molecular distillation process.

(2)Extraction and Reaction Process: Crude palm oil contains the highest natural level of mixed carotenoids and palm TRF in nature (500 part per million (ppm) and 800 ppm of carotenoids and TRF, respectively). In addition to these two phytonutrients, there are other minor components naturally found in palm oil such as sterols, squalene, and Co-Q10. These phytonutrients (minor components) make up about 1% in total of the oil. The remaining 99% is basically the oil (triglycerides). A patented process (US Patent No. 5,157,132) extracts and concentrates this 1% of minor components in the fruits of oil palm. In the process, crude palm oil is converted to methyl esters and glycerol through

transesterification via the addition of methanol in the presence of sodium methoxide (catalyst). The minor components such as the mixed carotenoids, vitamin E, squalene, sterol, etc. are not involved in this reaction and are not changed chemically or structurally. The ratio, chemical structure, and characteristics remain the same in the starting natural crude palm oil. The reaction process targets and involves only the oil part (triglycerides).

(3) Molecular Distillation Process: Molecular distillation is a unique and well-known process for distilling heat-sensitive materials such as essential oils and antioxidants. It uses an extremely high vacuum and low temperature technology to distill materials such as methyl esters and antioxidants. In the molecular distillation process and with such an extreme vacuum, all the methyl esters will be distilled out to leave a concentrated residue of phytonutrients (including carotenoids and vitamin E). With the vigorous hot water wash, vacuum drying, and molecular distillation, any residue or unreacted compounds are taken out. The concentrated residue then is subjected again to molecular distillation (varying vacuum and temperature) to recover the individual components – mainly TRF, mixed carotenes, and phytosterols. To manufacture high concentrations and purities of T3, conventional high performance liquid chromatography (HPLC), involving an organic solvent mobile phase (a mixture of hexane, ethyl acetate, and methanol) and a stationary phase, silica, can be used. Then, ultra high vacuum rotary evaporation removes the solvents used in the manufacturing process.

Alternatively, the following process can be used: Crude palm oil is degummed by treating with food grade phosphoric acid to remove phospholipids. The degummed oil then is mixed with bleaching earth (activated calcium montmorillonite) to absorb pigments and particles as well as any metal contamination and oxidizing materials that

may be in the oil. The oil-earth mixture then is passed through filters where the spent earth is removed together with the precipitated materials from degumming. The bleached oil is first de-aerated and then collected separately as a process by-product. The T3 in the resultant material is concentrated using a multistage physical process without the use of solvents. To manufacture higher concentrations of T3, standard solvent-based chromatography may be used to further separate and fractionate the materials, thus increasing the concentration and purity.

2. Production of Palm TRF, Water Soluble Emulsion: TRF liquids (>50% tocols) are the starting materials for the formulation of water-soluble emulsions of palm TRF. Glycerine and a proprietary blend of emulsifiers, such as mono- and diglycerides, sorbitan (GRAS; 21CFR173.110 and 172.842), sugar esters, starch derivatives, and surfactants) are added into palm TRF liquids. The mixture then is homogenized into an emulsion.
3. **Non-water Dispersible Powder: TRF liquids (>30% tocols) are the starting materials for the formulation of a cold water dispersible powder of palm TRF.** A powder carrier (modified corn starch, maltodextrin, tapioca dextrin, corn starch, or sodium caseinate (GRAS; 21CFR182.1748)), with and without an anti-caking ingredient such as 5% calcium silicate), is added into palm TRF. The mixture is dried and milled or alternatively spray dried into a dry powder.
4. Production of Palm TRF, Beadlets: TRF liquids (>50% tocols) are the starting material for the formulation of palm TRF beadlets. Gelatin, corn starch, sucrose (GRAS; 21CFR184.1854), and ascorbyl palmitate (GRAS; 21CFR184.3149) are added into palm TRF liquids. The mixture is spray dried into dry beadlets.

5. Production of palm TRF, Bioenhanced Suspension: Palm TRF liquids (>50% tocols) are the starting material for the formulation of palm TRF, Bio-enhanced Suspension. A proprietary blend of emulsifiers (including glycerides and starch derivatives) are added into palm TRF liquids and undergo various physical processing such as high-speed stirring and homogenization to produce Tocomin® Suprabio™.

F. Specifications

Tables 2-1 to Table 2-31 list specifications of palm TRF. In general, specifications have the following common characteristics. (1) Palm TRF is a mixture of T3 and α-T and the content of α-T is 20-30% of the total tocols present in palm TRFs. For example, palm TRF of Carotech and Davos contain approximately 20% and 25-30% of α-T, respectively. (2) Any number in the product name indicates the concentration (as a percentage) of total tocols; for example, DVL 30 means the product contains 30% tocols and 70% (100%-30%) of other components. (3) The maximum moisture concentration is 0.5-1% in a liquid form of palm TRF. (4) Palm TRFs are available in many forms: liquid (oil suspension), powder, water-soluble emulsion, beadlets, and bio-enhanced suspension. (5) Among components other than T3-T, the liquid form contains mostly palm oil (GRAS; 21CFR184.1259). The powder form of palm TRF contains maltodextrin (GRAS; 21CFR184.1444), modified corn starch (GRAS; 21CFR172.892), cyclodextrin (GRAS; 21CFR170.36, GRN 000074), tapioca starch, or corn starch instead of palm oil. Water soluble TRF contains a mixture of water, sucrose (GRAS; 21CFR184.1854), propylene glycol (GRAS; 21CFR184.1666), glycerol (GRAS; 21CFR182.1320), palm oil, and sucrose monopalmitate (GRAS; 21CFR172.859), instead of palm oil. The emulsion form of palm TRF contains a mixture of glycerine (GRAS; 21CFR182.1320) and mono-and diglycerides instead of palm oil.

The beadlet form contains a mixture of gelatin, corn starch, sucrose, and ascorbyl palmitate (GRAS; 21CFR184.3149) instead of palm oil.

Palm TRFs also contain phytosterols and squalene as minor components. Vegetable oil sterol esters (including beta-sitosterol, campesterol, and stigmasterol; 21CFR170.36, GRN000048) and squalene (from olive oil and other oils; 21CFR170.36, GRN000048) are recognized as GRAS substances.

Unless noted otherwise, the palm TRFs have the following common specifications: peroxide value <10 meq/kg, lead <0.5 ppm, standard plate count <1000 CFU/g, yeast and mold <100 CFU/g, and *E. coli* and Salmonella-negative. Examples of analytical data for each T3 preparation are shown in the appendix.

Table 2-1. Specifications of Carotech Palm TRF - Tocomin® 30% (oil suspension)

Item	Minimum	Maximum
α-T3, %	6.5	9.0
β -T3, %	1.0	
γ-T3, %	12	14
δ-T3, %	3.3	5.0
α-T, %	6.0	9.0
Total T3 and α-T, %	30.0	
Palm oil, %		65.0
Moisture, %		1.0
Plant squalene, %	4.0	
Phytosterols, %	1.0	
Mixed carotene, %	0.03	

Table 2-2. Specifications of Carotech Palm TRF - Tocomin® 50% (oil suspension)

Item	Minimum	Maximum
α-T3, %	11.0	14.0
β -T3, %	1.5	
γ-T3, %	20.0	24.0
δ-T3, %	5.5	7.0
α-T, %	10.0	14.0
Total T3 and α-T, %	50.0	
Palm oil, %		39.0
Moisture, %		1.0
Plant squalene, %	8.0	
Phytosterols, %	3.0	
Mixed carotene, %	0.05	

Table 2-3. Specifications of Carotech Palm TRF - Tocomax™ 20% (powder)

Item	Minimum	Maximum
α-T3, %	4.5	6.0
β -T3, %	0.5	
γ-T3, %	8.0	10.0
δ-T3, %	2.0	3.0
α-T, %	4.0	6.0
Total T3 and α-T, %	20.0	
Palm oil, %	20.0	
Corn starch, %	52.0	
Moisture, %		8.0
Plant squalene, %	3.0	
Phytosterols, %	1.0	

Table 2-4. Specifications of Carotech Palm TRF - Tocomin Suprabio ™ 20% (bio-enhanced suspension)

Item	Minimum	Maximum
α-T3, %	4.4	6.0
β -T3, %	0.6	
γ-T3, %	8.0	9.6
δ-T3, %	2.2	2.8
α-T, %	4.0	5.6
Total T3 and α-T, %	20.0	
Palm oil, %	20.0	
Emulsifiers, plant-based, %	55.0	
Moisture, %		1.0
Plant squalene, %	3.0	
Phytosterols, %	1.5	
Mixed carotene, %	0.02	

Table 2-5. Specifications of Carotech Palm TRF - Tocomin Suprabio ™ 25% (bio-enhanced suspension)

Item	Minimum	Maximum
α-T3, %	5.5	7.0
β -T3, %	0.75	
γ-T3, 5	10.0	12.0
δ-T3, %	2.75	3.5
α-T, %	5.0	7.0
Total T3 and α-T, %	25.0	
Palm oil, %	25.0	
Emulsifiers, plant-based, %	44.0	
Moisture, %		1.0
Plant squalene, %	4.0	
Phytosterols, %	1.5	
Mixed carotene, %	0.02	

Plant based emulsifiers: Modified corn starch, maltodextrin, cyclodextrins, or corn starch.

Table 2-6. Specifications of Carotech Palm TRF - Tocosem ™ 1.5% (water-soluble emulsion)

Item	Minimum	Maximum
α-T3, %	0.3	0.36
β -T3, %	0.05	
γ-T3, %	0.5	0.52
δ-T3, %	0.35	0.36
α-T, %	0.28	0.29
Total T3 and α-T, %	1.48	
Glycerine, %	82.0	
Emulsifiers, plant-based, %	15.0	

Plant based emulsifiers: Modified corn starch, maltodextrin, cyclodextrins, or corn starch.

Table 2-7. Specifications of Carotech PalmTRF - Tocobeads® 7.5% (beadlets)

Item	Minimum	Maximum
α-T3, %	1.65	2.1
β -T3, %	0.15	
γ-T3, %	3.0	3.6
δ-T3, %	0.8	1.05
α-T, %	1.5	2.1
Total T3 and α-T, %	7.5	
Palm oil, %	7.5	
Alginate, %	40.0	
Gum Arabic, %	5.0	
Pea starch, %	34.0	
Moisture, %		8.0

Table 2-8. Specifications of Davos palm TRF - Tocotrienol Concentrate DVL 30 (oil suspension)

Item	Typical content	Minimum	Maximum
α-T3, %	8.3		
β -T3, %	0.6		
γ-T3, %	11.6		
δ-T3, %	3.4		
α-T, %	9.1		
Total T3 and α-T, %		30.0	49.9
Palm oil, %		60.1	70
Moisture, %			1.0
Peroxide value, meq/kg			5.0

Table 2-9. Specifications of Davos palm TRF - Tocotrienol Concentrate DVL 50 (oil suspension)

Item	Typical content	Minimum	Maximum
α-T3, %	13.4		
β -T3, %	1.4		
γ-T3, %	18.5		
δ-T3, %	5.7		
α-T, %	14.7		
Total T3 and α-T, %		50.0	59.9
Palm oil, %		40.1	50
Moisture, %			1.0
Peroxide value, meq/kg			5.0

Table 2-10. Specifications of Davos palm TRF – Tocotrienol Concentrate DVL 70 (oil suspension)

Item	Typical content	Minimum	Maximum
α-T3, %	18.8		
β -T3, %	2.0		
γ-T3, %	25.9		
δ-T3, %	8.0		
α-T, %	20.6		
Total T3 and α-T, %		70.0	79.9
Palm oil, %		20.1	30.0
Moisture, %			1.0
Peroxide value, meq/kg			5.0

Table 2-11. Specifications of Davos palm TRF powder- Tocotrienol Powder - DVL 7.5

Item	Typical content	Minimum	Maximum
α-T3, %	2.0		
β -T3, %	0.2		
γ-T3, %	2.8		
δ-T3, %	1.0		
α-T, %	2.0		
Total T3 and α-T, %		7.5	10.0
Moisture, %			5.0

Table 2-12. Specifications of Davos palm TRF powder- Tocotrienol Powder - DVL 10

Item	Typical content	Minimum	Maximum
α-T3, %	3.0		
β -T3, %	0.4		
γ-T3, %	4.1		
δ-T3, %	1.3		
α-T, %	3.5		
Total T3 and α-T, %		10.0	19.9
Moisture, %			5.0

Table 2-13. Specifications of Davos palm TRF powder - Tocotrienol Powder DVL 20

Item	Typical content	Minimum	Maximum
α-T3, %	5.5		
β -T3, %	0.6		
γ-T3, %	6.7		
δ-T3, %	2.1		
α-T, %	6.3		
Total T3 and α-T, %		20.0	29.9
Moisture, %			5.0

Table 2-14. Specifications of Davos palm TRF powder - Tocotrienol Powder DVL 30

Item	Typical content	Minimum	Maximum
α-T3, %	7.0		
β -T3, %	0.8		
γ-T3, %	9.5		
δ-T3, %	3.8		
α-T, %	9.6		
Total T3 and α-T, %		30.0	39.9
Moisture, %			5.0

Table 2-15. Specifications of Davos palm TRF – Water Soluble (WS) Tocotrienol 1.5%

Item	Typical content	Minimum	Maximum
α-T3, %	0.4		
β -T3, %	<0.1		
γ-T3, %	0.5		
δ-T3, %	0.2		
α-T, %	0.5		
Total T3 and α-T, %		1.5	
Moisture, %			5.0

Table 2-16. Specifications of Carotino Palm TRF 10% (oil suspension)

Item	Minimum	Maximum
Total T3 and α-T, %	10.0	
Palm oil, %		90
Moisture, %		1.0

Table 2-17. Specifications of Carotino Palm TRF 20% (oil suspension)

Item	Minimum	Maximum
Total T3 and α-T, %	20.0	
Palm oil, %		80
Moisture, %		1.0

Table 2-18. Specifications of Carotino Palm TRF 50% (oil suspension)

Item	Minimum	Maximum
Total T3 and α-T, %	50.0	
Palm oil, %		50
Moisture, %		1.0

Table 2-19. Specifications of Carotino Palm TRF 80% (oil suspension)

Item	Minimum	Maximum
Total T3 and α-T, %	80.0	
Palm oil, %		20
Moisture, %		1.0

Table 2-20. Specifications of SD Bioganic Palm TRF Bio Tri.E 20

Item	Average	Minimum	Maximum
A mixture of T3, %	15		
α-T, %	5 (25% of the total tocols)		
Total T3 and α-T, %	20	19.5	
Palm oil, %			80.5
Moisture, %			1.0

Table 2-21. Specifications of SD Bioganic Palm TRF Bio Tri.E 30 or Gold Tri.E 30 (amber liquid, bland taste)

Item	Average	Minimum	Maximum
A mixture of T3, %	22.5		
α-T, %	7.5		
Total T3 and α-T, %	30.0	29.5	
Palm oil, %			70.5
Moisture, %			1.0

Table 2-22. Specifications of SD Bioganic Palm TRF Gold Tri.E 50

Item	Average	Minimum	Maximum
A mixture of T3, %	37.5		
α-T, %	12.5 (25% of the total tocols)		
Total T3 and α-T, %	50.0	49.5	
Palm oil, %			50.5
Moisture, %			1.0

Table 2-23. Specifications of SD Bioganic Palm TRF - Gold Tri.E 70

Item	Average	Minimum	Maximum
A mixture of T3, %	52.5		
α-T, %	18.75 (25% of the total tocols)		
Total T3 and α-T, %	70.0	69.5	
Palm oil, %			30.5
Moisture, %			1.0

Table 2-24. Specifications of P SD Bioganic Palm TRF - Gold Tri.E 20 Powder

Item	Average	Minimum	Maximum
A mixture of T3, %	15		
α-T, %	5		
Total T3 and α-T, %	20	19.5	
Palm oil, %			30
Matodextrin, %			40

SD Bioganic Palm TRF powder products contain Na caseinate and plant-based emulsifiers (5%) and calcium silicate (5%) as an anticaking agent.

Table 2-25. Specifications of SD Bioganic Palm TRF - Gold Tri.E 30 Powder

Item	Average	Minimum	Maximum
A mixture of T3, %	22.5		
α-T, %	7.5		
Total T3 and α-T, %	30.0	29.5	
Palm oil, %			30
Matodextrin, %			30

Table 2-26. Specifications of SuperVitamins Palm TRF - Super T25

Item	Minimum	Maximum
α-T3, %	5.6	
β -T3, %	0.4	
γ-T3, %	7.9	
δ-T3, %	3.8	
α-T, %	6	
Total T3 and α-T, %	25.0	
Palm oil, %		75
Moisture, %		1.0

Table 2-27. Specifications of SuperVitamins Palm TRF - Super T30

Item	Minimum	Maximum
α-T3, %	5.6	
β -T3, %	0.4	
γ-T3, %	7.9	
δ-T3, %	3.8	
α-T, %	6	
Total T3 and α-T, %	30.0	
Palm oil, %		70
Moisture, %		1.0

Table 2-28. Specifications of SuperVitamins Palm TRF - Super T50

Item	Minimum	Maximum
α-T3, %	12.5	
β -T3, %	0.5	
γ-T3, %	17.0	
δ-T3, %	7.0	
α-T, %	12	
Total T3 and α-T, %	50.0	
Palm oil, %		50
Moisture, %		1.0

Table 2-29. Specifications of SuperVitamins Palm TRF - Super T80

Item	Minimum	Maximum
α-T3, %	20.0	
β -T3, %	1.0	
γ-T3, %	26.0	
δ-T3, %	12.5	
α-T, %	18.5	
Total T3 and α-T, %	80.0	
Palm oil, %		20
Moisture, %		1.0

Table 2-30. Specifications of SuperVitamins Palm TRF - Super T Powder 20%

Item	Minimum	Maximum
α-T3, %	5.0	
β -T3, %	0.2	
γ-T3, %	6.8	
δ-T3, %	2.8	
α-T, %	4.8	
Total T3 and α-T, %	20.0	
Moisture, %		8.0

Table 2-31. Specifications of SuperVitamins Palm TRF - Super T Powder 25%

Item	Minimum	Maximum
α-T3, %	6.2	
β -T3, %	0.2	
γ-T3, %	8.5	
δ-T3, %	3.5	
α-T, %	6.0	
Total T3 and α-T, %	25.0	
Moisture, %		1.0

G. Analytical methods

Tocopherols and T3 can be analyzed by high performance liquid chromatography (HPLC) with an ultraviolet (UV), liquid chromatography-mass spectrometry (LC-MS), or fluorescent detector (Yap et al. 1999; Sanagi et al. 2005; Schwartz et al. 2008). Appropriate sample preparation methods are to be employed depending on the various matrices. For example, oil samples are dissolved in heptane (50–1000 mg/10 ml depending on the expected concentration range), filtered in the case of highly concentrated or cloudy solutions, and injected into the HPLC system. Margarines and rolling fats are saponified in the presence of 100 mg of ascorbic acid as an antioxidant. The vials are flushed with nitrogen for 20 sec before and after addition of KOH. Subsequent to saponification and addition of 12 ml of water, analytes are extracted three times with 12 ml of heptane–diethyl ether (50:50, v/v). The combined organic extracts are washed with 5 ml of 10% aqueous sodium chloride solution (w/v), 10 ml of water and 10 ml of 0.01M hydrochloric acid, dried over 3 g of sodium sulfate, and evaporated at 30 °C. The solid residue can be taken up in 10 ml of heptane and the solution is syringe-filtered prior to HPLC analysis.

The following methods were used in the chemical and microbiological analysis: peroxide values by the AOAC 965.33 method; total plate count and yeast and mold by the USP 2021 method; *E.coli* and *salmonella* by the USP 2022 method; and lead by inductively coupled plasma mass spectrometry (ICP/MS) or atomic absorption (AA) spectrophotometry.

II. Natural occurrence and exposure to T3 and α-T.

A. Food sources of T3 and T

Table 3 lists the T3 and α-T content of foods eaten by Americans (Franke et al. 2007). Intakes of T3 were mainly associated with the consumption of cereal products, margarine, and oils (Chun et al. 2006; Franke et al. 2007; Schwartz et al. 2008). The relative abundance of the vitamin E homologues (T and T3) in a specific preparation depends on the species of plant and the extraction procedure used. Palm oil contains significant quantities of T3. Other sources of T3 are coconut oil, cereal grains including rice, barley, rye, wheat, and nuts. Palm, rice bran, oat, and barley contain vitamin E mostly as the T3 (Ong 1993). The common sources of commercial vitamin E such as soy, corn, cottonseed, canola, and sunflower oil distillates contain little or no T3. Unlike water-soluble vitamins, T is relatively stable during processing; vitamin E losses after bread making were close to 30% (Nandith and Prabhasankar 2009). During frying at 175 C, tocopherols have a slow degradation rate with a half-life over 72 h (Normand et al. 2001).

Table 3. Sources of T3 and α-tocopherol (α-T) in foods; mg/kg

Foods	T3	α -T
Nuts		
almonds, honey-roasted	8.3	185.3
macadamia nuts, dry-roasted	20.9	0.8 ± 1.1
mixed nuts, oil-roasted, with peanuts	9.5	69.6 ± 6.6
peanuts, dry-roasted	12.1	69.1 ± 0.8
peanuts, boiled	5.3	36.9
Oils and fats		
corn oil	81.3	222.1 ± 66.2
corn oil	25	
margarine, 60% fat	18.3	33.7 ± 6.7
margarine, 70% fat	20.7	63.4
palm oil	738	160.8 - 279
safflower oil	59.1	391.2 ± 190.9
sesame oil	<2	65.0 ± 2.4
sunflower oil	<2	541.8
Salad dressing		
fat-free mayonnaise	1.8	0.7
blue cheese salad dressing	4.0	16.3 ± 8.1
French salad dressing	4.7	19.3 ± 26.0
Italian salad dressing	15.1	40.7 ± 8.8
Oriental salad dressing	8.3	22.1 ± 25.1
ranch salad dressing	1.6	2.4 ± 0.7
sesame and miso salad dressing	15.6	57.9
sesame ginger salad dressing	28.7	9.0 ± 10.6
thousand island dressing	8.9	16.3 ± 12.0
Fish and meats		
ahi (block) raw	<0.04	6.4 ± 2.4
ahi (filet), raw	<0.04	1.6
meat ball	0.075	0.75
manapua (char siu)	7.8	1.2
manapua (pork)	2.6	2.8
salmon (filet), raw Atlantic	0.33	37.7 ± 13.3
salmon (steak), raw Atlantic	0.13	32.4

Table 3. Sources of T3 and α-tocopherol (α -T) in foods; mg/kg - continued

Foods	T3	α- T
Grain based products		
wheat	8.51	2.3 ± 1.0
cereals	11.9	7.7 ± 4.2
chocolate chip cookies	16	18.7 ± 11
fig bars	4.8	8.4 ± 3.5
fried noodles (chow mein)	5.2	9.2 ± 9.2
fried noodles (top ramen)	52.7	16.3 ± 4.8
spaghetti/marinara pasta sauce	4.2	10.9 ± 3.1
pie crust, Graham cracker	15.1	12.6
pie crust, frozen, baked	6.5	15.0
popcorn, Microwave	13.2	25.4 ± 2.4
potato chips, plain, salted	11.1	37.5 ± 14.3
potato chips, plain, salted, hydrogenated oils	18.6	76.5 ± 25.3
Fruits and products		
jack fruit	0.4	10.4
mango	<0.32	7.9 ± 0.9
papaya (green)	<0.33	0.7
papaya (red)	<0.33	1.1 ± 0.4
papaya (yellow)	2.6	6.8 ± 8
Tamarind, raw	0.33	<0.01
Vegetable and products		
bell pepper, red	1.13	20.8 ± 14.1
beet greens	4.2	13.9
broccoli, frozen	0.13	4.8
carrot juice	0.8	1.0
chili, hot pepper paste	3.2	12.1
Chinese cabbage	0.33	2.6 ± 0.8
collard, frozen	1.9	18.5
dandelion greens	1.2	7.0
spinach, canned, whole leaf	9.5	20.9 ± 3.7
spinach, fresh	1.7	11.4 ± 2.0
sweet potatoes	0.13	2.1
taro root	1.2	4.1 ± 14.1
tomato	1.4	5.5
tomato soup, canned	1.33	2.1 ± 0.2
turnip greens	1.9	19.6
turnip greens, frozen	2.1	19.2
tofu, fried	3.0	18.1
Miscellaneous foods		
dairy products	0.14	1.16
poi	0.23	2.0 ± 0.4

Adopted from Chun et al. 2006, Franke et al. 2007, Heinonen 1991, Ong 1993, and Schwartz 2008.

The α -T and T3 content of T3 and T of palm oil and rice bran oil. These oils have similar TRF composition profiles.

Table 4. Content of T3 and T present in palm oil and rice bran oil (mg/kg)

	α -T	T3				
		Alpha (α)	Beta (β)	Gamma (γ)	Delta (δ)	total
Palm oil 1	256	143	32	286	69	530
Palm oil 2	279	274	-	398	69	741
Rice bran oil	324	236		349		>909

Adopted from EFSA 2008

B. Intended use

Table 5 presents primary applications of palm TRF that include fats or oils, such as margarine, and spread, designer fats and oils, salad dressing, mayonnaise, potato chips and salty snacks, bakery products, cookies and crackers, ready-to-eat cereals, cereal bars, granola bars, protein bars, and power bars, meal replacement and other functional beverages, meatless meat products, meatless soups and soup mix, meatless gravies, sauces, and pasta sauces, processed fruits, juice drinks and punch, soft drink, frozen dairy desserts and mixes, yogurt, pudding, and jello, chocolate milk, flavored milk, soy milk and products, chocolate, candy, confectionary, and sweets, and chewing gum. In this GRAS notice, designer fats and oils are defined as the oils that are claimed for antioxidant activities and/or vitamin content, and exclude common cooking oils. Palm TRF is not intended for use as a coloring agent.

Intended use levels for each food category are expressed as mg/serving as well as % Daily Values (DV) of vitamin E (α-T). However, it is not possible to express the dosage levels of T3 as %DV as there is no established DV for T3.

Table 5. Intended use of palm TRF.

	Recommended levels, mg/serving			Maximum levels, mg/serving			%DV of Vit. E (α-T)
Food category, serving size	tocols	T3	α-T	tocols	T3	α-T	
margarine and spread, 1 TSP=13.6 g	2.04	1.53	0.51	21.8	16.3	5.5	3-30% DV
designer fats and oils, 1 TSP=13.6 g	2.04	1.53	0.51	21.8	16.3	5.5	3-30%
salad dressing and mayonnaise, 27.2 g	1.36	1.02	0.34	13.6	9.8	3.4	2-20%
potato chips and salty snacks, 30 g	1.5	1.125	0.375	7.5	5.62	1.88	2-11%
bakery products (breads, rolls, croissants), 30-50 g	1.5	1.125	0.375	14.4	10.8	3.6	2-20%
Cookies, crackers, 25 g	1.25	0.94	0.31	7.5	5.62	1.88	2-11%
RTE cereals, cereal bars, granola bars, protein bars, and power bars, 30 g	7.5	5.6	1.9	75	56.25	18.75	10-100%
meal replacement and other functional beverage products (240 g) and mixes	7.2	5.4	1.8	72	54	18	10-100%
meatless meat products (soy based), 70 g	1.05	0.79	0.26	7.0	5.25	1.75	1.5-10%
meatless soups (240 g) and soup mix	2.7	2.02	0.68	5.4	4.04	1.36	3-8%
meatless gravies, sauces, and pasta sauces, 70 g	0.9	0.68	0.22	1.8	1.36	0.44	1-2.5%
processed juice, 240 g	7.2	5.4	1.8	72	54	18	10-100%
processed fruits, juice drinks, and punch, 240 g	3.6	2.7	0.9	14.4	10.8	3.8	5-20%
soft drinks, 240 g	2.4	1.8	0.6	7.2	5.4	1.8	3-10%
frozen dairy desserts and mixes, ½ C=68 g	1.36	1.02	0.34	2.04	1.53	0.51	2-3%
yogurt, pudding and jello, 240 g	4.8	3.6	1.2	7.2	5.4	1.8	7-10%
chocolate milk, flavored milk, soy milk and products, 240 g	7.2	5.4	1.8	14.4	10.8	3.6	10-20%
Chocolate, candy, confectionary and sweets, 43 g	2.4	1.8	0.6	7.2	5.4	1.8	3-10%
chewing gum, 1 stick	0.06	0.045	0.015	0.18	0.135	0.045	<1%

Daily Values of vitamin E are based on α –T values only and do not include any contribution from T3.

C. Current consumer intake levels

The Vitamin E intake status of the American population has been estimated using Recommended Daily Allowance (RDA) and Tolerable Upper Intake Levels (UL) outlined by the Institute of Medicine (IOM) of the U.S. National Academy of Sciences (IOM 2000; Table 6). Previous dietary recommendations for vitamin E intake were based on the α-tocopherol equivalent (TEV), which was derived by applying conversion factors to all 8 isomers of the vitamin E group. However, IOM recognized that α-T is the main form of vitamin E that exerts vitamin E activity and that other vitamin E isomers are poorly converted into α-T in humans. For dietary recommendation purposes, IOM (2000) has recently established RDAs, estimated average requirements (EARs), and ULs for α-T only. Thus, vitamin E intake refers to α-T intake in dietary recommendations unless noted otherwise, although other isomers also belong to the vitamin E family. The UL is defined as a safe upper level for consumption over a lifetime or the highest level of a daily nutrient intake that will most likely present no risk of adverse health effects in almost all individuals in the general population. The RDA is the intake level for a nutrient at which the needs of 97.5 % of the population will be met. The EAR is the intake level for a nutrient at which the needs of 50 percent of the population will be met; thus, the EAR is used (such as the percentage of people meeting EAR) when evaluating the nutrient adequacy of the population.

For adults, IOM recommends daily vitamin E (α -T) intake of 15 mg/d. Vitamin E intake (α -T) of up to1000 mg/d is considered as safe (IOM 2000). For T3, there are no established ULs.

Table 6. Vitamin E (α-T) intake recommendations (IOM 2000)

Age	RDA (mg/d)	UL (mg/d)
1-3	6	200
4-8	7	300
9-13	11	600
14-18	15	800
19+	15	1000

Based on food consumption data reported in the most recent National Health and Nutrition Examination Survey (NHANES; 2005-2006) compiled by the U.S. Department of Health and Human Services, National Center for Health Statistics and the Nutrition Coordinating Center, estimates of 2-d (d) average intakes of vitamin E (as α-T) and T3 were calculated from the food code list and the survey database of diet recalls. The usual intake estimation procedure requires multiple days of nutrient intake data for at least a representative subsample of the individuals in the sample in order to estimate variances (Goldman 2005). The NHANES provides the most current food consumption data available for the American population. The NHANES was conducted between 2005-2006 with non-institutionalized individuals in the U.S. In each of the two survey yrs, data were collected from a nationally representative sample of individuals of all ages.

The food and dietary supplement record for each individual includes the gram weight and nutrient data for all foods consumed during the day of the recall. All estimates were generated with USDA sampling weights to adjust for differences in representation of subpopulations.

The NHANES food code list includes the α-T content of each food, but not T3 values. Thus, the T3 content was developed by taking the α-T content for each food and multiplying it by a conversion factor to estimate the current consumption levels of T3. Based on published vitamin E composition tables (Chun et al. 2006, Franke et al. 2007, Heinonen 1991, Ong 1993,

and Schwartz 2008), the ratio of T3: α-T (i.e., a conversion factor) was determined for each food by category. The categories and conversion factors are listed in Table 7.

Table 7. Conversion factors of vitamin E

Food category	Conversion Factor: from α-T to T3
Breads, cookies, cereals, and cereal Bars	1.21358
Pasta	1.71
Desserts	0.7826
Nuts	0.1551
Popcorn	0.52
Potato chips and salty snacks	0.26
Margarine, fats, and oils	0.1357
Dressings, spreads, and mayonnaise	0.4723
Fruits and fruit products	0.1658
Vegetables, excluding sweet potatoes	0.1976
Sweet potatoes	0.062
Fried tofu	0.1657
Poi	0.115
Eggs and egg products	0.0238
Dairy products	0.1207
Mixed dish mostly grain	1.21
Mixed dish mostly beef or chicken	0.1
Fish products	0.00513
Pork products	0.9286
General foods (other foods)	0.55

The most up-to-date vitamin E (α-T) and T3 intake status for the U.S. population ages 1 yr and older are presented in Tables 8 and 9 (NHANES 2005-2006). The median intakes of T3 in the U.S. are estimated to be 2.2 mg/d for children aged 1-3 yr and 3.2 mg/d for the population aged 1 yr and above (the entire population)(Cho et al. 2009). The median intakes of vitamin E (α-T) are estimated to be 3.6 mg/d for children aged 1-3 yr and 5.8 mg/d for the population aged 1 yr and above; these intakes correspond to approximately 40-56% of RDAs. Over 98% of the U.S.

population did not meet the daily recommended intake of vitamin E and over 94% of the population did not meet the EAR. Based on the previous nutrient adequacy data that showed a similar trend as shown in this analysis, the USDA Dietary Guideline (DG) Committee has classified vitamin E as a shortfall nutrient low in the American diet (USDA 2004). None of population consumes vitamin E above UL levels; in the 2005-2006 NHANES, the 90th percentile intakes were estimated to be 5.1 and 12.5 mg/d in young children aged 1-3 yr and the entire population, respectively (Cho et al. 2009). These values represent less than 1.6-3% of the ULs established for these age groups.

Table 8. Tocotrienol and vitamin E (α-T) intakes before the intended use (NHANES 2005-2006)

Age, yr	Intake Type	N	Tocotrienol				Alpha Tocopherol			
			Median	SE	90th Pctl	SE	Median	SE	90th Pctl	SE
1+	Daily intake, mg/d	8792	3.1	0.03	8.1	0.14	5.8	0.04	12.5	0.18
1+	Daily intake, mg/kg BW/d	8720	0.05	0.00	0.15	0.00	0.09	0.00	0.22	0.01
1-3	Daily intake, mg/d	789	2.2	0.06	3.5	0.17	3.6	0.07	5.1	0.22
1-3	Daily intake, mg/kg BW/d	787	0.17	0.00	0.26	0.01	0.28	0.01	0.39	0.02

Pctl = Percentile; kg BW=kg body weight

Table 9. Nutrient adequacy of vitamin E (α-T) before the intended use (NHANES 2005-2006)

Age, yr	N	Usual Intake, mg/d		EAR		RDA		UL	
		Mean	SE	% Above	SE	% Above	SE	% Above	SE
1+	8792	6.4	0.05	6.4	1.8	1.6	0.9	0	0
1-3	789	3.9	0.05	10.5	8.4	2.5	4.3	0	0

D. Estimated Daily Intake of T3 and α-T from GRAS Uses

Using food intake data reported in the 2005-2006 NHANES, exposure levels to MPOB T3 and α-T that will result from the intended uses were estimated. Results of the exposure estimates after fortification with palm TRF in foods for the U.S. population ages 1 yr and older are presented in Table 10. The intended use models are reported at two different levels: The first set of estimates is based on the assumption that 20% of the products in each food category will be under the intended use (assuming 18% of users will consume the recommended levels and additional 2% of users will consume the maximum levels of intended use; total 20%). The second set of estimates is based on the assumption that 100% of the products are used at the maximum levels of T3 specified in the intended use (assuming 95% of users will consume the recommended levels and additional 5% of users will consume the maximum levels of intended use: total 100%). This is a highly unlikely scenario since it is not possible to use all the food groups under the intended use. Also wastage and other losses should be considered.

Assuming that 20% of the product will be used under the intended use, the median intakes including palm T3 from all GRAS proposed use categories by users of one or more foods is 6.4 mg/d for children aged 1-3 yr and 8.6 mg/d for the entire population. The 90th percentile intakes including MPOB T3 from all GRAS proposed use categories by users of one or more foods are 9.4 mg/d for children aged 1-3 yr and 17.4 mg/d for the entire population. These levels correspond to 0.68 mg/kg BW/d for children aged 1-3 yr and 0.35 mg/kg BW/d for the entire population (Table 11). These levels are more than 100x below the no-observed-adverse-effect level (NOAEL) values that have been found from sub-chronic and chronic toxicity studies in rats (Nakamura et al. 2001; Tasaki et al. 2008).

Even if 100% of the product will be used under the intended use, the median intakes including T3 from all GRAS proposed use categories by users of one or more foods is 19.1 mg/d for male children aged 1-3 yr and 23.8 mg/d for the entire population. The 90th percentile intakes including MPOB T3 from all GRAS-proposed use categories by users of one or more foods are 28.6 mg/d for children aged 1-3 yr and 49.2 mg/d for the entire population. These levels correspond to 2.07 mg/kg BW/d for children aged 1-3 yr and 0.99 mg/kg BW/d for the entire population (Table 10).

Estimated exposure to vitamin E (α-T) is presented in Tables 10 and 11. Intended use would significantly improve the vitamin E intake status of Americans. An assumption that 20 % of that population are consumers of MPOB α-T leads to estimates of dietary exposure of approximately 7.1 mg/d (or 0.54 mg/kg BW/d) for children aged 1-3 yr and 17.9 mg/d (or 0.35 mg/kg BW/d) for the entire population. Even if 100% of the product will be used under the intended use, the 95th percentile intakes including α-T from all GRAS-proposed use categories are 13.3 mg/d (or 1.0 mg/kg BW/d) for children aged 1-3 yr and 29.0 mg/d (or 0.63 mg/kg BW/d) for the entire population. These levels are far below the ULs established by IOM; 200 mg/d for children aged 1-3 yr and 1,000 mg/d for adults. While no population consumes vitamin E above the UL, intended use would increase the percentages of people meeting EAR and RDA (Table 11; Cho et al. 2009). Also, these exposure levels are within the Acceptable Daily Intake (ADI) of 0.15-2 mg/kg BW/d as defined by the Joint FAO/WHO Expert Committee on Food Additives (JECFA; EFSA 2008; WHO 1986).

Table 10. Estimated exposure under the intended use

Age	Intake Type	N	Tocotrienols				Vitamin E (α-T)			
			Median	SE	90th Pctl	SE	Median	SE	90th Pctl	SE
Assuming 20% of products are under the intended use										
1+	Daily Intake, mg/d	8792	8.6	0.06	17.4	0.23	7.30	0.04	14.6	0.19
1+	Daily Intake, mg/kg BW/d	8720	0.13	0.00	0.35	0.01	0.11	0.00	0.27	0.01
1-3	Daily Intake, mg/d	789	6.4	0.13	9.4	0.36	4.7	0.08	6.4	0.33
1-3	Daily Intake, mg/kg BW/d	787	0.48	0.01	0.68	0.03	0.35	0.01	0.49	0.02
Assuming 100% of products are under the intended use										
1+	Daily Intake, mg/d	8792	23.8	0.16	49.2	0.63	11.9	0.07	22.3	0.25
1+	Daily Intake, mg/kg BW/d	8720	0.36	0.00	0.99	0.02	0.18	0.00	0.43	0.01
1-3	Daily Intake, mg/d	789	19.1	0.41	28.6	1.18	6.23	0.10	11.2	0.38
1-3	Daily Intake, mg/kg BW/d	787	1.42	0.01	2.07	0.10	0.60	0.01	0.83	0.03

Table 11. Nutrient adequacy of Vitamin E (α-T) under the intended use

Age, yr	N	Usual intake,			EAR		RDA		UL	
		Mean, mg/d	SE	Mean, % RDA	% Above	SE	% above	SE	% Above	SE
Assuming 20% of products are under the intended use										
1+	8792	7.90	0.06	62.2	17.1	2.1	5.8	1.5	0	0
1-3	789	4.93	0.06	82.0	39.0	5.9	14.4	8.3	0	0
Assuming 100% of products are under the intended use										
1+	8792	12.70	0.08	98.2	66.1	1.9	40.7	2.1	0	0
1-3	789	8.41	0.10	139.7	97.9	3.2	90.1	7.2	0	0

% Above = percentage of people consuming more than EAR, RDA, or UL.

III. Basis for GRAS determination

A. Current regulatory status.

It should be noted that α-T (as a chemical preservative and as the nutrient, vitamin E), tocopherols (as a chemical preservative and as the nutrient, vitamin E), and α-T acetate (as the nutrient, vitamin E) are already listed as GRAS ingredients by the US FDA (21 CFR, Part 184.1890, 182.8890, and 182.8892) when used in accordance with good manufacturing practice. Also, T and α-T acetate are included in the *"Everything" Added to Food in the United States* (EAFUS) list. The EAFUS list of substances contains ingredients added directly to food that FDA has either approved as food additives or listed or affirmed as GRAS. The CAS number for mixed tocopherols is 1406-18-4, which is a mixture of the following T with their own CAS numbers: d-α-T, CAS number 59-02-9; d-β-T, CAS number 148-03-8; d-γ-T, CAS number 7616-22-0; d-δ–T, CAS Number 119-13-1. As mentioned earlier, IOM (2000) and the USDA Dietary Guidelines Committee (2004) have recommended the increased consumption of vitamin E for Americans of all ages.

Currently, T3 is not listed as an approved food additive or a GRAS-affirmed substance in the U.S. However, dietary supplements containing T3 have been on the market in the U.S. as dietary supplements since 1999 under the *Dietary Supplements Health and Education Act of 1994* (DSHEA).

Due to the current GRAS approval status of T, this review for GRAS self-affirmation has focused on T3. This GRAS determination for TRF is based upon scientific procedures in accordance with section 201(s) (21 U.S.C. §321(s)) of the Federal Food, Drug, and Cosmetic Act (21U.S.C.§301 et. seq.) ("the Act"), is set forth at 21 CFR 170.30. The following is provided to briefly set the stage regarding the GRAS process (US FDA 2008):

1) Any substance of natural biological origin that has been widely consumed for its nutrient properties prior to January 1, 1958, without known detrimental effect, for which no health hazard is known, and which has been modified by processes first introduced into commercial use after January 1, 1958, which may reasonably be expected significantly to alter the composition of the substance.
2) Any substance of natural biological origin that has been widely consumed for its nutrient properties prior to January 1, 1958, without known detrimental effect, for which no health hazard is known, that has had a significant alteration of composition by breeding or selection after January 1, 1958, where the change may reasonably be expected to alter the nutritive value or concentration of its constituents.
3) Distillates, isolates, extracts and concentration of extracts of GRAS substances.
4) Reaction products of GRAS substances.
5) Substances not of a natural biological origin, including those for which evidence is offered that they are identical to a GRAS counterpart of natural biological origin.
6) Substances of natural biological origin intended for consumption for other than their nutrient properties.

In general, materials of natural biological origin that have no significant detrimental effect and no known health hazard are eligible for GRAS status. Considering that TRF is of biological origin, that TRF exists naturally in many foods that have long histories of human consumption, and that TRF does not represent a known health hazard, TRF is considered as a GRAS substance.

Documentation qualifying a substance as GRAS has been compiled, where such documentation includes technical evidence and common knowledge of safety under the

conditions of intended use, as recognized by qualified experts (the Expert Panel). The safety requirement for GRAS substances can be supported by this historical consumption (prior to 1958) as well as by scientific studies. Technical evidence of safety includes the chemical identity of the substance, the method of manufacture, analytical data on composition and specifications, estimates of dietary exposure, safety data from animal and human clinical studies, and nutritional benefits from animal and human clinical studies.

The European Scientific Committee on Food (SCF) evaluated the safety of TRF for use as a food ingredient and concluded that the use of mixed T and T3 as a source of vitamin E in food supplements for the general population at the proposed levels of use is not a safety concern (EFSA 2008). Proposed levels include up to 1000 mg of T3 per daily dose, which would result in a daily intake of 16.7 mg T3/kg BW/d for a 60 kg person. At the international level, the ADI value was estimated to be 0.15 – 2 mg/kg BW, calculated as α-T (WHO 1986). Unlike the U.S. IOM and WHO, two European expert panels set UL based on TEV. The European SCF set a UL of vitamin E (as d-α-T) for adults of 300 mg TEV/d (SCF 2003). The U.K. EVM established a NOAEL and UL of 540-970 mg TEV (EVM 2003). In this GRAS self-affirmation, the U.S. IOM standard (α-T) is adopted in estimating dietary exposure levels of vitamin E since USDA Dietary Guidelines and other dietary recommendations in the U.S. are based on the IOM standards.

B. Intended technical effects

1. Natural vitamin E

Natural vitamin E includes eight chemically distinct molecules: T; and α-, β-, γ- and δ-T3 (IOM 2000; Traber and Sies 1996). They cannot be synthesized in the body; thus, vitamin E must be obtained from the diet (IOM 2000). α-Tocopherol is known to have the most powerful

vitamin E activity among these 8 isomers. Table 12 presents the relative biological activities of the various tocopherols and tocotrienols in the rat resorption-gestation test (Kamal-Eldin and Appelqvist 1996). The biological activities of vitamin E are usually compared based on their efficacy, when administered orally, that prevents resorption-gestation in 50 % of female rats deprived of vitamin E. Symptoms caused by α-T deficiency can be alleviated by T3 (Khanna et al. 2005a; Traber et al. 2004). For example, α-T3 restored fertility in TTP-deficient mice (Jishage et al. 2001). Thus, T3 may be viewed as being members of the natural vitamin E family, not only structurally but also functionally.

Table 12. Relative biological activities (in %) of vitamin E active compounds in the rat resorption-gestation test (Kamal-Eldin and Appelqvist 1996).

Form of vitamin E	Relative activity in prevention of fetal resorption (%)
d-α-tocopherol (RRR)	100
d-β-tocopherol (RRR)	25-50
d-γ-tocopherol (RRR)	1-11
d-δ-tocopherol	1-3
d-α-tocotrienol	29-30
d-β-tocotrienol	5
d-γ-tocotrienol	Not known
d-δ-tocotrienol	Not known

Adopted from EFSA 2008

2. Antioxidant effects

It is well known that vitamin E compounds have antioxidant properties (Sen 2007; Nesaretnam et al. 2008) and have been used as a food additive to prevent oxidation (Theriault et al. 2002; Romero et al. 2004; Rossi et al. 2007). Tocotrienols, like T, are capable of scavenging and quenching free radicals. Their antioxidative activity resides mainly with its chain breaking property, which neutralizes peroxyl and alkoxyl radicals generated during lipid peroxidation

(Nesaretnam et al. 2008) and T3 is more a potent antioxidant against lipid peroxidation than T (Serbinova et al. 1991, 1994; Qureshi et al. 2000; Ahmad et al. 2005). Peroxidation of membrane lipids is known to modify and inactivate cellular components, which can have damaging effects on crucial cellular factors, leading to disease.

In addition, antioxidant properties of T3 may have a role in protection against cancer and other ailments (Watson and Preedy 2008; Ahn et al. 2007; Cuddihy et al. 2004; Khanna et al. 2003, 2005a).

C. Review of safety data

1. Bioavailability and metabolic fate of T3

Once ingested, the T3 and α-T form chylomicrons in the intestine when they are released and mixed with human gastrointestinal fluid to enter the circulation from the intestine. The conversion of chylomicrons to remnant particles results in the distribution of newly absorbed T3 and α-T to all of the circulating lipoproteins and ultimately to tissues (Traber et al. 2004). In the liver, newly absorbed dietary lipids are incorporated into nascent very low-density-lipoproteins (VLDL). The liver is responsible for the control and release of α-T into blood plasma. α-Tocopherol transfer protein (TTP) is required for the release of α-T back into the plasma. α-Tocopherol transfer protein has the ability to bind to both α-T3 as well as α-T and TTP is known to bind to α-T3 with 8.5-fold lower affinity than that for α-T (Hosomi et al. 1997; Khanna et al. 2005a). The data indicate that tissue delivery of α-T3 is likely to be compromised when α-T and α-T3 are co-supplemented (Khanna et al. 2005a). However, a study of Khanna et al. (2005a) with the TTP-deficient mice indicates the existence of a TTP-independent mechanism for the tissue delivery of oral α-T3.

Recently it has been demonstrated that γ-T is more rapidly metabolized in women than in men (Leonard et al. 2005). In rat studies, delivery of oral α-T3 to the heart of females was more efficient compared to that of males (Khanna et al. 2005b; Patel et al. 2006). The accumulation of α-T3 in various organs over time was eliminated in 7 wk when the sub-group of rats was subjected to a vitamin E-deficient diet.

1) Human clinical trials

In a human study investigating the pharmacokinetics and bioavailability of T3 (Yap et al. 2001), eight volunteers were administered a single oral dose of mixed T3 (a 300 mg dose of α-, γ-, and δ-T3) under fed or fasted conditions. The bioavailability of α-T3 under the two conditions was compared using the outcomes, peak plasma concentration (Cmax), time to reach peak plasma concentration (Tmax), and total area under the plasma concentration-time curve (AUC). The absorption of each T3 was 100% greater in the fed state and Cmax was achieved between 3 and 5 h. A statistically significant difference was observed between the fed and fasted logarithmic transformed values of Cmax ($P < 0.01$) and AUC (0-infinity) ($P < 0.01$) for all three T3. However, no statistically significant difference was observed between the fed and fasted Tmax values of the three homologues. The mean apparent elimination half-life (t(1/2)) of α-, γ-, and δ-T3 was estimated to be 4.4, 4.3 and 2.3 h, respectively, being between 4.5- to 8.7-fold shorter than that reported for α-T. No statistically significant difference was observed between the fed and fasted t(1/2) values (Hayes et al. 1993).

Some TRF preparations, such as Tocomin® Suprabio™, contain emulsifiers, self-emulsifying drug delivery systems (Sen 2007). Emulsifiers are known to increase absorption of fat-soluble

compounds. A recent study using Tocomin® Suprabio™ examined the post-absorptive fate of tocotrienol isomers (Khosla et al. 2006). The peak plasma concentration of α-T3 (~3 uM) was 2–3 times more than the peak concentration reported in previous studies using TRF supplements not containing emulsifiers (O'Byrne et al. 2000; Yap et al. 2001).

2) Animal Studies

Orally supplemented α-T3 was successfully delivered to several vital organs among which the skin, adipose, ovaries, and the heart seemed to be preferred destinations within the body of rats (Khanna et al 2005b; Patel et al. 2006). α-Tocotrienol that accumulated in organs for over 2 yr was almost completely lost from the tissues in less than 2 mo after switching to a α-T3-deficient diet (Patel et al. 2006). The shorter elimination half life of T3 than that of T seems to be dependent on its higher metabolic rate and antioxidant property (Traber and Sies 1996; Birringer et al. 2002; Cuddihy et al. 2004). The ability of long-term oral supplementation to maintain and even increase α-T3 levels in vital organs, together with the rapid elimination from all organs studied, underscores the need for continuous oral supplementation of T3. The transport efficiency of α-T3 to tissues may be maximized by eliminating the co-presence of α-T in the oral supplement (Khanna et al 2005b).

a) Nine-generation rat study (Patel et al. 2006)

Oral supplementation of T3 (5 mg/kg BW; 5 d/wk) for 3 yr in 9 generations of female and male rat did not show any adverse effects. In this study, 10 vital organs (including skin, adipose, heart, lung, brain, spinal cord, ovary, and testes) were examined. To gain insight into the turnover of α-T3 in tissues, a subset of supplemented rats was moved to a vitamin E-deficient

diet for 7 wk. Orally supplemented α-T3 was delivered to all vital organs including the brain and spinal cord in significant amounts. In organs such as the skin, adipose, and gonads the maximum level of α-T3 achieved in response to supplementation was folds higher than baseline values of α-T3 in rats maintained on laboratory chow. The metabolic fate of T3 showed a gender-dependency. The level of α-T3 was approximately one magnitude higher in ovaries than in testes. Elimination of α-T3 from the enriched ovaries, under conditions of the vitamin E-free diet, was observed to be much sharper compared to that from the testes. Females had higher levels of α-T3 compared to matched tissues (including skin, heart, and lung) of corresponding males. To gain insight into how quickly α-T3 is metabolized in the tissues, washout of α-T3 from vital organs was examined. Unlike that for the transport of α-T, the liver does not seem to serve as the hub for α-T3 distribution in the body. Levels of α-T3 in the liver were much lower than the levels noted in most organs studied. α-Tocotrienol that accumulated in vital organs over more than 2 yr was almost completely lost in less than 2 mo when the supplementation was stopped. This is in sharp contrast with findings related to α-T retention.

b) Five-generation rat study (Khanna et al. 2005b)

A long-term study was conducted to examine the effects of α-T3 or α-T supplementation (5 mg/kg BW/d) over 60 wk, either alone or in combination, on tissue levels. Orally supplemented α-T3 was successfully delivered to several vital organs. In this study, rats were maintained on a vitamin E-deficient diet and gavaged with α-T3 or α-T alone or in combination. Five generations of rats were studied over 60 wk. α-Tocopherol transfer protein-deficient mice were supplemented with α-T3 and bred to examine tissue delivery of oral α-T3. Orally supplemented α-T3 was effectively delivered to most tissues over time. When co-supplemented,

α-T outcompeted α-T3 for transport systems delivering vitamin E to tissues. To evaluate the significance of TTP in α-T3 delivery to tissues, tissue levels of α-T3 in supplemented TTP-deficient mice were studied. α-T3 was transported to several vital organs in TTP-deficient mice. α-Tocotrienol restored fertility in TTP-deficient mice. The transport efficiency of α-T3 to tissues may be maximized by eliminating the co-presence of α-T in the oral supplement.

The gender-specific effect (better transport of T3 in females than in males) was noted as a general trend across all organs studied. The level of α-T3 in the ovary was over 5-fold higher than that in the testes from the corresponding males rats. No adverse effects were found.

2. Safety/Toxicity of Palm TRF—Preclinical studies

The results of various animal studies are summarized in Table 13. All the experiments were conducted under good laboratory practice (GLP). As shown in Table 13, a 52-wk chronic toxicity study (Tasaki et al. 2008) showed the no-observed-adverse-effect level (NOAEL) as 0.4% palm TRF in the diet (or 303 mg/kg BW/d for males, and 472 mg/kg BW/d for female rats). A subchronic toxicity study of Nakamura et al. (2001) showed no abnormality at the 0.19% TRF in diet (or 120 mg/kg BW/d for males, and 130 mg/kg BW/d for female rats).
A 5 week chick study (Yu et al. 2006) indicated that safe intake was estimated to be up to 1000 mg/day. Thus, the NOAEL of TRF appears to be 0.4% in diet, Also a subacute toxicity study (14 d) on the TRF from rice bran oil in rats showed that up to 0.5% TRF in the diet was safe (Sheppard et al. 1996). In addition, various studies reported cancer-preventive effects of palm TRF at lower levels.

Table 13. Studies on subacute, subchronic and chronic toxicity of palm TRF.

Species	Route	Dose of TRF	Duration	Result	Reference
Male rat	Feed	120 mg/kg/d or 0.19% in diet (T3-78.6%; α-T-21.4%)	13 wk	No abnormalities	Nakamura et al. 2001
Female rat	Feed	130 mg/kg/d or 0.19% in diet (T3-78.6%; α-T-21.4%)	13 wk	No abnormalities	Nakamura et al. 2001
Male rat	Feed	303 mg/kg/d or 0.4% in diet (T3-78.7%; α-T-21.3%)	52 wk	No abnormalities	Tasaki et al. 2008
Female rat	Feed	472 mg/kg/d or 0.4% in diet (T3-78.7%; α-T-21.3%)	52 wk	No abnormalities	Tasaki et al. 2008
Rat	Feed	0.75% and 3% in diet (T3-78.6%; α-T-21.4%)	13 wk	Abnormal pathological changes in male liver and hematological changes in females	Nakamura et al. 2001
Rat	Feed	1-2 % in diet (T3-78.7%; α-T-21.3%)	52 wk	Abnormal pathological changes in liver	Tasaki et al. 2008
Chick	Feed	1000 mg/d	5 wk	No abnormalities	Yu et al. 2006
Rat	Gavage	5000 mg/kg (T3-80%; α-T-20%)	14 d	No abnormalities	Sheppard et al. 1996*

*All the studies used palm TRF except the study of Sheppard et al. (1996). This study employed TRF from rice bran oil.

Table 14 summarizes the toxicity studies on palm oil and rice bran oil, which share similar TRF composition profiles. This review does not include other oils whose TRF compositions are different from that of palm TRF. For example, annatto oil TRF contains 85-92% δ-T3 and palm TRF has approximately10% δ-T3.

A subacute toxicity of palm oil extract (containing 80% T3) in young rats and mice demonstrated that up to 2500 mg/kg BW was safe (Oo et al. 1992). Also, repeatedly heated refined and crude palm oils showed no mutagenicity (Manorama et al. 1989). All the experiments were conducted under GLP.

In addition, various studies showed no teratogenic effects and genotoxicity of rice bran oil. A diet containing 10% rice bran oil, as compared to peanut oil, had no teratogenic effect

(Raghuram and Rukmini 1995). Rice bran oil concentrate representing the T3-T preparation up to 5000 ug/plate had no mutagenic activity in an Ames test (Polasa and Rukmini 1987).

Table 14. Toxicity studies of palm oil and rice bran oil

Species	Route	Test material	Duration	Result	Reference
Rat	Oral	Palm oil extract, 2.5 mg/kg BW/d	30 d	No abnormalities	Oo et al. 1992
Rat	Feed	5% red palm oil in diet	42 d	No abnormalities	Watkins et al. 2001
S. typhimurium TA100 and TA98	W/ & w/o metabolic activation	Heated (8-24 h) refined and crude palm oils	48 h	No mutagenic activity	Manorama et al. 1989
S. typhimurium TA100 and TA98	W/ & w/o metabolic activation	5000 ug/plate rice bran oil concentrate	48 h	No mutagenic activity	Polasa and Rukmini 1987
Rat	Feed	10% rice bran oil	Over 3 generations	Normal reproductive performance and no teratogenicity	Raghuram and Rukmini 1995

Details

1) A 13-week sub-chronic toxicity study (Nakamura et al. 2001)

Nakamura et al. (2001) performed a 13-wk oral toxicity study in 344 5-wk-old Fischer rats of both sexes (10 rats/sex/group) at dose levels of 0 (group 1), 0.19 (group 2), 0.75 (group 3) and 3% (group 4) of palm TRF (Eisai Co., Japan) in the diet. The high dose level of 3% was determined based on a pilot study in which 5%, but not 3%, TRF diet was refused by rats. The mixture used in this study had the following composition: α-T3 21.4%, β-T3 3.5%, γ-T3 36.5%, δ-T3 8.6%, α-T 20.5%, β-T 0.7%, γ-T 1.0% and δ-T 0.5%. The composition of palm TRF used in this study was similar to those of MPOB palm TRF: α-T3 20%, β-T3 3.3%, γ-T3 25%, δ-T3 8.3%, and α-T 23.3%. Because the chemical used in this study contained T, another group (group 5)

was fed a diet containing 0.69% α-T (Tama Biochemistry Co. Ltd), which was identical to the concentration of T in the high-dose (3%) group to assess the influence of T itself.

Suppression of body weight gain was observed in group 4 males. On hematological examination, a significant decrease in mean corpuscular volume (MCV) was observed in all treated males. Platelets were significantly reduced in group 3 and 4 males. Hemoglobin concentration, MCV, and mean corpuscular hemoglobin (MCH) concentration were significantly decreased in group 3 and 4 females (0.75-3% TRF in the diet) and hematocrit (Ht) in group 4 females. Increases in the albumin/globulin ratio (A/G) and alkaline phosphatase (ALP) in all treated males, elevated alanine transaminase in group 4 of both sexes, and increases in asparagine transaminase and g-glutamyl transaminase in group 4 females were observed. With regard to relative organ weights, liver weights in group 4 of both sexes and adrenal weights in all treated males demonstrated an increase, and ovary and uterus weights in group 4 females were reduced.

As a decrease in MCV, an increase in the A/G, elevation of ALP, and increase in adrenal weight were observed in all treated males, a NOAEL could not be determined in this examination. Histopathologically, slight hepatocellular hypertrophy in group 3 and 4 males and reduction of cytoplasmic vacuolation in the adrenal cortical region in group 4 males (3% TRF in the diet) were observed. Because of pathological changes in male liver and hematological changes in females, the authors concluded that the NOAEL was 0.19% in the diet (120 mg/kg BW/d for male rats and 130 mg/kg BW/d for female rats).

2) 52 week chronic toxicity study (Tasaki et al. 2008)

Tasaki et al. 2008 performed a 52-wk chronic study using Wistar Hannover rats of both sexes (10 rats/sex/group) given the compound at doses of 0, 0.08, 0.4, or 2% palm TRF (Eisai Co., Ltd.,Tokyo, Japan) in a powdered basal diet. Complete dissociation from T was impossible and the mixture used in this study had the following composition: α-T3 21.4%, β-T3 3.5%, γ-T3 36.5%, δ-T3 8.6%, α-T 20.5%, β-T 0.7%, γ-T 1.0%, and δ-T 0.5%. Since 6 animals in the 2% male group died of hemorrhage of several organs by week 50, the maximum dose level was changed to 1% in both sexes for the last 2 wk. A decrease in body weight gain was observed in the 2% males from week 5 and females from week 10, persisting to the end of the study. With the high dose, prolongation of prothrombin time (PT) and increase of serum ALT in males, as well as an increase of serum ALP in both sexes, were observed. In male and female rats receiving 0.4% or less, there were no toxicological changes in any of the criteria examined. At necropsy, multiple cyst-like nodules on the liver surface were macroscopically pronounced in both sexes receiving 2%. On histopathological examination, hepatocellular nodules were evident with distortion of hepatic cords and compression of the surrounding tissue, almost all including areas of spongiosis hepatis. The constituent hepatocytes were immunohistochemically stained with proliferation cell nuclear antigen at high rates. Nevertheless, they did not exhibit overt atypia and the basic lobular architecture remained intact. Additionally, they were consistently negative for glutathione *S*-transferase placental form (GST-P). Quantitative GST-P analysis of the liver sections overall showed numbers of GST-P foci in the high dose females to be significantly elevated as compared to the control value. Based on the data demonstrating nodular liver lesions only at the high dose of both sexes, the authors concluded that the NOAEL was 0.4% (303 mg/kg BW/d for males and 472 mg/kg BW/d for females).

3) 4 week chick study (Yu et al. 2006)

Diets supplemented with 50 (115 umol/kg diet), 100 (230 umol/kg), 250 (575 umol/kg), 500 (1150 umol/kg), 1000(2300umol/kg), or 2000 (4600 umol/kg) ppm of palmTRF, α-T, α-T3, γ-T3, or δ-T3 were fed to 204 White Leghorn female chickens (divided into 34 groups) weighing 40-50 g for 4 wk. Supplemental palm TRF produced a dose-response (50-2000 ppm) lowering of serum total and LDL cholesterol levels of 22% and 52% ($P < 0.05$), respectively, compared with the control group. Supplemental α-T3 within the 50-500 ppm range produced a dose-response lowering of total (17%) and LDL (33%) cholesterol levels. The more potent γ- and δ-isomers yielded dose-response (50-2,000 ppm) reductions of serum total (32%) and LDL (66%) cholesterol levels. A lowering of LDL cholesterol was achieved with higher levels of α-T; 2,000 ppm showed a modest response compared with responses to palm TRF and the individual T3, yielding a 6% reduction. HDL-cholesterol levels were minimally impacted by the T3; thus HDL/LDL cholesterol ratios were markedly improved (123-150%) by the supplements. Serum triglyceride levels were significantly lower in subjects receiving the higher supplements.

There were no supplement-induced changes in weight gain, weight of organs, or feed consumption. The study suggest that a T3 and/or α-T concentration up to 1000-2000 ppm (or 1000-2000 mg/kg diet) in the diet can show beneficial effects without having side effects.

4) Subacute toxicity studies of palm oil extract in mice and rats (Oo et al. 1992)

Palm oil extract (containing 80% T3 and 20% α-T) administered to young Sprague-Dawley rats (weighing 150-180 g) and albino mice (weighing 15-20 g) at a dose of up to 25,000 mg/kg BW for 30 d showed no appreciable adverse effects in animals with respect to physical

manifestations or behavioral changes (Oo et al. 1992). In this study, the animals were fed daily doses (250, 500, 1000, or 2500 mg/kg) of palm oil extract in refined, bleached and deodorized olein for 30 d. Results indicated that treated rodents did not exhibit any physical abnormalities or unusual behavior. However, some sluggish motor activity was manifested. Hematology, serum biochemistry, and histopathological observations were not made in this study. Interestingly, treated mice gained weight significantly whereas treated rats grew normally over the 30 d period. The authors concluded that palm oil extract, in extremely high doses, did not appear to produce appreciable adverse effects in the animals used.

Watkins et al. (2001) studied the effect of red palm olein on bone metabolism in growing rats. Rat growth was not affected by the dietary lipid treatments after 42 d of feeding (up to 5% red palm oil in diet). As compared to the soybean oil group (3.5% in diet), the concentrations of saturated fatty acids and monounsaturated fatty acids were higher but polyunsaturated fatty acids (PUFA) lower in tibia marrow of rats given the palm oil treatments compared with those given soybean oil. The reduction in total n-6 PUFA concentration in bone of rats given palm oil was associated with higher bone specific alkaline phosphatase activity and endosteal mineral apposition rate and bone formation rate in tibia. No difference was found in *ex vivo* prostaglandin E2 (PGE_2) production in femur from rats. The histomorphometric analyses indicated that red palm olein might support bone formation and bone modeling in growing rats. Palm oil or palm antioxidants may play a role in bone formation.

5) Carcinogenicity

At high concentration (2% in diet), T3 may be a hepatocarcinogen in female rats (Tasaki et al. 2008). However, long-term administration of T3 at a dose of 30 ppm was shown to exert a

preventive effect on liver tumor development in the Solt and Farber hepatocarcinogenesis model (Rahmat et al. 1993). This study investigated the effects of long-term administration of T3 on hepatocarcinogenesis in rats (28 male 7- to 8-wk-old *Rattus norwegicus* rats, weighing 120 to 160 g) induced by diethylnitrosamine (DEN) and 2-acetylaminofluorene (AAF). The T3 was composed of 80% γ-T3, 20% α- and β-T3 supplied by PORIM, Malaysia. The rats were killed after 9 mo and the livers were examined morphologically. Grayish white nodules (2/liver) were found in all the DEN/AAF-treated rats (n = 10), but only one of the rats treated with DEN/AAF and supplemented with T3 (n = 6) had liver nodules. The authors concluded that T3 supplementation attenuated the impact of the carcinogenesis in the rats.

Tocotrienols inhibit the growth of hepatoma cells but not that of hepatocytes from healthy rat liver (Sakai et al. 2004). Tocotrienols killed murine liver cancer cells but not normal cells (Har and Keong, 2005). Research indicates that T3 has an inhibitory effect on angiogenic factor secretion from cancer cells and mediates inhibition of growth factor-induced survival, migration, and angiogenesis signals (Miyazawa et al. 2008, 2009). Tocotrienols act on cell proliferation in a dose-dependent manner and can induce programmed cell death (Nesaretnam et al. 2008). At concentrations of 0.5-5.0 ug/mL, palm TRF dose-dependently protected against LPS-induced cell death (Ahn et al. 2007).

Consistent with observations made in other in vitro systems, T3 inhibits the growth of breast cancer cells in vitro and in vivo (Guthrie et al. 1997; Yu et al. 1999; Takahashi and Loo 2004; Nesaretnam et al. 1998, 2000, 2004; Elangovan 2008) as well as prostate cancer cells (Kumar et al. 2006; Srivastava and Gupta, 2006), gastric cancer cells (Sun 2008, 2009), and colon cancer cells (Shibata et al. 2008).

Tocotrienol is effective in suppressing mevalonate synthesis. By doing so, T3 can deplete tumor tissues of two intermediate products, farnesyl pyrophosphate and geranylgeranyl Pyrophosphate (Pearce et al. 1994).

6) Genotoxicity

The mutagenic potential of repeatedly heated (8- 24 h) refined and crude palm oils has been examined using the Ames test with *Salmonella typhimurium* strains TA100 and TA98 with and without metabolic activation (rat liver S9 fraction; Manorama et al. 1989). Benzo (a) pyrene (5 ug/plate) and sodium azide (1 ug/plate) were used as positive controls for TA 98 and TA 100 respectively. Dimethylsulfoxide (DMSO)-based extracts of the unheated crude and heated refined palm oils did not show mutagenic activity in these tests.

Oliveira et al. (1994) investigated the genotoxicity of red palm oil on bone marrow cells from 8-10 wk old Balb/C female mice. Experimental groups received daily doses of 4.5 g/kg supernatant and sediment of red palm oil (no information on TRF content). Corn oil served as the negative control and cyclophosphamide (20 mg/kg intraperitoneally) was used as the positive control. There were no differences in the frequency of chromosomal aberrations between the two groups of mice.

7) Related studies

a) Subacute toxicity study on the TRF from rice bran oil in rats

Exposure of rats up to 0.5% TRF in diet (rice bran oil source, representing the T3 and T preparation) by gavage resulted in no deaths and no abnormal clinical observations during the 14 d observation period (Sheppard et al. 1996).

b) The mutagenicity of rice bran oil concentrate.

A dimethylsulfoxide (DMSO)-based extract of rice bran oil (up to 5000 ug per plate) was reported to reveal no mutagenic activity in an Ames test using *Salmonella typhimurium* strains TA98 and TA100 with and without metabolic activation (Polasa and Rukmini 1987). Also snack foods fried in rice bran oil did not have any mutagenic effects in the bacterial screening system (Polasa et al. 1987; Raghuram and Rukmini 1995).

c) Reproductive and developmental toxicity

A review of Raghuram and Rukmini (1995) documented normal reproductive performance and lack of teratogenicity of rice bran oil in albino rats of NIN Wistar strain (15 males and 15 females in a three generation study). A diet containing 10% rice bran oil, as compared to peanut oil containing relatively lower amounts of T3, did not result in differences in conception, birth weights, litter size, weaning weights, and pre-weaning mortality, and had no teratogenic effect. There were no differences in body weight, feed efficiency ratio, and nitrogen, phosphorus, and calcium balances in the animals over three generations.

3. Safety/Toxicity of T3 and/or Palm TRF - Clinical studies

A number of clinical trials have been conducted with T3 and T and few side effects have been reported (EFSA 2008). Palm oil is a rich source of T3 that has been a traditional staple food in several Asian countries and also in some African countries. Table 15 summarizes human clinical studies on palm TRFs which had primary endpoints of DNA damage, blood lipid profiles, oxidative stress, and/or bioavailability. As shown in Table 15, daily consumption of up to 330-360 mg palm TRF was not associated with any adverse effects in healthy persons as well as in

subjects with hypercholesterolemia or non-insulin-dependent diabetes mellitus (NIDDM). In these studies, the percentages of T3 in palm TRF varied from 70% to 100%, and the proportion of α-T ranged from 0 to 30%.

Table 15. Human clinical studies reporting no adverse effects of palm TRF

Number and status of subjects	Dose of TRF	Ratio of T3: α-T	Duration	Reference
22 healthy	60 mg/d	70:30	6 mo.	Tan et al. 1991
36 healthy	80-320 mg/d	Mixed (15% δ-)	8 wk	Rasool et al. 2006
64 healthy	160 mg/d	74:26	6 mo	Chin et al. 2008
52 healthy	250 mg/d	Individual doses of α-, γ- or δ-tocotrienyl acetate (81%)	8 wk	O'Byrne et al. 2003*
8 healthy males	300 mg	84.1:2.3	Single dose	Yap et al. 2001
25 hypercholesterolemic	200 mg/d	70:30	6 wk	Qureshi et al. 1991
25 hypercholesterolemic	200 mg γ-T3/d	γ-T3 only	4 wk	Qureshi et al. 1991
36 hypercholesterolemic	260 mg/d	85:15	4 wk	Qureshi et al. 1995
36 hypercholesterolemic	200 mg γ-T3	γ-T3 only	4 wk	Qureshi et al. 1995
44 hypercholesterolemic	240 mg/d	70:30	20 wk	Walqvist et al. 1992
41 mildly hypercholesterolemic	240 mg/d	2:1	4 wk	Mensink et al. 1999
28 mildly hypercholesterolemic	73 mg/d, every other day	50:22.9	4 wk	Ajuluchukwu et al. 2007
50 carotid atherosclerosis	330 mg/d	72.7:27.3	18 mo	Tomeo et al. 1995
50 carotid atherosclerosis	56 mg/d	40:16	2-3 yr	Kooyenga et al. 1997, 2001
32 NIDDM	360 mg/d	80:20	60 d	Wan Nazaimoon et al. 1996

*Individual doses of tocotrienyl acetate

In addition, studies on rice bran TRF did not report any adverse effects (Qureshi et al. 1997, 2001, 2002; Kooyenga et al. 2001; Baliarshingh et al. 2005; Rubin et al. 2008).

Table 16. Human clinical studies reporting no adverse effects of rice bran TRF

Number and status of subjects	Dose of T3	Ratio of T3: α-T	Duration	Reference
28 hypercholesterolemic	3 mg/day T3; 0.04 mg/kg BW/day;	T3: α-T: δ-T= 86.9:8.7: 4.4	35-70 d	Qureshi et al. 2001
36 hypercholesterolemic	0.33mg/kg BW/d from a processed rice bran oil	25 mg α-T, 42 mg γ-T, 20 mg δ-T	4 wk	Qureshi et al. 1997
90 hypercholesterolemic	25, 50, 100, and 200 mg/d	T3: α-T: δ-T= 86.9:8.7: 4.4	5 wk	Qureshi et al. 2002
25 carotid atherosclerosis	112 mg/d	T3:α-T=52:60	3 yr	Kooyenga et al. 2001
19 NIDDM	3mg/kg BW/d	T3:α-T=92.5: 7.5	60 d	Baliarshingh et al. 2005
23 familial dysautonomia	100 mg/d	NA	3–4 mo	Rubin et al. 2008

Details

1) DNA damage study—at 160 mg/d for 6 mo

Chin et al. (2008) evaluated the effect of T3 on DNA damage. In this study, a daily dose of 160 mg of commercially available palm TRF (a mixture of α -, β-, γ-, and δ-T3 and α-T) was given for 6 mo to 64 healthy subjects aged 37-78 yr. The total T3 isomer ratio compared with T is approximately 74:26. No adverse effects were reported. Instead, results showed a significant reduction in DNA damage as measured by the comet assay after 3 mo ($P < 0.01$) and remained low at 6 mo ($P < 0.01$).

2) Lipid profile studies- 140-360 mg/d for up to 36 mo.

In general, the palm TRF lowered blood cholesterol concentrations without showing any adverse effects in healthy volunteers and subjects with hypercholesterolemia or NIDDM. For example, administration of 60 mg palm TRF in 22 healthy Malaysian subjects (with no age limitation) for 30 d lowered both serum total cholesterol (TC) and low-density-lipoprotein

cholesterol (LDL-C) concentrations. The magnitude of reduction of serum TC ranged from 5 to 35.9% whereas the reduction of LDL-C values ranged from 0.9 to 37.0% when compared with their respective starting values. No adverse effects were reported (Tan et al. 1991).

Rasool et al. (2006) assessed the effects of TRF supplementation (up to 320 mg/d of TRF composed of 34.6% α-T3, 24.6% γ-T3, 15% δ-T3, and 26.2% α-T: Golden Hope Plantations, Malaysia) for 2 mo on blood lipid profiles and arterial compliance in 36 healthy Malaysian men. The authors stated that the palm TRF supplements at doses up to 320 mg/d were well-tolerated with no serious adverse events.

The palm TRF was tested in 16-25 hypercholesterolemic Malaysian subjects aged 30-60 yr at a dose of 200 -300 mg/d over a 4-8 wk period (Qureshi et al. 1991, 1995). Significant reductions in serum total cholesterol, LDL cholesterol, apolipoprotein B, thromboxane B2, and platelet factor were observed. No adverse effects were reported.

In a study with 44 Australian subjects with hypercholesterolemia (aged 25-75 yr; Wahlqvist et al. 1992), administration of palm oil for 20 wk (up to 240 mg T3/d) did not show any changes in serum lipid profiles and plasma prostaglandins and platelet functions. No adverse effects were reported.

Mensink et al. (1999) reported that the TRF supplements (240 mg palm TRF/d for 4 wk) used had no adverse effects in 41 Netherlands men with slightly elevated lipid concentrations (aged 21-61 yr). Also, the study of Wan Nazaimoon et al. (1996) showed that administration of 360 mg palm TRF/d (a mixture of 288 mg T3 and 72 mg α-T) for 60 d did not cause adverse changes in 32 Malaysian subjects with NIDDM (aged 31-70 yr).

Ajuluchukwu et al. (2007) studied the effect of palm oil TRF on the serum lipid profiles of 28 individuals aged 18-80 yr with mild hypercholesterolemia and another cardiovascular risk

factor. Subjects were randomly assigned to consume tocotrienols as TOCOVID™ Suprabio™ capsules (consisting of 15.38 mg α-T3, 28.20 mg γ-T3, 6.42 mg δ-T3 and 22.90 mg α-T) or 500 mg or 1000 mg vitamin E (α-T) capsules per day or every other day (n=16) for 4 weeks. Compared to the T supplement, the T3 supplement significantly decreased total cholesterol and LDL-cholesterol, but not HDL-cholesterol or triglyceride concentrations. No information about adverse effects was reported.

Kooyenga et al. (1997, 2001) investigated the effect of supplementation with an extract from palm oil (52 mg of T3, 60 mg of α-T) for 2-3 yr on carotid atherosclerosis. The authors stated that there were no reports of headache, intestinal upset, muscle weakness, or persistent liver weakness abnormalities in the 50 Malaysian subjects studied.

In another placebo controlled study, α-, γ-, and δ–T3 acetates were administered individually to 52 American healthy subjects at a daily dose of 250 mg for 8 wk (O'Byrne *et al.* 2000). No adverse effects of T3 ester ingestion were observed in this study.

As shown in Table 16, several studies reported no adverse effects with the supplementation of TRF or a processed oil extracted from rice bran oil. Qureshi *et al.* (1997) performed a randomized double blind placebo controlled trial in which 41 hypercholesterolemic Malaysian subjects were first placed on the low fat diet for 4 wk and then given 200 mg/d of TRF from rice bran oil or corn oil as placebo. No adverse effects due to T3 ingestion were observed.

Qureshi et al. (2001) investigated the efficacy of TRF of rice bran alone and in combination with the cholesterol lowering drug, lovastatin. The TRF consisted of 8.7% α-T, 4.4% δ-T, and 86.9% T3. The combination of TRF and lovastatin significantly reduced lipid metabolites by 20 - 25% ($P < 0.001$) in these subjects. The authors stated that subjects reported no side effects during the 25-wk study.

Qureshi et al. (2002) investigated the effect of 4 doses (25, 50, 100, and 200 mg/d) of a TRF (8.7 % α-T, 15.5 % α-T3, 1.6% β-T3, 39.4 % γ-T3, 5.2 % δ-T3, and 4.4 % δ-T, 20.9% D-desmethyl (D-P_{21}-T3), plus D-didesmethyl (D-P_{25}-T3) T3, 4.3 % unidentified tocotrienols) extracted and purified from rice bran oil on the suppression of serum cholesterol in Malaysian adults. No information on adverse effects was reported in the study.

Baliarsingh et al. (2005) reported the therapeutic impacts of palm TRF (3 mg palm TRF/kg BW/d, 60 d) on serum lipoprotein lipid levels in 19 Indian subjects with NIDDM. The composition of tocols in palm TRF was 7.5% α-T, 14.6% α-T3, 2.2% β–T3, 38.8% γ-T3, 4.6% γ-T, and 29.9% δ-T3. No adverse effects were reported.

Kooyenga et al. (2001) investigated the effect of supplementation with an extract from rice bran for 1 yr on carotid atherosclerosis. Each rice bran oil capsule (produced by Rangsit Biotech, Bangkok) provided 52 mg of T3 and 60 mg of α-T. The authors stated that there were no reports of headache, intestinal upset, muscle weakness, or persistent liver weakness abnormalities in the 50 Malaysian subjects studied.

3) Oxidative stress and biomarker test- 240 mg/d T3 and 90 mg/d α-T for 18 mo

In a randomized, placebo-controlled study, 50 subjects (aged 49-83 yr) with carotid atherosclerosis received palm TRF (240 mg T3 and 90 mg T/d) or placebo in addition to standard medical therapy over 18 mo (Tomeo et al. 1995). Serum measures of oxidative stress (TBARS) showed a significant decrease with no appreciable change in serum lipids or other biochemical markers. Both T3 and placebo groups displayed attenuated collagen-induced platelet aggregation responses ($P < 0.05$) as compared with entry values. Serum total cholesterol, low density

lipoprotein cholesterol, and triglyceride values remained unchanged in both groups, as did the plasma high density lipoprotein cholesterol values. No adverse effects were reported.

4) Bioavailability study - 300 mg, a single dose

In a study reported by Yap et al. (2001), healthy volunteers were administered a single oral dose of 300 mg TRF (841 mg T3 and 23.3 mg α-T, Tocovid, Hovid Pte.Ltd, Malaysia) under fed or fasted conditions to study their bioavailability. No adverse effects were reported.

4. Safety of vitamin E

Three international expert groups have recently evaluated the safety of vitamin E: the Antioxidant Panel of the Food and Nutrition Board at the IOM of the U.S. National Academy of Sciences (IOM 2000), the EU Scientific Committee on Food (SCF 2003), and the UK Expert group on Vitamins and Minerals (EVM 2003). The IOM set the tolerable upper intake level (UL) for adults at 1000 mg/d of any supplemental form of α-T (IOM 2000). Unlike the IOM's recommendations that are based on α-T only, two European expert groups set the ULs of vitamin E based on TEV. The SCF, based on a placebo controlled, dose-response supplementation study in 88 healthy humans (Meydani et al.1998), set the NOAEL at 540 mg TEV, the highest dose used in the study. Considering an uncertainty factor of 2 to cover for inter-individual differences in sensitivity, the SCF established an UL of 270 mg TEV for adults, which was rounded to 300 mg TEV (SCF 2003). The EVM established a NOAEL of 540-970 mg TEV, based on three placebo controlled human studies (Gillilan et al. 1977, Meydani et al. 1998, Stephens et al. 1996, EVM 2003). An uncertainty factor to account for inter-individual differences was not considered since the results of the larger trial by Stephens et al. (1996) support a NOAEL of 540 mg TEV, so that the UL (defined by the EVM as a

safe UL for consumption over a lifetime) was established as 540 mg TEV for supplemental vitamin E (EVM 2003).

Most of the safety studies on vitamin E were carried out in the past few decades. Table 17 summarizes subacute and subchronic toxicity studies that have been carried out in animals (Demole 1939; Weissberger and Harris 1943; Levander et al. 1973; Dysma and Park 1975; Krasavage and Terhaar 1977; Abdo et al. 1986; Diplock 1995). There is no evidence that there is any detrimental effect attributable to vitamin E up to 500 mg/kg diet.

Table 17. Studies on subacute and subchronic toxicity of vitamin E.

Species	Route	Dose	Duration	Result	Reference
Rat	Oral	130 mg/kg/d	2 mo	No abnormalities	Demole 1939
Rat	Oral	100 mg/d	4 mo	Small increase in phosphorus turnover in the femur. Otherwise, no abnormalities	Weissberger and Harris 1943
Rat	Oral	10 mg/d	4 mo	No abnormalities	Weissberger and Harris 1943
Rat	Feed	500 mg/kg diet	1 mo	Small effect on liver TG	Levander et al. 1973.
Rat	Feed	875 and 1750 mg/kg	3 mo	No abnormalities	Dysma and Park 1975
Rat	Feed	0.0002 and 0.2% in diet	3 mo	No abnormalities	Krasavage and Terhaar 1977
Rat	Gavage	125-500 mg/kg 2000 mg/kg	3 mo 3 mo	No abnormalities Extended prothrombin time	Abdo et al. 1986

Adopted from Diplock 1995

Table 18 shows that there is no adverse effect of vitamin E (up to 2% in diet) with respect to the teratogenicity and reproductive toxicity (Hook et al. 1974; Krasavage and Terhaar 1977; Diplock 1995).

Table18. Studies on teratogenesis and reproductive toxicity of vitamin E.

Species	Route	Dose	Duration	Result	Reference
Rat	Feed	0.00, 0.2, and 2.0%	Days 6-16 of pregnancy	No difference between treatment and placebo groups	Krasavage and Terhaar 1997
Rat	Feed	0.002, 0.2, and 2.0%	F_1 generation: mated on days 112 and 175 of dosage: F_0 generation: 268 d	No effects on reproductive toxicity, teratogenicity, clinical and hematologic indexes	Krasavage and Terhaar 1997
Mouse	Gavage	591 IU/mouse	Days 7-11 of pregnancy	One malformation in 91 mice	Hook et al. 1974

Adopted from Diplock 1995

As shown in Table 19, vitamin E has no carcinogenic activity (up to 0.2% in diet for 24 mo; Weldon et al. 1983; Yang and Desai 1977; Diplock 1995). Table 20 shows that α-T intake of up to 600 mg/d for 12 wk did not show adverse effects as evaluated by clinical chemical indices, liver, kidney and thyroid functions, serum lipids, PT time, and urinary indices (Farrell and Bieri 1975; Corrigan et al. 1982; Stampfer et al. 1983; Kitagawa et al. 1989; Diplock 1995). These data support the IOM's assessment that up to a vitamin E (as α-T) intake of 1000 mg/d is safe for human consumption.

Table19. Studies on chronic toxicity and carcinogenicity of vitamin E

Species	Route	Dose	Duration	Result	Reference
Rat	Feed	500, 1000, and 2000 mg/kg/d	24 mo	No significant effects on a range of clinical chemical indexes; slight increase in liver weight and in liver enzymes in serum; hemorrhage (anti-vitamin K effect) at 2000 mg/kg/d; decrease in mammary tumors	Weldon et al. 1983
Rat	Feed	25, 250, 2500, 10,000, and 25,000 IU/kg	8-16 mo	No abnormalities at low doses; increase in serum alkaline phosphatase and relative heart and spleen weight at high doses (>10,000 IU/kg); no change in prothrombin time; no carcinogenicity	Yang and Desai 1977

Adopted from Diplock 1995

Table 20. Human clinical studies of vitamin E.

Subjects	Dose	Duration	Result	Reference
Adults, *n* = 28	100-800 IU/day: T	4 mo to 21 yr (mean 2.9 yr)	No evidence of toxicity judged by clinical chemical blood indexes (blood coagulation, liver enzymes, and thyroid hormones)	Farrell and Bieri 1975
Volunteers, n = 30 (20)	800 IU/d vitamin E	16 wk	No differences between groups	Stampfer et al. 1983
Volunteers, n = 19	600 mg/d α-T	12 wk	No adverse effects (clinical chemical indices, liver, kidney, and thyroid function, serum lipids, and prothrombin time, and urinary indices all normal)	Kitagawa and Mino 1989

Adopted from Diplock 1995

IV. Conclusions

The information/data provided by NutraSource/MPOB in this report and supplemented by the publicly available literature/toxicity data on T3 and T provide a sufficient basis for an assessment of the safety of T3 for the proposed use as an ingredient in food, when prepared according to appropriate specifications and used according to GMP. Key findings are summarized here:

1. T3 and T are well characterized and of consistent quality across lots and free from chemical and microbial contamination.
2. T3 and T have a long history of use in foods and beverages in the U.S.
3. T3 and T extraction processes have been safely used for many yrs in the dietary supplement industry.
4. The safety and nutritional benefits of T3 and T are well established by human clinical trials and animal studies of T3. There are no indications of significant adverse effects related to T3 consumption in the publicly available literature at the proposed use levels.
5. Increased consumption of vitamin E has been recommended by the USDA Dietary Guidelines Committee and IOM.
6. Intended use of T3 as part of the proposed food use results in levels of exposure significantly below those associated with any adverse effects and provides a reasonable certainty of safety.

Therefore, not only is the proposed use of TRF safe within the terms of the Federal Food, Drug, and Cosmetic Act (meeting the standard of reasonable certainty of no harm), but because of this consensus among experts, it is also *Generally Recognized as Safe* (GRAS).

V. Discussion of Information Inconsistent with GRAS Determination

We are not aware of information that would be inconsistent with a finding that the proposed use of T3 preparations in foods and beverages, meeting appropriate specifications and used according to GMP, is GRAS.

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