Request for scientific evaluation of “Substantial equivalence” for Prima Pharm’s phytosterol product, intended to be used in specified foods and under regulation EC 258/97 of the European Parliament

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SUMMARY

Under Regulation EC/258/97 on Novel Foods and Novel Food Ingredients, Teriaka received the approval of the use of its phytosterol product as Novel Food Ingredient under European Commission Decision 2004/336/CE.

Prima Pharm's product is manufactured by DRT under the brand name of «Beta Sitosterol» which is also one of the sources of Teriaka's phytosterol product.

Prima Pharm's product is extracted from tall oil pitch from pine Trees (Pinus Maritima L.).
It is a mixture of Sistosterol, Sistostanol, campesterol and campestanol with a small percentage of minor phytosterol and stigmasterol. The certificates of analysis provided demonstrate a composition in sterols in compliance with the commission decision 2004/336/CE.

The manufacturing process of Prim Pharm's product is the same as the one described in the Teriaka Novel Food Application (for sterols from tall oil of Pinus Maritima L.).

The sterols are also very little contaminated in term of Dioxins, PCB, PAH, heavy metals, pesticide residues. The purity and composition of the presented phytosterol allows considering the product as safe for human consumption.

The Prima Pharm's product is intended to be consumed in a manner identical to the Teriaka product. **The intended uses of Prima Pharm's product are thoses specified in Commission Decision 2004/336 and in the two Opinions of the Novel Food Board (FIN) "on substantial equivalence in the case of a milk and soya drink with added Diminicol Ingredient" and "in the case of fermented milk products with added Diminicol" expressed respectively the 1st of July 2004 and the 4th of October 2004.**

The labeling of the product will follow the requirement of the relevant commission regulation (608/2004).

This document provides evidence to confirm that Prima Pharm's product is substantially equivalent to Teriaka Novel food Approval 2004/336/CE and to the two Opinions of the Novel Food Board (FIN) in composition, manufacturing process, nutritional value, metabolism, level of undesirable substances and intended use.

1-INTRODUCTION

The European regulation nº 258/97 on Novel Foods and Novel Food Ingredients sets out rules for authorization of GM food products and other categories of novel foods. Phytosterols and phytosterols esters fall under the scope of the above mentioned regulation and are identified as "novel" food under article 1 [(1)].

Phytosterols and phytosterol esters were authorized to be placed on the market for the EU on 24 July 2000 (Commission Decision 2000/500/EC) [(2)]. Since then other applications were deposed who were authorized under the European commission decision 2004/333/CE [(3)], 2004/334/CE [(4)], 2004/335/CE [(5)], 2004/336/CE [(6)] and 2004/845/CE[(7)].

The purpose of the document is the demonstration that Prima Pharm's product is substantially equivalent within the terms of article (3) of the regulation nº258/97[(1)] to already approved ones and more specifically the Teriaka (2004/334/CE) [(4)] one. The Prima Pharm's product fall under the scope of class1.1 as defined in the chapter 4 of the recommendation [(1).]

Based on these decisions, Prima Pharm applies for a favorable opinion in order to notify its phytosterol as ingredient in the range of product types into which phytosterols may be added, according to decision 2004/336/CE[(6)] and according to the two Opinions of the Novel Food Board (FIN) "on substantial equivalence in the case of a milk and soya drink with added Diminicol Ingredient" and "in the case of fermented milk products with added Diminicol" expressed respectively the 1st of July 2004 and the 4th of October 2004. The opinion of the Food Standards Agency will be used to support notifications to be made either by Prima Pharm, on its own name for the benefit of its future customers or to support notifications by its customers.

This application is also based on the relevant SCF opinion on the safety of phytosterols and phytosterols esters [(8,9,10)] in relation to the authorisations that we are claiming substantial equivalence to.

2-ADMINISTRATIVE INFORMATION

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The supplier of Prima Pharm's product is:

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Prima Pharm's product is manufactured by DRT under the brand name of «Beta Sitosterol».

3-COMPOSITION

The Prima Pharm's phytosterol is isolated from tall oil from *Pinus maritima L*.

According to SCF opinion[8], Teriaka proposed to obtain their sterols from Tall oil (*Pinus maritima)* or vegetable oil. *Pinus maritima* (Synonym *Pinus Pinaster)* is a Mediterranean tree and can neither be found in Finland nor in Northern European countries. The biggest forest of *Pinus maritima* is located in the south west of France (Landes) where DRT is based.
According to DRT, the source of Prima Pharm's product ("Beta-Sitosterol" product manufactured by DRT) is also one of the sources of Teriaka's product, reinforcing thus the substantial equivalence claimed.

The source of the product is tall oil pitch that was also used for the obtainment of Teriaka phytosterols. The Prima Pharm's product is tall oil derived sterol with betasitosterol as main component. Specification sheet and analytical results on sterol composition are given in Appendix 1 and 4.

3.1 Manufacturing process

The manufacturing process described by the supplier (see Appendix 2), includes 3 major steps that are summarized here below

1-Extraction
The tall oil pitch is extracted at pH superior 7 to obtain crystals. These crystals are washed thoroughly with water in order to remove tall oil impurities which are water soluble.

2- Crystallization
The primary crystals are then redisolved in a hydrocaronated solvent and then methanol is used to recrystallise the product. After cooling, the pure crystals are recovered by filtration.

3- Drying
The crystals of product are dried under vacuum, milled and sieved to obtain pure crystals.

This process is the same as the one described in the Teriaka Novel Food Application (for sterols from tall oil of Pinus Maritima L.). There is no difference between this process and the process described in Teriaka Novel food Application. This statement is also reflected in the point 3.2 (page 4) of the SCF Opinion [8].
Prima Pharm's product as well as one of the sources of Teriaka' product are the phytosterols called "Beta Sitsoterol" that are manufactured by DRT. The two products come from the same and unique source, thus the manufacturing process is the same.

Adequate quality control and methods such as HACCP, performed by the supplier warrant the control of the process

A statistical analysis on the phytosterols purity level was performed on all the batches produced during a one year period (19/04/04 – 25/04/2005). The data are presented in Appendix 3. The purity mean obtained on 317 samples was 99.4% with a standard deviation of 0.70 demonstrating the very good control of the manufacturing process.
DRT will only supply to Prima Pharm lots of Prima Pharm's product with a purity of more than 99% in phytosterol/phytostanol.

3.2. Specifications

The sterol profile is defined in annex 2 of decisions 2004/336/CE[(6)] under the following section "Specifications of phytosterols and phytostanols for the addition to foods and food ingredients".

To be considered as "substantially equivalent", the Prima Pharm's product must meet the same requirements. A comparative evaluation of the EU requirements for Teriaka[(6)] versus Prima Pharm's specifications is presented here below:

Composition (with GC-FID or equivalent method)	**Requirements 2004/336/CE[(6)] Teriaka**	**Prima Pharm's product Specifications**
β- sitosterol (%)	<80	70-80
β- sitostanol (%)	< 15	10-15
campesterol (%)	<40	8-11
campestanol (%)	<5	1-4
Stigmasterol (%)	< 30	<2*
Brassicasterol (%)	<3	<2*
other sterols/stanols (%)	<3	<3
Purity (%)	>99	>99

*In the certificate of analysis of the Prima Pharm's product, stigmasterol and brassicasterol are expressed under the title "other minor sterols" (see Appendix 1). To be in compliance with the requirements, the stigmasterol, as isolate compound, must be inferior to 30% and the brassicasterol under 3%. In the case of the Prima Pharm's product, the sum of stigmasterol

plus brassicasterol plus other minor sterols is under 2% allowing *de facto* levels under the specified ones.

According to the new analytical data defined by the commission decision 2004/336/CE[(6)] the total sterols contain must be superior to 99%. As already demonstrated in point 3.1 and in Appendix 3, the mean purity obtained on the Prima Pharm's product batch is superior to the required specification. In any case Prima Pharm will only buy to DRT lots of Prima Pharm's product with a purity of more than 99% in phytosterol/phytostanol.

In the following table, a compilation of the GC-FID analyses performed on different batches of Prima Pharm's product are presented versus approved specifications:

	Composition				
	b- sitosterol (%)	**b- sitostanol (%)**	**Campesterol (%)**	**Campestanol (%)**	**Other sterols /stanols (%)**
Requirements 2004/336/CE	<80	<15	<40	<5	<3
Batches					
036486*	76.6	11.6	8	1.1	2.5
038722*	76.4	11.5	8.6	1.1	2.4
033650*	75.9	11.3	9.1	1.2	2.5
033652*	75.9	11.1	9.3	1.2	2.5
011607	76.7	11.1	8.6	1.1	2.5
011575	76.9	10.9	8.6	1.1	2.5
11104	76.3	12.0	8.6	1.0	2.1
030661	76.8	11.4	8.3	1.1	2.4
034043	75.9	11.3	9.3	1.1	2.4
21041	74.0	14.3	8.4	1.4	1.9
21035	75.3	12.8	9.0	1.4	1.5
21032	74.8	13.0	9.0	1.5	1.7
21027	75.2	12.7	8.9	1.3	1.9

The batches identified by the * correspond to the certificate of analysis presented in Appendix 4). The others correspond to the batches used for the determination of the undesirable substance (Appendix 5,6,8). They are all representative of the quality of the Prima Pharm's product: all the data are in compliance with the requirements[(6)] demonstrating the equivalence.

4-NUTRITIONAL VALUE / METABOLISM

4.1 Anticipated intake

Prima Pharm's product will be added to the same products as those already approved in decision 2004/336 and in the two Opinions of the Novel Food Board (FIN) "on substantial equivalence in the case of a milk and soya drink with added Diminicol Ingredient" and "in the case of fermented milk products with added Diminicol" expressed respectively the 1st of July 2004 and the 4th of October 2004. For this reason the daily intake of sterols will not be increased within the European Community population.

4.2 Nutritional information

Two potential nutritional effect are linked to the consumption of phytosterol: reduction in circulating cholesterol and reduction of vitamin and nutriment absorption

4.2.1 Reduction in circulating cholesterol

Phytosterols are widely found in the plant kingdom and are chemically similar to cholesterol.

Phytosterols are present in diets. Typical daily dietary intakes of phytosterols range from 100 to 300 milligrams. It is higher in vegetarians. There are over 40 phytosterols, but beta-sitosterol is the most abundant one, comprising about 50% of dietary phytosterols. The next most abundant phytosterols are campesterol (about 33%) and stigmasterol (about 2 to 5%). Other phytosterols found in the diet include brassicasterol, delta-7-stigmasterol and delta-7-avenasterol.

The consumption of phytosterols was demonstrated to lower blood levels of cholesterol, by inhibiting the absorption of dietary and endogenous produced cholesterol from the small intestine. The maximum phytosterol intake recommended by the SCF[(14)] is 3 grams per day. This recommendation will be followed by Prima Pharm and its customers in the foods and beverages enriched with Prima Pharm's product in order to be essentially equivalent to Teriaka[(6)].

Additionally and accordingly to Commission Regulation 608/2004, Prima Pharm and its customers will advice on the labelling of the foods and beverages enriched with Prima Pharm's phytosterols that these products are intended exclusively for people who want to lower their blood cholesterol level, that patients on cholesterol lowering medication should only consume these products under medical supervision, that these products may not be nutritionally appropriate for pregnant and breastfeeding women and children under the age of five years and that the consumption of more than 3 g/day of added plant sterols/plant stanols should be avoided.

The lowering effect of the plant sterols on the blood cholesterol was demonstrated during the last 20 years. Thousands of people were enrolled in clinical trials which showed the efficacy and safety of the preparation based on phytosterols.

Exhaustive results compiled from recent studies are summarized in the following table: It is important to note that no adverse events were collected during the clinical trial, demonstrating thus the safety of the phytosterols.

Food type	**Number of subject** **duration**	**Diary dose**		**Ref erence**
500 mL Sterol enriched Milk blend,	71 Healthy subjects 4 weeks for each product	- Placebo 0 g/day; - Lo group 1.2 g/day - Hi group 1.6 g/day	*Double blind, cross over placebo controlled study.* Substantial reduction of LDL cholesterol and the two treated group: no significative difference between the two administered doses.	Thomsen et al, 2004
300ml/d sterol enriched milk	39 Healthy subjects 12 weeks	- Placebo, - 2.0 g/day plant sterol ester alone or combined with 25 g/day of placebo or spread.	*single blind crossover design with 4 phases of 3-week interventions* Sterol enriched milk and sterol enriched spread were equally efficacious in lowering total and LDL-cholesterol as compared to placebo by 6-8% and 8-10%, respectively.	Noakes et al 2004
low-fat milk-based beverage	26 Healthy subjects 1 week for each product	Placebo, 2.2 g plant sterol equivalents or 2.2 plant sterol ester equivalent	*Double-blind, randomized, crossover study.* Both milks containing plant sterols and plant sterol ester reduced beta-carotene and alpha-tocopherol bioavailability and cholesterol absorption in normocholesterolemic men.	Richelle et al, 2004
4 phytosterol ester-enriched low-fat foods: bread, breakfast cereal, milk and yoghurt.	58 Healthy subjects 3 weeks each product	1.6 g/day of phytosterols as sterol esters.	*randomized, incomplete crossover, single-blind study* Serum total and LDL cholesterol levels were significantly lowered by consumption of phytosterol-enriched foods: milk (8.7 and 15.9%) and yoghurt (5.6 and 8.6%). Serum LDL cholesterol levels fell significantly by 6.5% with bread and 5.4% with cereal. Lipid-adjusted beta-carotene was lowered by 5-10% by sterols in bread and milk, respectively. Plant sterols in low-fat milk were almost three times more effective than in bread and cereal.	Clifton et al, 2004
Orange juice	72 mildly hypercholesterolemic subjects 8 weeks	- placebo orange juice -or plant sterol fortified orange juice (2g/d)	*placebo-controlled, double-blind, randomized trial.* Sterol supplemented orange juice significantly decreased total (7.2%), LDL (12.4%), and non-high-density lipoprotein (HDL) cholesterol (7.8%) compared with baseline and compared with placebo. Apolipoprotein B levels were significantly decreased (9.5%) with sterol orange juice. There were no significant changes in HDL cholesterol or triglycerides with the sterol orange juice.	Devaraj et al, 2004

Margarine containing sterol	42 healthy subjects	30 g/day in 2 servings	*Randomized double-shielded trial* The subjects consuming margarine with sterols showed a significant (11%) decrease in LDL-C (P<.001). After the consumption of margarine with sterols, the adhesion and aggregation time of blood platelets was significantly prolonged after collagen-epinephrine activation.	Kozlowska-Wojciechowska et al, 2003
Phytosterol-enriched margarines	85 subjects with type 2 diabetes mellitus 12 weeks	2 x 10 g/day of spread with or without 8 % phytosterol-esters.	*Randomized, placebo-controlled, double-blind clinical trial in two parallel groups.* After 4 weeks, total and LDL cholesterol were significantly reduced in the phytosterol group by 5.2 % and 6.8 %, respectively, compared to baseline ($p < 0.05$). After 8 and 12 weeks, these reductions became smaller and were not significant any more compared to baseline or between the groups, but a repeated measurement analysis demonstrated a significant difference for both variables between the two groups (each $p < 0.05$). HDL cholesterol was significantly increased in the phytosterol group compared to the placebo group after 8 and 12 weeks demonstrating the difficult maintenance under free-living conditions over time.	Lee et al, 2003
polyunsaturated spread	50 Healthy subjects total of 11 weeks	25 g of poly unsaturated spread with or without 2 g of plant sterols for 4 weeks, crossing over in the last 4 weeks to the alternate spread.	*Parallel butter phase followed by double-blind, randomized, cross-over polyunsaturated spread phases* Replacing butter with a standard polyunsaturated fat spread reduced mean plasma total cholesterol concentrations by 4.6% and low-density lipoprotein cholesterol by 5.5%. Replacing butter with a polyunsaturated spread containing plant sterols reduced plasma total cholesterol by 8.9% and low density lipoprotein cholesterol by 12.3%. Plasma high density lipoprotein cholesterol concentration was the same on the three diets.	Cleghorn et al, 2003
spreads enriched with plant sterols	42 healthy subjects 8 weeks	Sterol content of the enriched spread 8.3%. Intake of 25 g/day	*randomized double-blind placebo-controlled cross-over study* Serum total and LDL-cholesterol concentrations lowered by 7% and 10%, respectively, with the plant sterol-enriched compared to the control spread. Serum HDL-cholesterol concentration did not significantly differ between the two spreads. Apolipoprotein B concentrations lowered by 8% with the plant sterol-enriched spread.	Temme et al, 2002

4.2.2 Reduction of vitamin and nutriment absorption

The metabolism of ingested phytosterols and their influence on the fat soluble vitamin absorption had been investigated, especially, vitamins A, D, E, K and beta carotens. The SCF adopted an opinion in 2002 upon "the long term effects on the intake of elevated levels of phytosterols from multiple dietary sources, with particular attention to beta carotens"[15]. The efficiency of absorption of such compound varies depending on the product. It is around 40% for the cholesterol, vitamin D, E, K, between 50 and 70% for the retinol when the beta carotene absorption can range between 10 and 90%. The bioavailability of carotenoids can be affected by the meal contents of dietary fiber and fat, the food matrix, the cooking procedure and cholesterol-lowering medication. The latter has been reported to reduce the levels of circulating beta-carotene by 30 to 40%. Beta-carotene being a source of a vitamin A, it is important not to lower its intake especially in the case of people with diets with poor intake of animal products. But it is accepted that doses up to 10 mg/day of beta-carotene from fruits and vegetable can confer health benefits.

Accordingly to Commission Regulation 608/2004, Prima Pharm and its customer will advice on the labelling that the foods or beverages enriched with Prima Pharm's product are to be used as a part of a balanced and varied diet, including regular consumption of fruit and vegetables to help maintain carotenoid levels.

5-INTENDED USE

The intended use of Prima Pharm's product is claimed in accordance with the food applications covered by decisions 2004/336/CE [6].

That is to say:

- Yellow fat spreads as defined by Council Regulation (EC) No 2991/94 [10], excluding cooking and frying fats and spreads based on butter or other animal fat.
- Milk based fruit drinks,
- Yoghurt type products,

- Cheese type products (fat content $\leq$ 12 g per 100 g) in which milk fat and or protein has been partly or fully replaced by vegetable fat and/or protein.

The 1st of July 2004 and the 4th of October 2004, the Novel Food Board (FIN) extended the intended use of Teriaka product to

- Milk type products
- Soya drinks
- Fermented milk products.

The presentation of the food products with added Prima Pharm's phytosterols will comply with article 2 of the here cited decision(6).

The products shall be presented in such a manner that they can be easily divided into portions that contain either a maximum of 3 g (in case of one portion a day) or a maximum of 1 g (in case of three portions a day) of added phytosterols/phytostanols.
The amount of phytosterol added to a container of beverage will not exceed 3 g.

6-LEVEL OF UNDESIRABLE SUBSTANCES

6.1 Chemical contaminants

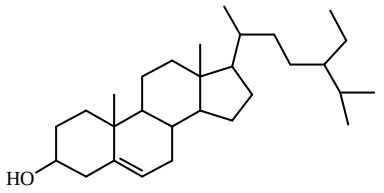
A special focus must be brought on the high purity of the product. The raw material supplier realized several studies on the phytosterols in order to demonstrate the low levels of contaminants in the product.

No levels of contaminants were included in the Decision in relation with Teriaka application(6), but it is important to demonstrate the potential contamination stays within acceptable levels in compliance with European regulation. Some data have been compiled from the already cited SCF Opinions to allow a comparison between an approved phytosterol and Prima Pharm's one demonstrating thus the high purity level of the Prima Pharm's product.

Analyses were performed on the Prima Pharm's product that demonstrated the purity of the material in relation to the presence of PCDD's and PCDF's (appendix 5), PAH's (appendix 6), herbicides and pesticides (appendix 7) and heavy metals (appendix 8).

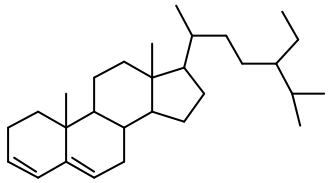
Using an effective GC-FID method, no sterenes or sterol esters have been found by the raw material supplier.

Sterenes are the products of the dehydration of the 3-hydroxy sterols as shown below.



Sitosterol (stigmast-5-èn-3β-ol)

High temperature
or acidic medium



Main Sterene from sitosterol (stigmasta-3,5-dien

Important contaminants are more specifically discussed here below.

Dioxins (see Appendix 5)

According to the new directive ITEC/OMS 1997, the total amount of ITEC must be less than 0.51 pg/g expressed as toxicity equivalents of 2,3,7,8-TCDD[11]. The analysis report presented in Appendix 5 shows value of 0.40 pg/g. This value is inferior to the ones contained in Teriaka commission opinion[4] (0.51 pg/g)

PAH (polycyclic aromatic hydrocarbons) (see Appendix 6)

- Benzo (a) pyrene

The level of Benzo (a) pyrene is under 10 µg/kg as mentioned in the SCF opinions and complies with the requirement of the SCF report of June 1993 about smoke flavouring [12].

-Acenaphthylene

The levels are inferior to 10 µg/kg less than 150 ppb, pyrene less than 30 ppb, the other 13 PAHs showing less than 1-10 µg/kg depending on the individual compound.

- Benzo antharcene.

The benzoanthracene levels are also inferior to 10 µg/kg corresponding of detection limit and comply with the requirement of the SCF report of June 1993 about smoke flavouring [12].

- Other products:

Other PAH(s) classified as priority pollutants by the US environmental Protection agency are all under 40 µg/kg.

All the presented values are very close to the ones described in the SCF opinions of Teriaka products [6]

Herbicides and Pesticides (see Appendix 7)

The polychlorinated biphenyls (PCBs) and pesticides residues researched in compliance with European Pharmacopea are below the detection limit. The possible contaminants such as pyrethrines, organochlorine and organophosphorus were also analyzed and were under the detection limits allowing levels inferior to pharmacopea limits for such product family.

6.2 Heavy Metals

Heavy metals were evaluated in one batch and they are individually all under 2 ppm, the sum being largely under 20 ppm. In four batches, lead and arsenic were specifically quantified. (See Appendix 8) They were under 0.1 ppm (100 ppb). All the obtained values are similar to the one presented by Teriaka according the already cited SCF Opinion[8].

6.3 Microbiological contamination

The microbiological quality of the phytosterols was clearly demonstrated on more than 35 batches (see Appendix 9). The bacterial contamination was always under 15 CFU /g of product and mould and yeast were always under 20 CFU/g. The levels of contamination presented were always inferior to the levels commonly accepted in food products that are under 1 000 CFU/g for the bacterial contamination and under 100 CFU for fungal contamination.
Thus the absence of specific bacteria such as *enterobacteries, E. coli, Salmonella, P. aeruginosa* and S. *aureus* establishes the strict control of the manufacturing process and packaging steps in regard of microbiological contamination.

This high microbiological quality ensures that the incorporation of Prima Pharm's product in further manufacturing process is safe.

7-OTHER RELEVANT DATA

7.1 Labelling

The labelling of the product will be done in accordance with the commission regulation 608/2004 of 31 march 2004 concerning the labelling of food with added phytosterols[13] and more specifically to article 2 of this regulation.

The importance of the consumer information is focused within this regulation, in order to avoid excessive intake of additional phytosterols /phytostanols and inform the consumer that the product is intended exclusively for people who want to lower their plasmatic cholesterol level. It is also important to inform consumers that the product may not be nutritionally appropriate for pregnant and breastfeeding women and children under the age of five years and that the consumption of more than 3 g/day of added plant sterols/plant stanols should be avoided.

7.2 Toxicological assessment

The SCF stated on the safety of phytosterols esters as novel food ingredient, especially for yellow fat enriched spread (SCF, 2000)[14] and other products (SCF, 2002)[15].

As a substantially equivalence is claimed, the safety doesn't have to be demonstrated because the first applicant of the novel food did it [2,16,17]

However a brief bibliographical overview of the important points concerning long term safety is performed here below in order to assess the toxicological status of the product.

- The oral absorption of a radiolabelled samples of phytosterol has been demonstrated as very low in rats, thus permitting to consider the systemic effect as very low [18].

- Using no-observed-adverse-effect-level (NOAEL) methodology, no sub chronic toxicity was evidenced after daily oral administration for 90 days into rats. This was equivalent to a dose of 4.1 g/kg/day phytosterol[19]

- No effects were observed on the reproductive system[20] and no major abnormalities in growth, food and water consumption, routine haematological and clinical chemistry values, composition of the urine, appearance of the faeces, oestrus cycle length, organ weights and histopathological findings[21] The absence of oestrogenic activity was demonstrated[22].

- The absence of adverse effects was demonstrated on reproduction and foetus development on one [23] and two generations[24]

- An in vitro mammalian cell gene mutation assay and two in vivo mutagenicity studies, namely rat bone marrow micronucleus and liver unscheduled DNA synthesis assays shows the absence of genotoxicity[25]

Specific toxicological tests were performed on Prima Pharm's product to reinforce its knowledge on the product (Appendix 10):

- In primary irritation test performed in 3 rabbits (OCDE 404) a dose of 0.5 g of the product applied under occlusion has no irritation properties[26]

- During an acute toxicity test (OCDE 401), 5 rats received an oral dose of 2g/kg body weight of the product: no mortality was observed [27].
- the sensitizing potential (OCDE 406) was evaluated in guinea pig model with product diluted at 10 and 5%. 5 animals received an unique application and did not demonstrate cutaneous irritation. 10 other guinea pigs were first sensitized by intradermal injection of the product with the Freund complete adjuvant coupled with a cutaneal application under occlusion. After a rest time, the product diluted at 10 and 5% was applied on other skin localization under occlusion. After 24 and 48 hours, all the animals were free of local irritation and sensitizing sign. The product can be considered as non sensitizing[28].

The SCF opinion on Teriaka application[(8)] mentions in its point 6 about the Toxicological Information (page 5) the three reports presented in Appendix 10. These three reports were provided by DRT to Teriaka to support its Novel Food Application. Theses three reports are about the toxicological assessment of DRT's "Beta-Sitosterol" which is the source of Prima Pharm's product and one of the sources of Teriaka's product, reinforcing once again the demonstration of the Substantial Equivalence between the two products.

The Material Safety Datasheet is presented in Appendix 11 of this document.

All the precedent studies were performed on animals. The European scientific committee of food evaluated the data of the first Novel Food Application and concluded that the human consumption of sterols up to 3 grams per day was safe.

To this date, the long term effects of the intakes of phytosterols are not known.
A particular concern must be made on the possible consumption of the product by pregnant women and lactating women and also by young children [(14-15)]

The assessment made by the European Scientific Committee on Food upon Teriaka[(8)] consider the product to be safe, and this conclusion can be applied to Prima Pharm's product because it is identical in term of specification, quality, purity and daily dose (with a maximum of 3g/day).

8-BIBLIOGRAPHY

(1) Regulation 258/97 of the European Parliament and of the Council of 27 January 1997 concerning Novel Foods and Novel Food Ingredients. *OJ 43; 14.2.1997*

(2) Commission Decision 2000/500/EC 24 July 2000 authorizing the placing on the market of "yellow fat spreads with added phytocholesterol esters".

(3) Decision 2004/333/CE of 31 March 2004 authorizing the placing on the market of yellow fat spreads, salad dressings, milk type products, fermented milk type products, soya drinks and cheese type products with added phytosterols/phytostanols as novel foods or novel food ingredients under Regulation (EC) No 258/97 of the European Parliament and of the Council.

(4) Decision 2004/334/CE of 31 March 2004 authorizing the placing on the market of yellow fat spreads, milk type products, yoghurt type products, and spicy sauces with added phytosterols/phytostanols as novel foods or novel food ingredients under Regulation (EC) No 258/97 of the European Parliament and of the Council.

(5) Decision 2004/335/CE of 31 March 2004 authorizing the placing on the market of milk type products and yoghurt type products with added phytosterol esters as novel food ingredients under Regulation (EC) No 258/97 of the European Parliament and of the Council.

(6) Decision 2004/336/CE of 31 March 2004 authorizing the placing on the market of yellow fat spreads, milk based fruit drinks, yoghurt type products and cheese type products with added phytosterols/phytostanols as novel foods or novel food ingredients under Regulation (EC) No 258/97 of the European Parliament and of the Council.

(7) Decision 2004/845 of 12 November 2004on authorizing the placing on the market of milk based beverages with added phytosterols/phytos-tanols as novel foods or novel food ingredients under Regulation (EC) No 258/97 of the European Parliament and of the Council

(8) Opinion of the scientific committee on food on applications for the approval of a variety of plant sterol enriched foods_ SCF/CS/NF/DOS/15 Add 2 final, 13 march 2003

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