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Opinion of the Scientific Committee on Food on Applications for Approval of a Variety of Plant Sterol-Enriched Foods

(expressed on 5 March 2003)

B-1049 Bruxelles/B-1049 Brussels - Belgium
Telephone: direct line (+32-2) 29 599.10, exchange 299.11.11. Fax: (+32-2) 299.48.91
Telex: COMEU B 21877. Telegraphic address: COMEUR Brussels.
http://europa.eu.int/comm/food/fs/sc/scf/index_en.html

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1. TERMS OF REFERENCE

With reference to the initial assessments carried out by the authorities of Finland, taking into account the relevant comments/objections presented by Member States and pursuant to Article 11 of Regulation 258/97/EC, the Committee is asked to assess the safety, from the point of view of consumer health of

(a) plant sterol-enriched bakery products, grain-based snack products and gum arabicum pills,

(b) plant sterol-enriched frankfurters, sausages and cold cuts, and

(c) the plant sterol-enriched fat ingredient Diminicol planned to be added to yoghurt, fresh cheese, margarine and fruit-milk drinks.

2. BACKGROUND

The first application for the approval of plant sterols in food concerned the use of phytosterol esters of fatty acids in yellow fat spreads as a means of reducing blood cholesterol levels in hypercholesterolaemic individuals. The Scientific Committee on Food (SCF) assessed the safety of these plant sterol derivatives and concluded that the use of phytosterol esters in yellow fat spreads at a maximum level corresponding to 8% free phytosterols is safe for human use, provided the phytosterol profile is as follows: 30-65% β-sitosterol, 10-40% campesterol, 6-30% stigmasterol and a total of 5% other phytosterols, based on the total sterol content (SCF, 2000).

In the meantime, further applications have been submitted for the approval of bakery products, snack products and gum arabic pastilles (Oy Karl Fazer AB), meat products (Pouttu Ltd.) and the fat ingredient Diminicol for use in yoghurt, fresh cheese, margarine and fruit-milk drinks (Teriaka Ltd.), all of them enriched with non-esterified phytosterols containing some phytostanols.

In addition to these food products and food ingredients, for which approval is requested, margarine and other types of products with stanol esters obtained from phytosterols by hydrogenation and esterification are on the market, but have not been specifically evaluated by the Committee.

The Committee has recently expressed a general view on the long-term effects of the intake of elevated levels of phytosterols from multiple dietary sources. The Committee concluded that a numerical upper level for the total daily intake of phytosterols could not be established. On the basis of the available data, including information on stanol esters, the Committee came to

the following conclusion: in consideration of dosages found to be effective for cholesterol-lowering, without evidence of additional benefits at higher intakes and the possibility that high intakes might induce undesirable effects, it is prudent to avoid plant sterol intakes exceeding a range of 1-3 g/day (SCF, 2002).

The major concerns and suggestions raised by the Member States were as follows:

1. Need for a detailed specification.

2. Potential contamination of tall oil derived sterols by constituents and impurities of crude tall oil from the wood processing industry.

3. Insufficient toxicological data on non-esterified tall oil derived sterols.

4. Cumulative consumption of plant sterols from different products.

5. Possible consumption by children and pregnant and lactating women.

6. Effects on the absorption of fat-soluble vitamins and carotenoids.

7. Potential hormonal effects.

8. Effects on the balance between endogenously produced and absorbed cholesterol.

9. Effects on the bile acid metabolism.

10. Effects on prostate, kidney and malign diseases.

11. Need for appropriate labelling.

3. COMPOSITIONAL DATA

3.1 Phytosterol profile

In contrast to other plant sterol-enriched foods already on the market, the sterols used in the new products are not hydrogenated and not esterified. Raw materials for the plant sterol preparations intended to be used are tall oil from pine trees, mainly *Pinus maritima L.*, and in the case of Oy Karl Fazer AB and Teriaka Ltd. also edible vegetable oils of different origins.

As originally described, the tall oil derived plant sterol mixture had a sterol content of >95% and contained 75-80% β-sitosterol, 10-14% β-sitostanol, 6-9% campesterol, 0-2% campestanol and 0-2.5% other sterols. According to new analytical data, the sterol mixture extracted from tall oil now intended to be used is of higher purity and contains >99% total plant sterols/stanols including 70-80% β-sitosterol, 10-15% β-sitostanol, 8-11% campesterol, 1-4% campestanol and <2% minor sterols.

The composition of the vegetable oil derived plant sterol mixture is described as >90% sterols, 40-58% β-sitosterol, 0-5% β-sitostanol, 20-30% campesterol, 14-22% stigmasterol and 0-6% brassicasterol.

3.2 Potential impurities

Concerns may arise from using tall oil as raw material because it is a by-product of the cellulose production from pine trees (pulping process). Crude tall oil is removed from the paper-making process before any cellulose bleaching step. After distillation of the tall oil the remaining fraction (tall oil pitch) is extracted and the resulting sterol mixture is purified by recrystallization from a hydrocarbon solvent and an aqueous methanol solution.

In an analytical study three samples of tall oil derived sterols were found to contain 99.6, 99.4 and 99.2% total plant sterols/stanols (Holmbom *et al.*, 2002). According to the applicants, other constituents of the tall oil, such as terpenes, terpene alcohols, resins or resin acids or any oxidised tall oil residues are removed by the process. An industrial sample of tall oil derived sterols (Beta Sitosterol) contained 5000 mg/kg methanol as solvent residue, less than 20 mg/kg heavy metals, 0.51 pg/g dioxin, expressed as toxicity equivalents of 2,3,7,8-TCDD, and less than 1 μg/kg benzo(a)pyrene (detection limit). The concentrations of 15 other polycyclic aromatic hydrocarbons (PAHs), classified as priority pollutants by the US Environmental Protection Agency, and polychlorinated biphenyls (PCBs) were also below the detection limits of the methods used (Oy Karl Fazer AB, 2002; Teriaka Ltd., 2001). In analyses of a tall oil sample for pesticide residues the levels of all 55 compounds analyzed were below the detection limits.

4. ANTICIPATED INTAKE

The plant sterol mixtures are intended to be used in amounts up to 2 g in 100 g of novel bakery products, 50 g of novel grain-based snack products, and 50 g of novel gum arabic pastilles. As estimated by the Finnish Novel Food Board on the basis of average consumption data from Finland (224 g bakery products and 8 g sweets) and high levels of consumption from Sweden (322 g of bakery products and 39 g of sweets), the phytosterol intake could be 4.8 or 8 g per day, respectively.

In the case of frankfurters, sausages and cold cuts containing 2 g plant sterols in 75 g, the applicant anticipates an intake of 2 g plant sterols at normal level of consumption of these products and 5.9 g at high level of consumption (mean + 2 x standard deviation).

Based on an effective daily dose of 1.5 g plant sterols, the concentration of plant sterols in Diminicol varies from 17% to 30% depending on the recipe and the portion sizes of the final product. The recommended daily intake of plant sterols of 1.5 g will be delivered by 20 g of margarine, 30 g of soft cheese, 240 g of yoghurt or 450 g of fruit-milk drinks.

If consumers ingest not only yellow fat spread or margarine with phytosterol or stanol esters, but also bakery, meat and milk products as well as snacks and sweets enriched with plant sterols, the total average intake of phytosterols would be much higher than the intended or recommended intake through each of these products.

5. NUTRITIONAL INFORMATION

In addition to previous studies with plant sterol and stanol esters already evaluated by the Committee (SCF, 2000 and 2002), double-blind, placebo-controlled clinical trials were performed with three different food items (bread, jam in yoghurt, meat products containing

slightly elevated levels of calcium, magnesium and potassium) enriched with a tall oil-derived plant sterol mixture (79% β-sitosterol, 11% sitostanol, 7.5% campesterol). In one of these trials, 71 subjects who completed the study (10 men and 26 women in the sterol group and 9 men and 26 women in the placebo group) had a total intake of 0.91 g, 1.86 g, and 4.17 g of plant sterols from the enriched food items per day during a first, second and third 5-week period, respectively. Serum total cholesterol was reduced by 6, 6 and 8% and LDL-cholesterol by 10, 10 and 13%, respectively (Tikkanen *et al.*, 2001).

A second study with the same study design and 73 subjects who completed the study (53 women and 20 men) revealed a similar decrease in serum total cholesterol and LDL-cholesterol as well as an increase in the HDL/total cholesterol ratio. The levels of retinol and β-carotene did not change significantly during the trial. The serum concentration of α-tocopherol decreased significantly. After adjusting the change in α-tocopherol for the change in LDL-cholesterol, there was no difference between the two randomisation groups. Differences in serum sex-hormone levels (FSH, LH, prolactin, oestradiol, testosterone, progesterone, SHBG) were not seen (Tuomilehto *et al.,* 1999).

Three clinical trials were performed with spreads and cooked and baked foodstuffs containing a wood-based plant sterol mixture (75-80% β-sitosterol, 10-14% β-sitostanol, 6-11% campesterol/campestanol). In the first one, 155 hypercholesterolaemic subjects received 0, 1.5 and 3 g plant sterols/day in a spread for 6 months, (Christiansen *et al.*, 2001). In the second study, 13 volunteers received 1 g plant sterols/day in cooked/baked lunch dishes for 4 weeks (Isoherranen *et al.*, 2000). In the third trial, 34 subjects with mild to moderate hypercholesterolaemia were given 0, 0.8, 1.6 and 2.4 g plant sterols/day in spreads over 4 three-week periods (Sarkkinen *et al.*, 2001). Apart from the low-dose study with only 1 g plant sterols/day, plasma total- and LDL-cholesterol concentrations were significantly reduced compared to the control groups. Slight reductions in the serum levels of β-carotene and other carotenoids were statistically not significant. In the third study, the mean serum vitamin E concentration was significantly reduced at doses of 1.6 and 2.4 g/day. The differences were not significant, when the concentrations were standardized with serum total cholesterol concentrations.

6. TOXICOLOGICAL INFORMATION

In addition to the studies already included in the previous evaluations (SCF, 2000 and 2002), the following studies with the plant sterols intended to be used have been submitted:

In an acute toxicity test of tall oil-derived “Beta Sitosterol” preparations with rats, mortality was not observed at an oral dose of 2 g/kg body weight. The same material did not reveal irritating properties when tested in rabbits and did not show a sensitisation potential in a guinea pig maximization test (EVIC-CEBA, 1990, 1989 and 1997).

Groups of 10 rats each were fed a diet with 5% “β-sitosterols” from tall oil and from cottonseed oil for periods of 8, 18 and 22 months, respectively. There was no detectable alteration in growth, blood cell counts, blood urea nitrogen, serum proteins and gross or microscopic appearance of any organ or tissue (Shipley *et al.*, 1958).

Groups of 6 or 7 male Wistar rats received 0, 0.5 and 1% β-sitosterol (containing 7% campesterol) and 0, 0.5 and 1% β-sitostanol (containing 7% campestanol) in the diet for periods up to 23 days. There was no demonstrable negative effect on growth and weight of

kidneys, heart, lung, spleen, adrenals and testes. Liver weight appeared somewhat lower in rats fed β-sitostanol when cholesterol was simultaneously included in the diet (Sugano *et al.*, 1977).

A total number of 11 dogs were fed a diet with "β-sitosterols" at doses of 0.5 and 1 g/kg body weight for periods of 8 and 22 months. Body weight, haematological parameters, serum composition and the results of gross and microscopic pathological examination were not significantly different from those of two control dogs (Shipley *et al.*, 1958).

Groups of 6 New Zealand rabbits of both sexes were placed on diets with 3% cottonseed oil and 4% "β-sitosterols" derived from either tall oil or cottonseed oil. None of the rabbits showed any gross or microscopic abnormality of the blood vessels or other tissues after consuming 4 g "β-sitosterols"/day for periods of 348 to 842 days. Total liver and aorta lipid concentration and free and total liver and aorta sterol concentrations were essentially the same as those observed in two control rabbits (Shipley *et al.*, 1958).

Clinical studies with continuous administration of "β-sitosterols" to patients for periods exceeding 4 years did not show any harmful effect as determined by laboratory tests of kidney and liver function, blood and urine composition, electrocardiogram, and gall bladder visualization. Also, there were no symptoms indicating a contribution of "β-sitosterols" to vascular lesions (Shipley *et al.*, 1958). Details on the number of patients and the dose are not given.

Furthermore, information has been submitted that the vegetable oil derived phytosterol esters administered in the studies on subchronic toxicity and reproductive toxicity, on which the considerations of the Committee were largely based (SCF, 2000), have been reanalysed. According to the results of the reanalysis, the phytosterol ester preparations used in these studies contained not only the originally reported sterols in amounts of 46.5 and 46.8% ß-sitosterol, 26.5 and 27.2% campesterol, 20.1 and 17.3% stigmasterol, 1.0 and 3.0% brassicasterol, but also 1.8 and 1.7% campestanol, 0.2 and 0.5% D5-avenasterol, 0.2% D7-stigmastenol and 2.8 and 2.3% sitostanol, respectively (Unilever, 2002).

In a recently published study (Kim *et al.*, 2002), undiluted phytosterol esters were administered to Sprague-Dawley rats by gavage at dose levels of 0, 1000, 3000 and 9000 mg/kg body weight/day for 13 weeks. The phytosterol esters were obtained from soy bean sterols by esterification with unsaturated fatty acids (oleic acid ≥70%) from olive oil, had a purity of more than 95.4% and a sterol profile of 49.4% sitosterol, 27.9% campesterol and 18.5% stigmasterol. In the highest dose group, body weight gains were reduced significantly in both sexes and the incidence of cardiomyopathy with mononuclear cell infiltration was significantly increased in males (2/16, 2/10, 2/10, 8/16). It must be noted that the animals in all groups had a high background incidence of liver inflammation, suggesting that their general health status was compromised. In addition, the effects on the heart were only seen at the highest dose level in males given as a bolus dose. These aspects limit the relevance of the study for risk assessment of phytosterol esters in the human diet.

7. CONCLUSION

The toxicological information available on non-esterified phytosterols is less extensive than that on phytosterol esters with fatty acids evaluated for the use in yellow fat spreads (SCF, 2000) and on the phytostanol esters used in margarine and other types of products (SCF,

2002). Phytosterol esters, however, are hydrolysed by pancreatic cholesterol esterase (Swell *et al.*, 1954; SCF, 2000) and are similar to free phytosterols in the ability to lower cholesterol absorption in rats, suggesting that the liberated phytosterols are the active moieties of the esters (Best and Duncan, 1958; Mattson *et al.*, 1977). In the gut of rats, β-sitosterol linoleate has been shown to undergo extensive hydrolysis to liberate the free sterol and vice versa the free β-sitosterol to be esterified with fatty acids (Minter and Sanders, 1997). Therefore, the available toxicological data on phytosterol esters are considered relevant for the evaluation of the free phytosterols as well. Thus, the safety assessment of the plant sterol-enriched foods can largely be based on the data reviewed and evaluated by the Committee in its opinions on phytosterol esters in yellow fat spreads (SCF, 2000) and on long-term effects of elevated levels of phytosterols from multiple dietary sources (SCF, 2002).

The concentrations of phytosterols in the enriched foods result in an intake of 1.5 to 2 g phytosterols per day if only one of these products is consumed daily in the amounts suggested by the applicants (100 g bakery products, 50 g snack products, 75 g meat products, 20 g margarine, 30 g soft cheese, 240 g yoghurt or 450 g fruit-milk drinks). Provided that these enriched food products are not consumed in amounts resulting in total phytosterol intakes exceeding 3 g/day, the Committee concludes on the basis of the available toxicological and nutritional data on phytosterol esters and phytosterols in accordance with the previous opinions (SCF, 2000; SCF, 2002), that the use of phytosterols in these foods is safe.

The Committee, however, reiterates the recommendations (SCF, 2000):

- that the small number of people with inborn error of phytosterol metabolism (phytosterolaemia) should be made aware of the presence of higher levels of phytosterols in the product,

- that patients on cholesterol-lowering medication should only consume the products under medical supervision, and

- that the potential β-carotene lowering effect should be communicated to the consumer, together with appropriate dietary advice regarding the regular consumption of fruits and vegetables.

In addition, the Committee notes again, that the consequences of a persistent decrease of blood concentrations of β-carotene on human health are largely unknown and that situations where vitamin A requirements are greater than normal as in pregnancy, lactation or infancy may be of concern (SCF, 2002).

In the case that higher amounts of enriched products than recommended by the applicants and /or more than only one of these products are consumed, the total intake of phytosterols can be much higher than 3 g/day, for example about 6 g/day by consumption of 200 g enriched bread together with 25 g margarine/day. Therefore, the Committee considers that appropriate risk management measures should be developed to minimize the likelihood of a daily intake exceeding 3 g phytosterols/phytostanols, in particular from cumulative intakes of different types of products.

The Committee encourages the Commission to initiate a programme monitoring the total intake of phytosterol-enriched products, in particular who the consumers are and how often and which amounts of these products are consumed.

The sterol mixture extracted from tall oil differs from the phytosterol profile already accepted by the Committee (SCF, 2000) in that it has a higher content of β-sitosterol, β-sitostanol and campestanol and a lower content of campesterol, stigmasterol and other sterols. The higher content of β-sitosterol can be accepted, because β-sitosterol esters and in some cases β-sitosterol were the main constituents in the mixtures tested for subchronic toxicity, genotoxicity, reproductive toxicity and oestrogenic activity, without showing effects causing concern (SCF, 2000). The higher contents of ß-sitostanol and campestanol appear acceptable as well. Their esters were main constituents of the stanol ester mixtures tested in studies on subchronic toxicity, genotoxicity, reproductive toxicity, developmental toxicity and oestrogenic activity (SCF, 2002). Therefore, the phytosterol profile of the tall oil-derived plant sterol mixture specified by the applicants on the basis of new analytical data can be accepted. Any contamination of the sterol mixture with non-sterol constituents from crude tall oil, however, should be avoided. Consequently, phytosterol preparations derived from tall oil should contain more than 99% sterols/stanols and comply with the maximum limits of the Council Directive on extraction solvents (EC, 1992) and the recommendations on potential contaminants in the Report of the Committee on smoke flavourings (SCF, 1993).

The vegetable oil-derived phytosterol mixtures comply largely with the phytosterol profile accepted by the Committee (SCF, 2000). They contain, however, up to 5% β-sitostanol and up to 6% brassicasterol resulting in a higher content of total other phytosterols than the accepted 5%. The higher content of β-sitostanol is acceptable for the same reason as in the case of the tall oil-derived sterol mixture. Brassicasterol esters of fatty acids, however, were only minor constituents of the phytosterol esters tested for subchronic (1%) and reproductive toxicity (3%). Therefore, the content of brassicasterol in the vegetable oil-derived phytosterol mixtures should not exceed 3%.

On the basis of these considerations and the data provided by the applicants, the recommendation of the Committee for the phytosterol profile of phytosterol esters of fatty acids (SCF, 2000) can now be updated and extended to unesterified phytosterols/phytostanols resulting in the following phytosterol/phytostanol profile acceptable in general: up to 80% β-sitosterol, 15% β-sitostanol, 40% campesterol, 5% campestanol, 30% stigmasterol, 3% brassicasterol and 3% other phytosterols.

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