FEB 2.3 / FDA

NEW DIETARY INGREDIENT NOTIFICATION FOR CARDIABEAT™

Submitted to: Division of Standards and Labeling Regulations
Office of Nutritional Products, Labeling and Dietary Supplements
Center for Food Safety and Applied Nutrition
Food and Drug Administration
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January 17, 2004

NEW DIETARY INGREDIENT NOTIFICATION FOR CARDIABEAT™

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NEW DIETARY INGREDIENT NOTIFICATION FOR CARDIABEAT™

In accordance with the Dietary Supplement Health and Education Act of 1994 (DSHEA), 21 U.S.C. §350b (a) (2), and with final regulations published in the Federal Register (1997, 62:49886-49892, 21 C.F.R. § 190.6) "Requirement for Premarket Notification", the following information is submitted by Enzymotec Ltd. (Enzymotec) in support of a New Dietary Ingredient Notification for car*D*iabeat™ (carDiabeat). Enzymotec intends to market carDiabeat as a dietary supplement in the United States. As per the statutes of the DSHEA, 21 U.S.C. § 350b (a) (2), Enzymotec will not introduce, market, distribute or sell carDiabeat until at least 75 days following official acknowledgement of the receipt of this notification by the U.S. Food and Drug Administration (FDA).

SECTION 1

The name and complete address of the manufacturer of the dietary supplement that contains the dietary ingredient, or the dietary ingredient.

The manufacturer of the dietary ingredient will be:

Enzymotec Ltd.
Hataasia 5 St.
P.O. Box 6
Migdal HaEmeq 23106
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Contact: Iris Meiri-Bendek, M.Sc.
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The name and complete address of the importer-distributor of the dietary supplement that contains the dietary ingredient, or the dietary ingredient.

The importer and distributor of the new dietary ingredient will be:

Buckton Scott Nutrition Inc
Leo R, Cullen
Executive Vice President
24 Stewart Place
Fairfield, NJ
07004
Ph.: (973) 882-0322
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SECTION 2

CONSTITUTING

PAGES 3 THROUGH 5

REDACTED IN ITS

ENTIRETY

CONTAINS

TRADE SECRET

CONFIDENTIAL

COMMERICAL

INFORMATION

SECTION 3

Description of the dietary supplement or dietary supplements that contain the dietary ingredient including (i) the level of dietary ingredient in the dietary supplement, and (ii) the conditions of use recommended or suggested in the labeling of the dietary supplement, or if no conditions of use are recommended or suggested in the labeling of the dietary supplement, the ordinary conditions of use of the supplement.

CarDiabeat is a dietary supplement containing DHA and EPA phytosterol esters and diglyceride containing DHA/EPA enriched fish oil. CarDiabeat is a yellow to brown liquid

SECTION 4

The history of use or other evidence of safety establishing that the dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe, including any citation to published articles or other evidence that is the basis on which the distributor or manufacturer has concluded that the dietary supplement will reasonably be expected to be safe.

4.1 Metabolism

The metabolism of phytosterol esters has been reported to begin with the hydrolysis of the ester bond, separating the molecule into its component phytosterol and fatty acid (Mattson *et al.*, 1977). This process is carried out by intestinal esterase, which have been reported to act at the same rate on both cholesterol and phytosterols, regardless of the chain length of the fatty acid (Mattson *et al.*, 1977). Due to the fact that phytosterol esters will be rapidly hydrolyzed in the gastrointestinal tract, the metabolism of the phytosterol component of carDiabeat will be discussed separately from the metabolism of DHA and EPA, the major fatty acid component of carDiabeat.

Phytosterols have been reported to be poorly absorbed from the gastrointestinal tract, and as a result low levels of phytosterols are present in the blood (Weststrate *et al.*, 1999). Of the phytosterols that are absorbed, the majority are quickly secreted back into the bile, where they are re-circulated back to the gastrointestinal tract (Salen *et al.*, 1970). In a study conducted by

Sanders *et al.* (2000), the absorption and retention of various radiolabeled phytosterols was examined in rats. Of the administered dose, 13% of campesterol and 4% of β-sitosterol and stigmasterol was absorbed from the gastrointestinal tract. Phytosterols were reported to be widely distributed throughout the body, with a tendency to be present in most tissues at a very low level. After 96 hours, the greatest retention of the absorbed phytosterols was reported to occur in the adrenal gland, intestinal epithelia and the ovaries. Sanders *et al.* (2000) reported that the predominant route of excretion was the feces, in which 75 to 96% of the administered dose was recovered within 24 hours of dosing.

While human absorption of phytosterols has been reported to be similar to that of laboratory animals, an exception exists for individuals with a condition known as phytosterolemia, also referred to as sitosterolemia (Miettinen *et al.*, 1990; Heinemann *et al.*, 1993; Ratnayake and Vanasour, 2004). Phytosterolemia is caused by a genetic mutation that is estimated to be present in approximately 50 families worldwide (Patel, 2001). Phytosterolemic individuals have blood phytosterol levels that are 50 to 60 times greater than those of normal individuals, resulting from an increase in phytosterol absorption, reduced cholesterol synthesis, and impaired biliary secretion of phytosterols (Ikeda *et al.*, 2001; Ratnayake and Vanasour, 2004; Yu *et al.*, 2004). Individuals with phytosterolemia are at a greater risk of developing xanthomas (cholesterol deposits in the skin and tendons) in the first decade of life and premature coronary heart disease (CHD) (Stalenhoef *et al.*, 2001).

The metabolism of EPA and DHA is very different from that of phytosterols, as approximately 60% of an administered dose of EPA or DHA is absorbed from the gastrointestinal tract, with absorption improving to almost 90% when consumed with other fats (Nordøy *et al.*, 1991; Banno *et al.*, 2001). Once EPA and DHA are absorbed from the gastrointestinal tract they are incorporated into plasma phospholipids and circulate in the blood stream (Hansen *et al.*, 1993a). From plasma phospholipids, DHA and EPA can distribute to cellular membranes in a variety of tissues in the body including the brain, the eye, the liver, the kidney, red blood cells, and adipose tissue (Vidgren *et al.*, 1997; Fenton *et al.*, 2001; Yasui *et al.*, 2001; Berson *et al.*, 2004). Further metabolism of DHA and EPA begins with their removal from the membrane phospholipids, after which they are used in the synthesis of eicosanoids, which are endogenous compounds involved in blood clotting and immune responses.

4.2 Animal Studies

Animal studies relative to the safety of carDiabeat and its components are discussed below. CarDiabeat has a phytosterol distribution consisting of 2.51, 21.08, 25.23, and 44.10% of brassicasterol, stigmasterol, campesterol, and β-sitosterol, respectfully. The composition of phytosterol oils employed in the animal trials discussed below is similar to that of carDiabeat as the oils are all derived from vegetable oils such as soybean oil. For this reason, these trials are considered relevant to the safety of carDiabeat.

4.2.1 Acute Single Dose Toxicity

One acute toxicity study was identified in which a single dose of 3.2 g sitosterol/kg body weight was orally administered to mice (Gupta *et al.*, 1980). No effects were observed following the administration of the dose and therefore the oral LD_{50} for sitosterol was reported to be >3.2 g/kg body weight.

4.2.2 Repeated Dose Toxicity

4.2.2.1 Phytosterols

Repeated dose toxicity studies involving phytosterols and phytosterol esters have been conducted in rats, rabbits, dogs, and guinea pigs (Shipley *et al.*, 1958; Hepburn *et al.*, 1999; Ewart *et al.*, 2002; Russell *et al.*, 2002; Kritchevsky *et al.*, 2003).

Several studies were identified in which the toxicity of phytosterols was examined in normal rats, the earliest of which was conducted by Shipley *et al.* (1958). In this study, rats were administered diets containing 5% sitosterol derived from tall oil for periods of 8, 18, or 22 months, with 10 rats receiving each dosing regime. Additionally, 2 groups of 10 rats each received diets containing 5% sitosterol derived from cottonseed oil for periods of 18 or 22 months. The authors reported that at the end of all dosing regimes, there were no detectable changes in the growth, blood cell counts, blood urea nitrogen, serum protein levels, or in the gross or microscopic appearance of any organ or tissue after consuming sitosterol derived from either cottonseed or tall oil (Shipley *et al.*, 1958).

A 13-week subchronic toxicity was conducted by Kim *et al.* (2002) in which male and female Wistar rats were administered phytosterol ester doses of 0, 1,000, 3,000, and 9,000 mg/kg body weight/day by gavage. The phytosterol esters administered to the rats contained sitosterol (49.4%), campesterol (27.9%), and stigmasterol (18.5%) esterified to oleic acid from olive oil. A significant decrease in body weight was observed in rats of both sexes receiving 9,000 mg phytosterol ester/kg body weight/day, and cardiomyopathy was observed in the male rats administered 9,000 mg phytosterol ester/kg body weight/day. Several differences were observed in individual treated rats, including changes in erythrocyte counts, liver enzyme levels, and clinical and pathological abnormalities; however, these were determined by the authors to be of no toxicological significance because they were either within historical controls, not dose-dependent, or occurred at a very low incidence. No significant differences were observed when the organ weights of male rats, or the opthalmoscopy and urinalysis of all rats in the treatment groups was compared to those of the negative controls group. Kim *et al.* (2002) determined that the no-observed-adverse effects level (NOAEL) for phytosterol esters was 3,000 mg/kg body weight/day for both male and female Wistar rats.

As a part of an ongoing safety evaluation of phytosterol esters, Hepburn *et al.* (1999) conducted a 90-day oral toxicity study involving phytosterol esters in rats. In this study, male and female

Wistar rats were administered diets containing 0 (control), 0.16, 1.6, 3.2, or 8.1% phytosterol esters, with each dosing group containing 20 animals of each sex. The phytosterol ester mixture added to the diet was reported to contain 62.0% phytosterols, of which 8.4% were present as free phytosterols, and 38.2% fatty acids, of which 0.3% were present as free fatty acids. The phytosterols present in the mixture were primarily β-sitosterol, stigmasterol, and campesterol, with minor amounts of brassicasterol and cholesterol. To assess the effects of the phytosterol ester diets, Hepburn *et al.* (1999) examined the clinical condition and behavior, body weight, food consumption, clinical pathology, and histopathology of the rats.

Administration of diets containing up to 8.1% phytosterol ester to male and female Wistar rats for 90 days was reported to have no adverse effect on animal survival, mean body weights, food consumption, and food utilization (Hepburn *et al.*, 1999). Additionally, no toxicologically or biologically significant changes were observed in the ophthalmological, histological, hematological, and clinical chemistry parameters observed. Minor changes were reported in some hematological and clinical chemistry parameters; however, due to a lack of accompanying organ weight changes, histopathological changes, or abnormal macroscopic findings at necropsy, the authors did not consider these to be significant. Hepburn *et al.* (1999) determined that at the highest dose level, consumption of phytosterol esters did not produce any adverse effects, and thus was equivalent to the NOAEL. Consumption of the diet containing 8.1% phytosterol esters corresponds to a daily intake of 6.6 g of phytosterol ester/kg body weight/day, equivalent in this instance to approximately 4.1 g phytosterols/kg body weight/day.

To examine the potential estrogenic affects of phytosterol consumption, female Wistar rats were orally administered phytosterol doses of 0, 5, 50, or 500 mg/kg body weight/day for 3 consecutive days (Baker *et al.*, 1998). Additionally, some control animals were administered a weak phytoestrogen and a strong phytoestrogen to provide several positive controls. No significant difference between the uterine weight of phytosterol and negative control animals was observed, while the administration of both weak and strong phytoestrogens was reported to induce a significant increase in uterine weights (Baker *et al.*, 1998).

In addition to normal rats, the safety of phytosterol consumption has been extensively examined in stroke-prone spontaneously hypertensive (SHRSP) rats, which are considered to be a model for human hypertension and hemorrhagic stroke. In a study conducted by Ratnayake *et al.* (2000a), SHRSP rats consuming diets containing canola oil and various vegetable oils were reported to have a lifespan that was 8.9% shorter then animals consuming diets containing soy bean oils. Of the factors examined, the authors concluded that only the phytosterol content of the oils could be correlated to the decrease in lifespan. In 2 follow up studies, Ratnayake *et al.* (2000b, 2003) demonstrated that SHRSP rats consuming higher quantities of phytosterols had a greater risk of hemorrhagic stroke, and therefore had shorter lifespans. Ratnayake *et al.* (2000b) hypothesized that the decrease in cholesterol absorption associated with increased phytosterol consumption was the reason for the shortened lifespans. Analysis of the

erythrocytes of the rats consuming approximately 1,350 mg/kg body weight/day of phytosterols indicated that sterol had replaced cholesterol in the erythrocyte membrane, making the membrane less deformable. Ratnayake *et al.* (2000b) concluded that the increase in rigidity resulted in more fragile erythrocytes and increased the risk of hemorrhagic stroke.

The conclusions of Ratnayake *et al.* (2000a,b, 2003) were contradicted by the studies of Tatematsu *et al.*, 2004 who again fed different vegetable oil, oil fractions enriched in phytosterols and vegetable oils with added phytosterols isolated from soybean oil to groups (12 male rats) of male SHRSP rats for up to 180 days. Whereas canola oil again significantly shortened the life span of the rats, free fatty acid fractions (containing the phytosterols) did not shorten the life span of the rats indicating that a different component of the canola oil was responsible for this shortening of life span. The addition of large quantities of purified phytosterols to the canola oil group had no impact on life span whereas the addition of purified phytosterols to the soy oil group reduced the life span compared to the soy oil group but did not reduce the life span of this group to that of the canola oil.

One study was identified in which the consumption of phytosterol esters was examined in hamsters (Ntanios *et al.*, 2003). Syrian golden FB hamster were administered diets containing 30% fat, 0.12% cholesterol and 0, 0.24, 0.48, 0.96, 1.92, or 2.84% phytosterol esters, equivalent to approximately 288, 576, 1,152, 2,304, or 3,408 mg phytosterol esters/kg body weight/day (FDA, 1993), for 12 weeks. Consumption of phytosterol esters was reported to result in a significant decrease in plasma total cholesterol and low-density lipoprotein (LDL)-cholesterol, which is considered to be a beneficial effect of phytosterol consumption. No significant differences between the body weight gain or macroscopic and hepatic microscopic examination of the hamsters fed phytosterol esters were observed when compared to those of the control hamsters. The authors concluded that feeding Syrian hamsters up to 2.84% phytosterol esters/day (3,408 mg/kg body weight/day) presented no safety concerns.

Two studies were identified in which the effects of dietary consumption of phytosterols were examined in rabbits. In the first study, conducted by Shipley *et al.* (1958), several experimental protocols were used to demonstrate the safety of phytosterol consumption. Rabbits were fed doses of up to 4 g of phytosterols/day, representing an intake of approximately 2 g/kg body weight/day (FDA, 1993), for periods as long as 2 years. Shipley *et al.* (1958) reported that dietary consumption of phytosterols did not produce any gross or microscopic abnormalities in the blood vessels, heart, thyroid, spleen, liver, or intestinal tract. Additionally, the authors reported that 2 separate experimental protocols demonstrated that sterols do not deposit in the vasculature or accumulate in the tissues of white New Zealand rabbits consuming up to 4 g phytosterols/day.

In the second study, Kritchevsky *et al.* (2003) examined the atherogenic properties of diets enriched with 500 mg of phytosterol-rich diet preparations (spreads) containing sitosterol. The rabbits were fed a semi-purified, cholesterol free diet containing 295 mg of phytosterols/100 g of

food, with or without the phytosterol-rich spread, for a period of 60 days. No adverse effects or signs of toxicity were reported to result from consumption of the diet containing the phytosterol-rich spread. Additionally, no aortic lesions were reported in any of the animals involved in the study. In fact, at the end of the experimental period the average serum cholesterol level of the rabbits in the control group was 105 mg/dL, while the average serum cholesterol level of the rabbits exposed to sitosterol was 78 mg/dL.

One study was identified in which the effect of phytosterol consumption was examined in dogs. As part of their assessment of phytosterol safety, Shipley *et al.* (1958) examined the effects of dietary consumption of β-sitosterol by dogs for periods of 8 to 22 months. The dogs were administered diets providing β-sitosterol doses of 1.0 mg/kg body weight/day, and were given monthly exams which included measurements of physical, hematological, and blood chemistry parameters. No changes in body weight, serum composition, or formed blood elements were observed throughout the experimental period. Upon sacrifice, a complete pathological examination of the dogs was performed, which revealed no gross or macroscopic changes to any of the tissues examined. Additionally, there were no incidences of gross or macroscopic aortic lesions, and the free and total liver sterol levels of the dogs were not significantly different from those of the control dogs.

4.2.2.2 DHA and EPA phytosterol esters

Two studies were identified in which the safety of phytosterols esterified to fish oil fatty acids, producing a product similar to carDiabeat, was examined. In the first study JCR:LA-cp rats, who exhibit the symptoms of obesity and insulin resistance syndrome, were administered diets containing phytosterol esters produced from canola oil and marine oil (Russell *et al.*, 2002). The rats consumed diets providing 86, 500, or 2,600 mg/kg body weight/day of phytosterol esters containing DHA and EPA for a period of 4 weeks. The phytosterol fraction was composed of 85% stigmasterol. No differences were observed in the food consumption or body weights of the rats consuming phytosterol esters and the control animals. Consumption of DHA and EPA phytosterol esters was reported to have a beneficial effect on serum triglycerol levels and on the vasculature.

In the second study, phytosterol esters containing DHA and EPA were administered through the diet to guinea pigs (Ewart *et al.*, 2002). These phytosterol esters were produced from corn oil phytosterols and marine oil, in the presence of sodium methoxide, to produce phytosterol esters in which the fatty acid components were primarily DHA and EPA. The phytosterol component again was 85% stigmasterol. A diet containing 0.8 g of cholesterol/kg was supplemented with 25 g/kg of the phytosterol esters and 5 g/kg of corn oil, while 30 g/kg of corn oil was added to the control diet. Over the course of the 28 day study the guinea pigs consumed an average of 28 to 36 g of food per day, providing 700 to 900 mg of phytosterol esters/day, equivalent to approximately 933 to 1,200 mg phytosterol esters/kg body weight/day (FDA, 1993), 597 to 768 mg of phytosterols/kg body weight/day, and 202 to 260 mg DHA and EPA/kg body

weight/day. No adverse effects or signs of toxicity were reported to result from the guinea pigs phytosterol ester consumption. Additionally, the authors reported a beneficial effect on blood levels of triacylglycerol, total cholesterol, and non-high-density cholesterol in the guinea pigs consuming phytosterol esters containing DHA and EPA (Ewart *et al.*, 2002).

4.2.2.3 DHA and EPA studies

Several studies were identified in which the safety of DHA and EPA consumption was examined in rats and pigs. Of the studies conducted in rats, one was conducted in type 2 diabetic model rats, 1 was conducted in ovariectomised rats, and 2 were conducted in normal rats. In the study conducted in type 2 diabetic model rats, 5-week-old male Otsuka Long-Evans Tokushima Fatty (OLETF) rats were provided with diets containing 1.0 g EPA/kg body weight/day, 1.0 g Oleic acid/kg body weight/day or a non-supplemented rat chow diet until they were 30 weeks of age (Minami *et al.*, 2002). Consumption of the diet containing EPA was reported to significantly lower plasma triacylglycerol levels and abdominal fat accumulation, as well as improve insulin resistance. All of these effects were considered by the authors to have a beneficial impact on insulin sensitivity in type 2 diabetes. No adverse effects or signs of toxicity were reported in OLETF rats consuming a diet providing 1.0 g EPA/kg body weight/day.

In the study conducted in ovariectomised rats, diets containing corn oil, corn oil and 0.1 g EPA/kg body weight/day, or corn oil and 1.0 g EPA/kg body weight/day were fed to the rats for 9 weeks (Poulsen and Kruger, 2004). No significant difference was noted between the bone breaking strength and serum type-1 collagen concentrations of the treatment and control rats. Ovariectomised rats consuming 1.0 g EPA/kg body weight/day were reported to have a significantly lower femur bone density. Poulsen and Kruger (2004) suggested that the decrease in bone density was the result of an increase in bone resorption, which was stimulated by the high intake of unsaturated EPA. It should be noted that in clinical trials conducted by Kruger *et al.* (1998), the administration of EPA to older women (mean age 79.5 years) produced beneficial effects on bone.

In the first study conducted in normal rats, a diet containing 2% DHA or EPA esters, representing a dose of approximately 1,000 mg/kg body weight/day (FDA, 1993), was administered for a period of 3 weeks (Hung *et al.*, 2000). Consumption of EPA and DHA were reported to have beneficial effects on lipid metabolism and leukotriene synthesis, although the effects elicited by EPA consumption were stronger than those elicited by DHA consumption. No adverse effects were reported to result from the consumption of either DHA or EPA by normal Sprague-Dawley rats.

In the second study conducted in normal rats, CD rats were administered 0 (control), 0.5, or 1.25 g/kg body weight/day of an oil containing 40 to 50% DHA by gavage for 90 days (Arterburn *et al.*, 2000). Examinations of the rats during and after the experimental period included hematology, clinical chemistry, pathology, ophthalmology, neurobehavioral, and

neuropathological parameters. No significant differences were observed between the treatment and control groups in any of the parameters examined. The authors concluded that the NOAEL for normal CD rats was the highest dose level, providing approximately 0.5 to 0.625 g DHA/kg body weight/day.

One study was identified in which the safety of the dietary consumption of DHA from tuna oil was examined in pigs (Meritt *et al.*, 2003). Newborn piglets were administered formula containing 0.34 or 1.6 mg DHA/g formula, providing approximately 387 and 653 mg DHA/kg body weight/day, respectively, for male piglets and 412 and 691 mg DHA/kg body weight/day, respectively, for female piglets. The consumption of DHA was reported to have no significant effects on the body weights, clinical signs, food consumption, clinical chemistry, hematology, organ weight, or gross or histopathology of any the piglets receiving either dose of DHA. The authors concluded that the study findings support the safety of DHA supplementation, and specifically provide support for the use of DHA from tuna oil in infant formulations.

4.2.2.4 carDiabeat Studies

4.2.3 Genotoxicity

The results of studies examining the mutagenic and genotoxic potential of vegetable or plant phytosterols and vegetable or plant phytosterol esters have been summarized in Table 4.2.3-1.

Table 4.2.3-1 Summary of Studies Evaluating the Genetic Toxicity of Vegetable or Plant Phytosterols and Vegetable or Plant Phytosterol Esters

Test System	Assay	Dose	Results	Reference
Vegetable or Plant Phytosterols				
Salmonella typhimurium TA1535, TA1537, TA98, and TA100	Bacterial Mutation	5 to 5,000 µg/plate (±S9)	Negative	Wolfreys and Hepburn, 2002
Human Peripheral blood lymphocytes	Chromosomal Aberration	40, 80 and 160 µg/plate (-S9) 40, 80 and 160 µg/plate (-S9)	Negative Negative	Wolfreys and Hepburn, 2002
Vegetable or Plant Phytosterol Esters				
S typhimurium TA1535, TA1537, TA98, and TA100	Bacterial Mutation	50 to 5,000 µg/plate (±S9)	Negative	Wolfreys and Hepburn, 2002
Escherichia coli WP2 uvrA (pKM101)	Bacterial Mutation	50 to 5,000 µg/plate (±S9)	Negative	Wolfreys and Hepburn, 2002
Human peripheral blood lymphocytes	Chromosomal Aberration	40, 80 and 160 µg/plate (-S9) 40, 80 and 160 µg/plate (-S9) 50, 100 and 200 µg/plate (±S9)	Negative Negative Negative	Wolfreys and Hepburn, 2002
L5178Y mouse lymphoma cells	Gene Mutation	0, 2.5, 5, 10, 20, 40, and 80 µg/mL	Negative	Wolfreys and Hepburn, 2002
Rat bone marrow polychromatic erythrocytes	Micronucleus	500, 1,000, and 2,000 mg/kg body weight/day	Negative	Wolfreys and Hepburn, 2002
Rat hepatocytes	Unscheduled DNA synthesis	0, 800, and 2,000 mg/kg body weight/day	Negative	Wolfreys and Hepburn, 2002

Abbreviations: (-S9) = without metabolic activation by rat liver post-mitochondrial fraction; (+) = with metabolic activation; (±S9) = with and without metabolic activation

Phytosterols derived from common edible vegetable oils, primarily soybean oil, were employed in 2 *in vitro* mutagenicity assays conducted by Wolfreys and Hepburn (2002). In the first assay, *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 were exposed to

phytosterol concentrations of 5 to 5,000 µg/plate, both with and without metabolic activation by S9 to mimic metabolism. No significant increase in the number of mutant *S typhimurium* colonies was observed following their exposure to phytosterols. In the second assay, human peripheral blood lymphocytes were exposes to 40, 80, and 160 µg of phytosterols/plate in the absence of metabolic activation, for periods of 20 or 48 hours. No increase in the incidence of chromosomal aberrations was reported in the lymphocytes following either exposure period.

Wolfreys and Hepburn (2002) examined the mutagenic potential of vegetable or plant phytosterol esters as part of a safety evaluation of phytosterol esters. Both phytosterol esters and phytosterols were subjected to several *in vitro* and *in vivo* assays to determine their mutagenic potential. The phytosterol ester solutions employed in the *in vitro* assays were reported to consist of 51.0% β-sitosterol, 26.7% campesterol, 17.7% stigmasterol, 2.9% brassicasterol, and 0.2% cholesterol. In bacterial mutation assays conducted in *S typhimurium* strains TA1535, TA1537, TA98, and TA100, and *E coli* WP2 uvrA (pKM101), phytosterol ester concentrations of up to 5,000 µg/plate were reported to have no mutagenic activity, with and without metabolic activation. In a chromosomal aberration assay conducted in human peripheral blood lymphocytes, exposure to up to 200 µg of phytosterols/plate for as long as 48 hours, with and without metabolic activation, did not result in an increase in the percentage of cells with chromosomal aberrations. The *in vitro* cell mutation assay conducted in L5178Y mouse lymphoma cells by Wolfreys and Hepburn (2002) indicated that with or without metabolic activation, phytosterol ester concentrations of up to 80 µg/mL did not result in cytotoxicity or an increase in mutation frequency.

The *in vitro* mutagenicity assays conducted by Wolfreys and Hepburn (2002) employed the same phytosterol ester solution suspended in corn oil. In the micronucleus assay, rats were administered doses of 0 (control), 500, 1,000, and 2,000 mg/kg body weight/day twice, 24 hours apart. Examination of bone marrow collected from the femur of the rats 24 hours after the second dose indicated that exposure to phytosterol esters was not toxic to the bone marrow. In the second *in vitro* assay, rats were administered 0 (control), 800, and 2,000 mg/kg body weight/day of phytosterol esters *via* gavage. Cytoplasmic graining was observed in rat hepatocytes collected 2 to 4 hours following exposure to phytosterol esters; however, no cytoplasmic graining was reported in the hepatocytes collected 12 to 14 hours after exposure. The authors concluded that the cytoplasmic graining was likely to be spurious and therefore unrelated to exposure to phytosterol esters and of no toxicological significance. No further indications of an increase in cellular proliferation were observed in the hepatocytes of rats exposed to phytosterol esters. Overall, Wolfreys and Hepburn (2002) concluded that phytosterol esters demonstrated no mutagenic activity when assessed in five assays conducted in accordance with internationally accepted standards.

4.2.4 Developmental Toxicology

As part of a safety evaluation of vegetable or plant phytosterol esters derived from vegetable oils, a 2-generational reproductive study was carried out in rats (Waalkens-Berendsen *et al.*, 1999). Diets containing 0 (control), 1.6, 3.2, or 8.1% phytosterol esters, corresponding to 1.0, 2.0, or 5.0% phytosterols, were administered for a 10-week premating period. Both male and female Wister rats were administered the diet in the premating period, after which they were consuming the same percentage of phytosterol esters in their diets. Subsequently, the F_1 offspring consumed the same diet as their parents, as did the F_2 offspring. Offspring were weaned from their mothers at postnatal day 21, and randomly selected pups from each diet group began consuming premating diets when they were 5 weeks of age. The effect of phytosterol esters on the treatment animals was assessed through clinical observation and pathological examination of the animals, as well as statistical analysis of fertility and reproductive performance.

Overall, no treatment-related mortality, or differences between the appearance, general behavior, and general condition of the treatment rats and the control rats were observed in any generation. Additionally there were no organ weight changes, no differences in macroscopic observations at necropsy, and no histopathological changes observed in the tissues examined between the treatment groups and the control animals. At several time points during the experimental period, the average body weight of F_0 and F_1 males in the 8.1% dose group was significantly lower than that of other dosing groups. The effect of phytosterol ester consumption on food consumption was varied, with some treatment groups exhibiting significantly higher and lower food consumption during premating and gestational and lactation periods respectively. Phytosterol ester consumption was determined to have no effect on the fertility and reproductive parameters including sexual maturity, estrous cycle length, precoital time, and the histopathology of reproductive tissues. Overall, phytosterol ester consumption was reported to have no developmental or reproductive effects, and no estrogenic activity. The authors concluded that the NOAEL was the highest dosing level of 8.1% phytosterol esters in the diet, which is equivalent to 2.5 to 9.1 g of phytosterol esters/kg body weight/day, or 1.54 to 5.62 g phytosterol/kg body weight/day, depending on the phase of the study.

4.2.5 Special Studies

Several studies have indicated that β-sitosterol can act as an estrogenic agonist in female sheep and rats (El Samannoudy *et al.*, 1980; Malini and Vanithakumari, 1993; Rosenblum *et al.*, 1993; Mellanen *et al.*, 1996). As a result, Baker *et al.* (1998) examined the estrogenic potential of phytosterol esters both *in vitro* and *in vivo*. The *in vivo* study conducted by Baker *et al.* (1998) was previously discussed in Section 4.2.2. The *in vitro* study conducted by Baker *et al.* (1998) examined the ability of phytosterols to bind to estrogen receptors, and to activate estrogen-responsive gene transcription. For this purpose, estrogen receptors from 10-week-old Wistar rats were incubated with phytosterols for 18 hours, and recombinant yeasts carrying

human estrogen receptor genes were incubated with phytosterols for 3 days. Baker *et al.* (1998) reported that phytosterols were negative in the estrogen receptor-binding assay and did not induced transcription of human estrogen receptor genes. The authors concluded that the results of their *in vitro* and *in vivo* testing indicate that phytosterols lack estrogenic activity.

4.3 Clinical Studies

4.3.1 Phytosterols

Although the majority of these trials were not conducted specifically for the assessment of the safety of phytosterol consumption, they measured endpoints that can be used to lend support to the safety of phytosterols. Summaries of the clinical trails reviewed are presented in Table 4.3.1-1.

The clinical trials reviewed have administered phytosterols doses of up to 8.6 g/day and for periods as long as 52 weeks. None of the clinical trials reviewed reported any adverse effects resulting from the consumption of phytosterols by healthy, normolipidemic, hyperlidemic, normocholesterolemic, and hypercholestorolemic male and female adults, individuals heterozygous for phytosterolemia, adults with type 2 diabetes and children with a family history of hypercholesterolemia.

Table 4.3.1-1 Summary of Clinical Studies Evaluating the Effects of Phytosterol Consumption

Study	Population	Study Design	Phytosterol Dose	Endpoints	Results of phytosterol consumption
Hendriks *et al.*, 2003	Healthy males and females of 35 to 64 years of age	52 week randomized, double-blind, placebo controlled	1.6 g/day	Sex hormones, blood chemistry, and lipid soluble carotenoid and vitamin levels	Significant decrease in total and LDL cholesterol levels. Overall carotenoid concentrations were unaltered, although significant decrease in α- and β-carotene levels was observed.
Kwiterovich *et al.*, 2003	Individuals heterozygous for phytosterolemia	28 and 16 week treatment protocols	2.2 g/day	Plasma lipids, TG retinol, tocopherols, and carotenoids	Significant decrease in LDL-cholesterol levels and increase in stigmasterol and campesterol levels (both reversible)
Christiansen *et al.*, 2001	Hypercholesterolemic males and females of 25 to 64 years of age	6 month randomized, double-blind, placebo controlled	0, 1.5, or 3.0 g/day	Blood lipids, TG, and clinical chemistry	Significant decrease in LDL-cholesterol in both groups, no significant difference between 1.5 and 3.0 g/day.
Lee *et al.*, 2003	Male and female type 2 diabetes patients of 52 to 68 years of age	12 week randomized, double-blind, placebo-controlled	1.6 g/day	Plasma cholesterol levels and parameters of glycemic control	Significant decrease in total and LDL-cholesterol and no significant difference in parameters of glycemic control.
Noakes *et al.*, 2002	Hypercholesterolemic males and females of 20 to 75 years of age	12 week, 3-way, double-blind, randomized, crossover	2.0 to 2.3 g/day	Plasma lipids and carotenoids	Significant decrease in LDL-cholesterol, no change in plasma carotenoids.
Seki *et al.*, 2003	Healthy males with slightly elevated total cholesterol	12 week, randomized, double-blind, controlled	0.04 or 0.45 g/day	Plasma lipoproteins, lipid soluble vitamins	Significant decrease in total, VLDL-, and LDL-cholesterol. No change in lipid soluble vitamin levels
Nigon *et al.*, 2001	Hyperlidemic males and females of 22 to 76 years of age	2 month randomized, double-blind, placebo controlled Latin square	1.6 g/day (0.8 g β-sitosterol, 0.4 g campesterol, and 0.32 g stigmasterol/day)	Plasma lipids, lipoproteins and apolipoproteins	Significant decrease in plasma total and LDL-cholesterol.
Quílez *et al.*, 2003	normocholesterolemic males and females	8 week, randomized, double-blind, placebo controlled, repeated measures	3.2 g/day	Plasma lipids, lipoproteins, apolipoproteins, and lipid soluble antioxidants	Significant decrease in total and LDL-cholesterol, and plasma α-tocopherol but no effect on lycopene, carotenoid, or γ-tocopherol levels.

Table 4.3.1-1 Summary of Clinical Studies Evaluating the Effects of Phytosterol Consumption

Study	Population	Study Design	Phytosterol Dose	Endpoints	Results of phytosterol consumption
Amundsen *et al.*, 2001	Children with family history of hypercholesterolemia	8 week, double-blind, crossover	1.60 ± 0.13 g/day	Serum lipids, lipoproteins, carotenoids, and lipid-soluble vitamins	Decrease in plasma total and LDL-cholesterol, and apolipoprotein B levels. Lipid adjusted retinal and α-tocopherol concentrations increased
Stalenhoef *et al.*, 2001	Individuals heterozygous for phytosterolemia	8 week open feeding study with 2 week run-in and 2 week washout	~3 g/day	Plasma lipids and TG	Decrease in total plasma cholesterol levels. Blood phytosterol levels not significantly different from non phytosterolemia heterozygous individuals
Simons, 2002	Mean and women of ≥18 years of age with primary hypercholesterolemia	4 week, randomized, double-blind study with 4 parallel treatments	0, 0 + 400 µg cerivastatin, ~2 g, or ~2 g + 400 µg cerivastatin	Serum total, HDL- and LDL-cholesterol, and TG	Significant decrease in LDL-cholesterol was observed following phytosterol consumption.
Hallikainen *et al.*, 2000	Hypercholesterolemic males and females of 30 to 65 years of age	4 week, Latin square, double-blind, with 3 test periods	~0.1 or 2.0 g/day	plasma lipids and lipid soluble vitamins and carotenoids	Significant decrease in total and LDL-cholesterol, no change in lipid soluble vitamin or carotenoid levels when related to serum total cholesterol
Matvienko *et al.*, 2002	Hypercholesterolemic male college students	4 week randomized, triple-blind	2.7 g/day (4.0 g β-sitosterol, 2.2 g campesterol, and 1.7 g stigmasterol/day)	Plasma lipids, lipoproteins, and neutral sterols	Significant decrease in plasma total and LDL-cholesterol concentrations and the TC:HDL-cholesterol ratio
Ayesh *et al.*, 2000	Healthy normolipidemic males and females of 30 to 40 years of age	3 to 4 week double-blind, two-group, two-period, parallel dosing, randomized, placebo controlled	8.6 g/day (4.0 g β-sitosterol, 2.2 g campesterol, and 1.7 g stigmasterol/day)	gut microflora species typology, count, and activity, and fecal SCFAs and female sex hormones	Significant decreases in fecal lactic acid, azo reductase activity, progesterone levels, and total and LDL-cholesterol in phytosterol group.
Weststrate *et al.*, 1999	Healthy normolipidemic males and females of 30 to 40 years of age	21 days for males 28 days for females placebo controlled	8.6 g/day (4.0 g β-sitosterol, 2.2 g campesterol, and 1.7 g stigmasterol/day)	excretion of fecal bile acid and neutral sterols	Significant increase in cholesterol, β-sitosterol, campesterol, and stigmasterol excretion

Table 4.3.1-1 Summary of Clinical Studies Evaluating the Effects of Phytosterol Consumption

Study	Population	Study Design	Phytosterol Dose	Endpoints	Results of phytosterol consumption
Hendriks *et al.*, 1999	Males and females, healthy normocholesterolemic and mildly hypercholestorolemic, of 19 to 58 years of age	3.5 week, randomized, double-blind, placebo controlled balanced incomplete Latin Square	0.85, 1.62, or 3.26 g/day	Blood chemistry and plasma lipid soluble (pro)vitamins	Significant decrease in total, LDL-, and the HDL/LDL cholesterol ratio. Significant decrease in plasma (α + β)-carotene in 0.85 and 3.26 g/day groups, therefore authors support 1.62g/day.
Clifton *et al.*, 2004	Males and females, aged 20 to 75 years, with a moderately elevated plasma cholesterol level	3 week, single-blind, controlled, randomized, incomplete crossover with 4 test periods	1.6 g/day (0.8 g sitosterol, 0.3 g stigmasterol, and 0.3 g campesterol/day)	Serum lipids, plasma phytosterols and carotenoids	Decrease in total and LDL-cholesterol, and β-carotene levels. Results were not dependent on route of administration.
Judd *et al.*, 2002	Males and females of 25 to 65 years of age with normal to slightly elevated cholesterol levels	3 week, crossover, parallel design	0 or 3.6 g/day	Plasma lipids and carotenoids	Significant decrease in LDL-cholesterol and plasma TG. Significant decrease in total plasma carotenoids, however levels remained within normal levels.
Ntanios *et al.*, 2002	Normal and hypercholesterolemic Japanese men and women of 24 to 67 years of age	3 week, double-blind, crossover, with a one week washout	1.8 g/day	arteriosclerosis risk factors, lipid soluble vitamin and carotenoid levels, cholesterol levels	Significant decrease in plasma TG, and total and LDL-cholesterol, β-carotene, and apolipoprotein B. No change in vitamin A or E levels

Abbreviations: SCFA = Short Chain Fatty Acids; TG = triglyderides; LDL = Low-density-lipoproteins; HLD = High-density lipoproteins

The primary concern regarding phytosterol supplementation is the effect it may have on the absorption and circulating levels of lipid soluble vitamins and carotenoids. The majority of the clinical trials reviewed examined the effect of phytosterol consumption on lipid soluble vitamins and carotenoids. No trials were identified in which phytosterol supplementation resulted in a significant decrease in the circulating levels of lipid soluble vitamins following consumption of 0.83 to 3.6 g of phytosterols for periods of 3.5 to 52 weeks (Hendriks *et al.*, 1999; Hallikainen *et al.*, 2000; Christiansen *et al.*, 2001; Judd *et al.*, 2002; Ntanios *et al.*, 2002; Hendriks *et al.*, 2003; Seki *et al.*, 2003). Additionally, in a study conducted by Noakes *et al.* (2002), individuals who consumed one or more servings of carrots, sweet potatoes, pumpkins, tomatoes, apricots, spinach or broccoli per day while receiving 2.3 g of phytosterols/day were reported to exhibit no significant change in plasma carotenoid concentrations.

Individuals with phytosterolemia are known to absorb and store larger amounts of phytosterols than normal individuals, leading to an increased risk of premature CHD. Of the clinical trials reviewed, 2 examined the effect of phytosterol supplementation on individuals heterozygous for phytosterolemia (Stalenhoef *et al.*, 2001; Kwiterovich *et al.*, 2003). As phytosterolemia is an autozomal recessive disorder, heterozygotes do not show symptoms of phytosterolemia; however, the impact of large doses of phytosterols on them was previously unclear. In the first trial, consumption of 2.2 of phytosterol/day was reported to significantly increase plasma campesterol and sitosterol levels; however, the increases were not significantly different from those seen in control populations that were not phytosterolemia heterozygotes (Kwiterovich *et al.*, 2003). In the second trial, the consumption of approximately 3.0 g phytosterols/day was also reported to increase campesterol and sitosterol levels and again this increase was not significantly different from that seen in normal individuals (Stalenhoef *et al.*, 2001). The authors of both studies concluded that individuals heterozygous for phytosterolemia do not accumulate phytosterols and therefore do not experience the problems of phytosterolemic individuals.

All of the clinical trials reviewed indicated that consumption of phytosterols produced beneficial effects on plasma cholesterol levels. These beneficial effects were significant in individuals consuming as little as 450 mg of phytosterols/day. Phytosterol consumption was reported to be well tolerated in all the clinical trials reviewed and no side effects were reported to result from the consumption of as much as 8.6 g phytosterols/day. In a meta-analysis conducted by Katan *et al.* (2003) 41 clinical trials, many of which are summarized in Table 4.3.2-1, examining phytosterols and phytosterol esters were reviewed. The authors concluded that sufficient evidence was present to promote the use of phytosterols for the purpose of lowering LDL cholesterol levels in persons at risk for coronary heart disease (Katan *et al.*, 2003).

4.3.2 EPA/DHA

The FDA addressed the safety of DHA and EPA supplementation in the 1997 final rule on the approved use of menhaden oil as a direct food ingredient (FDA, 1997) and regarding the use of ω-3 fatty acids as a dietary supplement (FDA, 2000a). In both of these decisions, the FDA set a

maximum intake of 3 g DHA + EPA/day based on scientific literature. As the scientific literature is equivocal in reports of adverse events associated with DHA and EPA supplementation at or above the FDA upper limit, a large number of clinical trials were identified in this area.

In setting the upper limit for daily DHA + EPA intake, the FDA identified three areas of concern regarding DHA and EPA supplementation related adverse effects (FDA, 1997). Increased bleeding times were identified as an area of concern as a result of observational studies reporting they occur in population traditionally consuming fish-based diets containing high quantities of DHA and EPA (Bang and Dyerberg, 1980). Increases in low-density lipoprotein (LDL)-cholesterol levels were identified as an area of concern by the FDA as they are known to be a risk factor in the development of CHD (Balestrieri *et al.*, 1996). The third are of concern identified by the FDA was the effects on DHA and EPA supplementation on the control of fasting glucose levels in individuals with non-insulin dependent diabetes mellitus (NIDDM) (FDA, 1997, 2000a). Over 75 clinical trials were identified in which the safety of DHA and EPA supplementation was examined, and these trials are summarized in Table 4.3.2-1. The trials focused primarily on 3 areas of concern identified by the FDA when setting the maximum daily intake.

Due to the totality of evidence at the time of ruling concerning DHA and EPA intake, the FDA reported that increased bleeding time was a relevant determinant in limiting the consumption of DHA and EPA to 3 g/person/day; however, the FDA also acknowledged the equivocal nature of the results reported in the literature regarding increased bleeding time and dietary intake of DHA + EPA, and that the clinical significance of an increased bleeding time resultant from the consumption of DHA + EPA is lacking.

More than 40 of the clinical trials summarized in Table 4.3.2-1, examined the effect of DHA and EPA consumption on bleeding time and parameters related to blood clotting and fibrinolysis. Eleven of these trials reported that bleeding times were either unchanged from baseline or within normal ranges following consumption of DHA and EPA doses ranging from 3.3 g/day for 3 weeks, to 10 g/day for 6 weeks. Clinical trials examining parameters of blood clotting revealed that the incorporation of DHA and EPA into phospholipids membranes affected clotting mediators and responses (Leaf *et al.*, 1994; Prisco *et al.*, 1994; Turini *et al.*, 1994; Mori *et al.*, 1997). The physiological response to these changes was varied, as some studies reported decreases in platelet aggregation and clotting factor levels, while others reported that these parameters did not change significantly (Nilsen *et al.*, 1993; Scheurlen *et al.*, 1993; Eritsland *et al.*, 1995a, 1996; Luostarinen *et al.*, 1995; Prisco *et al.*, 1995; Freese and Mutanen, 1997a; Mori *et al.*, 1997; Hansen *et al.*, 2000).

In the clinical trials pertaining to the second area of concern identified by the FDA, an increase in LDL-cholesterol, the results are much more unified. Again more than 40 clinical trials were identified in which the effect on DHA and EPA supplementation on plasma lipids and triglycerols, and specifically LDL-cholesterol were examined. Of these trials, the majority report

that consumption of DHA and EPA did not significantly change, or resulted in a decrease in LDL-cholesterol levels. It should also be noted that in all of the studies in which an increase in LDL-cholesterol was observed, the subjects were those with established health conditions such as NIDDM, hypertriglyceridemia, IDDM, hypercholesterolemia, or hypertension (Connor *et al.*, 1993; Harris *et al.*, 1993, 1997; Gray *et al.*, 1996; Otto *et al.*, 1996; Rossing *et al.*, 1996; Swahn *et al.*, 1998; Mori *et al.*, 2000). Additionally, the physiological relevance of these results is uncertain as other CHD risk factors were unaffected by DHA and EPA consumption. Furthermore, of the clinical trials were no increase in LDL-cholesterol was reported, both healthy patients and patients with pre-existing conditions including CHD were not affected by doses as high as 10.1 g DHA + EPA/day, lending support to the safety of DHA and EPA doses >3 g/day.

The third area of concern regarding DHA and EPA consumption identified by the FDA was the control of fasting glucose levels in individuals with NIDDM. Approximately 20 clinical trials were identified in which the effect on DHA and EPA on variables related to glycemic control were examined. Of these trials, the vast majority reported that doses of >3 g DHA + EPA/day did not alter blood glucose or glycosylated hemoglobin concentrations. Furthermore, the majority of the trials also indicated that DHA and EPA had no effect on plasma insulin levels either.

While the primary focus of the majority of the clinical trials examined were the concerns identified by the FDA, several additional parameters relating to the immune system, and liver and kidney function were examined. The results of these trials indicated that DHA and EPA had no adverse effects on any of these parameters.

Overall the clinical trials presented in Table 4.3.2-1 examined the effects of DHA and EPA supplementation for as long as 4 years and provided doses as high as 10 g DHA + EPA/day. These demonstrate that doses of DHA and EPA significantly larger than the amount provided by the recommended use of carDiabeat do not have an adverse effect on homeostasis, LDL-cholesterol levels, glycemic control, immune responses, or the function of the kidney or the liver. Specifically, the reported significant effects of DHA and EPA on various parameters of homeostasis are inconsistent, no longer supported by the weight of evidence, and clinical effects indicative of adverse toxicological effects on health are absent.

Table 4.3.2-1 Summary of Clinical Studies Evaluating the Effect of DHA and/or EPA Consumption

Study	Population	Study Design	DHA/EPA Dose	Endpoints	Results
Berson *et al.*, 2004	Retinitis Pigmentosa patients of 18 to 55 years of age	4 years, randomized, controlled, double-masked trial	1.2g DHA/day and 15,00 IU Vitamin A/day or placebo and 15,00 IU Vitamin A/day	Visual acuity, retinal degeneration	No significant difference between treatment group and placebo.
Wheaton *et al.*, 2003	Male X-linked retinitis pigmentosa patients with a mean age of 16 years	4-year, placebo controlled	400 mg DHA/day or 400 mg placebo oil/day	Fatty acid and vitamin status, ALAT activity antioxidant capacity, platelet aggregation, lipoprotein cholesterol and TG profiles	Increase in plasma DHA levels, no negative effects on any of the parameters examined, and no difference in adverse events reported between groups.
Clark *et al.*, 1993	Patients with lupus nephritis randomized to receive 1 of 2 initial treatments, 22 to 66 years of age	2-year placebo-controlled, double-blind crossover of 2 treatments separated by a 10-week washout	1.7g DHA + 2.7g EPA/day in 1st year, 15 placebo capsules/day in 2nd year 15 placebo capsules/day in 1st year, 1.7 g DHA + 2.7 g EPA/day in 2nd year	Bleeding time, plasma lipids and TG, renal function, immunological markers	No significant effect on bleeding time, HDL or LDL cholesterol, renal function, or immunological markers. Significant decrease in plasma TG and VLDL cholesterol.
Yasui *et al.*, 2001	Male and female hypercalciuria patients of 25 to 71 years of age	18-month clinical trial	1,800 mg EPA/day	Urinary parameters, urinary calcium levels	A significant reduction in urinary calcium was observed.
Sacks *et al.*, 1995	Patients of 55 to 69 years of age, with CHD and normal plasma lipid levels	28 months, randomized, double-blinded, placebo-controlled	12 g fish oil (Promega)/day, providing 1.92 g DHA and 2.88 g EPA/day, or 12 g olive oil (control)	Plasma lipids and TG	Significant decrease in TG, no other effects observed.
Gogos *et al.*, 1998	Patients of 56 to 60 years of age, with generalized solid tumors	Until death; mean 213-481 days	2.07 g DHA + 3.06 g EPA/day	T-cell subsets, cytokine production, measured on day 40 of treatment, patient survival	Significant changes in T-cell subset levels and ratios were observed. No effects on cytokine production.
von Schacky *et al.*, 1999	Patients with coronary atherosclerosis	1 year, randomized, double-blinded, placebo-controlled	1.29 g DHA and 2.12 g EPA/day for 3 months, followed by 0.64 g DHA and 1.06 g EPA/day for 21 months	Plasma lipids and TG	No significant change in any parameter examined over the treatment period.

Table 4.3.2-1 Summary of Clinical Studies Evaluating the Effect of DHA and/or EPA Consumption

Study	Population	Study Design	DHA/EPA Dose	Endpoints	Results
Rossing *et al.*, 1996	Normotensive IDDM patients with diabetic nephropathy of 24 to 44 years of age	Randomized, double-blind, 1year	2.6 g DHA and 2.0 g EPA/day	Blood glucose, plasma lipids and TG, and parameters of kidney function	Glycemic control remained constant, no effect on kidney function. Significant decrease in TG and VLDL, and increase in LDL-cholesterol levels. No "serious side effects" reported.
Eritsland *et al.*, 1995a, b, 1996	610 patients with coronary artery disease and undergoing CAB surgery with mean ages of 59.9±8.7 years (fish oil) and 59.4±8.8 years (control)	1-year post-operation (until 5-7 days before angiographic evaluation)	Approx. 1.28 g DHA + 2.04 g EPA/day	Bleeding episodes, bleeding time, blood chemistry, plasma insulin and C-peptide, plasma glucose, plasma lipids and TG	No change in bleeding time or any measured haemostatic or glucose homeostasis parameters, blood chemistry, or plasma lipids and TG. Few incidences of gastrointestinal side effects were reported.
Kurabayashi *et al.*, 2000	Symptomatic menopausal Japanese women of 46 to 62 years of age	Double blind, placebo controlled, 48 week trial	1,737 mg EPA ethyl ester, 3.36 mg Vitamin E and 2 mg estriol per day	Plasma lipids and TG levels, hematology, urinalysis	EPA and estriol significantly decreased TG level and improved lipid metabolism as compared to EPA alone.
Palozza *et al.*, 1996	Healthy adults of 25 to 46 years of age	9 months; randomized, double-blind, placebo-controlled	1.1 g DHA + 1.4 g EPA/day, 2.4 g DHA + 2.7 g EPA/day, 3.6 g DHA + 4.4 g EPA/day, or placebo	Fatty acid composition, vitamin E status, in vitro lipid peroxidation of red blood cell (RBC) membranes	Significant, time-dependent increase in DHA and EPA concentrations in RBC membranes. Decrease in RBC lipid peroxidation
Almallah *et al.*, 2000	Patients with active distal proctocolitis	6 months	2.4g DHA + 3.2g EPA/day Placebo = 15 mL sunflower oil (2.6 g oleic acid + 7.9 g linoleic acid)	Clinical response and rectal mucosal biopsies for histological and immunohistochemical analyses	No adverse effects were reported.

Table 4.3.2-1 Summary of Clinical Studies Evaluating the Effect of DHA and/or EPA Consumption

Study	Population	Study Design	DHA/EPA Dose	Endpoints	Results
Eritsland *et al.*, 1994a, b	Hypertryglyceridemic patients undergoing CABG with mean ages of 61.0±8.1years (treatment) and 61.7±8.9 years (control)	6 months post operation	3.4 g DHA + EPA/day	Fibrinogen, PAI-1 activity and antigen, t-PA antigen, plasma D-dimer, fasting glucose, insulin, C-peptide, oral glucose tolerance test	Slight reduction in PAI-1 antigen, fasting glucose and fasting insulin. Significant decrease in TG levels. No other significant effects were reported.
Leaf *et al.*, 1994	Patients undergoing elective percutaneous intraluminal coronary angioplasty	12-14 days before through 6 months after angioplasty, randomized, double-blind, placebo-controlled	2.8 g DHA and 4.1 g EPA/day	Bleeding time, excretion of urinary eicosanoid metabolites, platelet count, plasma lipids and TG	All bleeding times were within normal ranges and no change observed in LDL-cholesterol levels. Significant decrease in TG levels.
Connor *et al.*, 1993	Patients of 46 to 72 years of age with NIDDM and hypertriglycidemia	Consecutive 6-month dietary intervention, randomized, double-blind, placebo-controlled cross-over	15 g fish oil (Promega)/day, providing 1.9 g DHA and 4.1 g EPA/day	Fasting glucose, glycosylated hemoglobin, urine C-peptide and glucose, plasma lipids and TG	No significant change in fasting glucose or glycosylated hemoglobin levels. Significant increase in LDL-cholesterol and decrease in TG, and total and VLDL-TG levels.
Tremoli *et al.*, 1995	Healthy volunteers, 23 to 39 years old	18 weeks	0.96 g DHA + 1.29 g EPA/day, or 1.92 g DHA and 2.58 g EPA/day for 6 weeks, followed by 0.96 g DHA + 1.29 g EPA/day for 12 weeks	Platelet aggregation, TxB_2 production, urinary thromboxane metabolites	No significant effect on platelet aggregation, TxB_2 production, or urinary thromboxane metabolites
Prisco *et al.* 1994; 1995	Healthy males with a mean age of 32±4 years	4 months, randomized, double-blind, placebo-controlled	1.4g DHA and 2.04g EPA/day or 4 g olive oil/day	Lipid and haemostatic parameters, fatty acid composition single platelet phospholipid fractions, fibrinolysis, and platelet changes after treatment	Inhibition of TXB_2 production and platelet aggregation. Decrease in urinary TXB_2 metabolites. No significant effects on parameters of coagulation, or fibrinolysis.
Kelley *et al.*, 1999	Healthy males, 20 to 40 years of age	120 days (basal diet for 30 days followed by 90 days with supplement)	Basal diet with safflower oil replaced by 6g DHA/day	Parameters of natural killer and peripheral blood mononuclear cells	Significant decrease in polymorphoneclear leukocytes. No changes in all other measured parameters.

Table 4.3.2-1 Summary of Clinical Studies Evaluating the Effect of DHA and/or EPA Consumption

Study	Population	Study Design	DHA/EPA Dose	Endpoints	Results
Kelley *et al.*, 1998	Healthy males, 20 to 40 years of age	120 days (basal diet for 30 days, 90 days of DHA)	Basal diet with or without 6 g DHA/day	Parameters of immune response	No significant effects were observed.
Nelson *et al.*, 1997	Healthy males with a mean age of 33 years	120 days	Stabilization diet for 30 days, followed by 6 g DHA/day for 90 days, or stabilization diet for 120 days, <0.05 g DHA/day	Platelet function *in vitro*, bleeding time, platelet fatty acid composition, and coagulation factors	No effect on bleeding time, platelet aggregation or soluble clotting factors.
Fenton *et al.*, 2001	Schizophrenia or schizoaffective disorder patients between the ages of 18 and 65	16 week, double blind	3 g EPA/day or 3 g mineral oil (placebo)/day	Schizophrenic symptoms	No significant improvement in schizophrenic symptoms, two adverse events – diarrhea and upper respiratory infection
Toft *et al.*, 1997	Patients of 43 to 63 years of age with untreated hypertension	16 week, randomized, double-blind, placebo-controlled	0.6 g DHA + 3.4 g EPA ethyl esters/day	Platelet count, plasminogen PAI-1, t-PA, fibrinogen, FVIIC proinsulin	No significant effect on platelet count, plasminogen PAI-1, t-PA, FVIIC, or proinsulin. No serious side effects reported.
Harris *et al.*, 1997	Patients with severe hypertriglyceridemia (5.65 mmol/L ≥TG< 22.6 mmol/L), 35 to 57 years of age	16-weeks randomized, double-blind, prospective parallel group	1.5 g DHA and 1.9 g EPA/day	Liver/kidney function: ASAT, ALAT, creatinine, uric acid, blood glucose, plasma lipids and TG	No effect on blood glucose levels or liver/kidney function. Significant increase in HDL and LDL-cholesterol and decrease in total cholesterol, VLDL and TG levels.
Christensen *et al.*, 1995	Patients with ventricular tachyarrythmia of 48 to 73 years of age	16 weeks	4.3 g DHA + EPA/day	Ventricular extrasystoles, plasma lipids and TG	Significant decrease in plasma TG, no further changes in parameters examined.
Smuts *et al.*, 2003	Women 24 to 28 weeks pregnant	Randomized, double-blind, controlled clinical trials lasting until the birth of the baby	Eggs supplemented with DHA, ~133 mg DHA/egg Control: Normal eggs ~ 33 gm DHA/egg	Gestational age and birth weight	DHA intake increased gestation by an average of 6 days

Table 4.3.2-1 Summary of Clinical Studies Evaluating the Effect of DHA and/or EPA Consumption

Study	Population	Study Design	DHA/EPA Dose	Endpoints	Results
Wigmore *et al.*, 1996	Patients with unresectable adenocarcinoma of the pancreas	median of 3 months	Dose escalation at weekly intervals from 0.24 g DHA + 0.36 g EPA/day to a maximum of 1.92 g DHA + 2.88 g EPA/day	Physical assessment, progression of cachexia, fatty acid composition of plasma phospholipids	Maximum tolerated dose of 4.8 g DHA + EPA/day. No serious toxicity reported in any patients.
Grundt *et al.*, 1999	Patients with combined hyperlipidemia (2.0 mmol/L ≤plasma TG≤ 15.0 mmol/L; total plasma cholesterol ≥6.0 mmol/L)	12 weeks, randomized, double-blind, placebo-controlled	3.4 g DHA and EPA/day	Bleeding time, platelet count, plasma Hcy, PAI, fibrinogen, TFPI and FVIIC	No change in bleeding time, fibrinogen, Hcy, TFPI, or FVIIC levels. A significant decrease in platelet cont and increase in PAI levels were observed.
Swahn *et al.*, 1998	Outpatients with history of myocardial infarction >3 months prior to study	12 weeks	3.0 g DHA and EPA/day	t-PA, PAI activity, PAI-1 antigen, antithrombin III, plasma lipid and lipoprotein analyses	Significant increase in LDL-cholesterol levels. Treatment had no significant effect on all other measured variables as compared to placebo group.
Mori *et al.*, 1997	Healthy, non-smoking males "having body mass index, blood pressure, and plasma cholesterol toward the higher end of the normal range" with a mean age of 45.7±0.6 years	12 weeks; 120 subjects randomized to 7 dietary groups	Diet of 40% energy from fat with: 1.5-2.4 g DHA and 1.32 g EPA/day, 0.8 g DHA and 1.32 g EPA/day, 2.3-3.2 g DHA and 2.64 g EPA/day, or 1.6 g DHA and 2.64 g EPA/day Diet of 30% energy from fat with: 1.5-2.4 g DHA and 1.32 g EPA/day	Platelet aggregation, TxA_2 (as TxB_2)	Significant decrease in collagen and PAI-1 induced platelet aggregation, and TxB_2 levels.
Morgan *et al.*, 1995	Patients with NIDDM and hyperlipidemia of 46 to 61 years of age	12 weeks, randomized, double-blind, placebo-controlled	9 g fish oil/day, providing 2.5 g DHA and 2.6 g EPA/day, or 9 g corn oil 18 g fish oil/day, providing 4.9 g DHA and 5.2 g EPA/day, or 18 g corn oil/day	Fasting plasma glucose, glycosylated hemoglobin (HbA_{1c}), plasma lipids and TG	No significant change in fasting glucose, glycosylated hemoglobin levels, or lipid parameters.

Table 4.3.2-1 Summary of Clinical Studies Evaluating the Effect of DHA and/or EPA Consumption

Study	Population	Study Design	DHA/EPA Dose	Endpoints	Results
Nilsen *et al.*, 1993	Patients with CHD and elevated plasma lipids	67 ± 30 days, randomized, double-blind, placebo controlled	5.1 g DHA + EPA/day, or 6 g corn oil (placebo)/day	Plasma TxB_2, PAI-1, TAT, TFPI, monocyte thromboplastin activity	Significant increase in all parameters observed with DHA + EPA and placebo
Silva *et al.*, 1996	Patients 47 to 58 years of age with hypertriglyceridemia (TG >200 mg/dL) or mixed hyperlipidemia (total cholesterol >200 mg/dL and TG >200 mg/dL)	2 months, randomized, double-blind	1.44 g DHA and 2.16 g EPA/day	Liver/kidney function: ALAT, ASAT, alkaline phosphatase, creatine kinase, creatinine, uric acid, blood glucose, plasma lipids and TG	Significant increase in fasting glucose levels. No significant effects on plasma lipids and TG, liver function, or kidney function. No major side effects were reported.
Bagdade *et al.*, 1996	Otherwise healthy IDDM patients of 30.4±3.7 years of age	2 months	1.0 g DHA and 3.6 g EPA/day	Fasting glucose, plasma fructosamine	No significant change in glucose or fructosamine levels
Miyajima *et al.*, 2001	Male outpatients with mild to moderate essential hypertension with a mean age of 44.8 ±6.4 years	8 week, randomized, double-blind, crossover trial	2.7 g EPA/day	Blood pressure, intracellular sodium content, and cell membrane fatty acid composition	A decrease in systolic blood pressure and intracellular sodium content, and an increase in erythrocyte membrane EPA content
Otto *et al.*, 1996	Patients with primary hypertriglyceridemia (FDL or FHTG)	8 weeks	0.6 g DHA + 0.9 g EPA/day for 2 weeks, followed by 1.2 g DHA + 1.8 g EPA/day for 6 weeks	Hemorrheological parameters, plasma lipid parameters	No significant effects on hemorrhealogical parameters, significant increase in mean LDL cholesterol levels in FHTG patients.
Gray *et al.*, 1996	Males of 52.8 to 60.4 years of age with hypertension not adequately controlled with antihypertensive drugs	8 weeks	1.28 g DHA and 2.16 g EPA/day	Blood chemistry and parameters of liver function, blood glucose, plasma lipids and TG	Blood glucose levels were unchanged, treatment was well tolerated. Significant increase in LDL-cholesterol and decrease in TG levels.

Table 4.3.2-1 Summary of Clinical Studies Evaluating the Effect of DHA and/or EPA Consumption

Study	Population	Study Design	DHA/EPA Dose	Endpoints	Results
Nestel *et al.*, 2002	Male and female dislipidemic volunteers aged 40 to 69 years	7 weeks, double blind, parallel design	3 g DHA/day, 3 g EPA/day or a placebo	Arterial functions, plasma lipids and plasma fatty acids	Significant decrease in plasma total and VLDL TG, an increase in SAC, and a trend of lower pulse pressure and total vascular resistance
Hansen *et al.*, 2000	Healthy, non-smoking men of 36 to 56 years of age	7 weeks, double blind, placebo controlled	3.8 g EPA, 3.6 g DHA, or 4 g corn oil/day	PAI-1 levels and activity, and it's relationship to plasma TG and phospholipid n-3 fatty acid levels	No significant changes in PAI-1 activity, and no relationship between TG and phospholipid n-3 fatty acid levels and PAI-1 activity after consumption of EPA or DHA.
Grimsgaard *et al.*, 1997	Healthy males of 36 to 65 years of age	7 weeks	3.6 g DHA ethyl ester/day, or 3.8 g EPA ethyl ester/day	Plasma lipids and TG	Significant decrease in plasma TG and total cholesterol.
Hansen *et al.*, 1993b	Healthy, non-obese, normotensive, normolipemic males of 21 to 47 years of age	7 weeks, randomized, double-blind, placebo-controlled	1.2 g DHA + 2.2 g EPA/day or 1.4 g DHA + 2.2 g DHA/day	Plasma fatty acids, platelet functions and hemostasis	Significant reduction in TXB_2 production and collagen-induced platelet aggregation. No significant effects on any other parameters and no adverse effects reported.
Mori *et al.*, 2003	Type 2 diabetes men and postmenopausal women aged 40 to 75	6 week, double blind, placebo controlled trial of parallel design	4 g EPA, 4 g DHA, or 4 g olive oil (placebo)/day	Urinary F_2-isoprotanes and markers of inflammation	A significant decrease in oxidative stress and no changes in markers of inflammation were reported
Leigh-Firbank *et al.*, 2002	Mildly hypertriacylglycerolemic males	6 weeks, double blind, placebo controlled crossover with a 12-week washout period	1.7 g EPA + 1.3 g DHA/day	Platelet EPA and DHA, lipoprotein concentrations, and LDL oxidation	Increased platelet EPA and DHA, decrease in CHD risk factors, and an increase in LDL, and LDL oxidizability

Table 4.3.2-1 Summary of Clinical Studies Evaluating the Effect of DHA and/or EPA Consumption

Study	Population	Study Design	DHA/EPA Dose	Endpoints	Results
Woodman *et al.*, 2002	Male and female hypertensive type 2 diabetic patients of 40 to 75 years of age	6 week, double-blind, placebo controlled, parallel design	4 g of EPA, DHA or olive oil (placebo)/day	Platelet aggregation, TXB_2 release, plasma t-PA and PAI-1 antigens, von Willebrand factor, p-selectin and brachial artery dilation	No significant changes in platelet aggregation, fibrinolytic function or vascular function. DHA significantly decreased collagen aggregation and TXB_2 levels
Mori *et al.*, 2000	Mildly hypercholesterolemic males of 20 to 65 years of age	6 weeks	3.68 g DHA ethyl ester/day, or 3.84 g EPA ethyl ester/day	Glucose and insulin levels, plasma lipids, LDL particle size, plasma and platelet phospholipid fatty acids	Significant increase in fasting glucose and insulin levels. Significant increase in LDL-cholesterol level and LDL particle size. No effect on total plasma cholesterol.
Conquer *et al.*, 1999	Healthy males of 28 to 31 years of age	6 weeks, randomized, double-blind	1.7 g DHA + 1.3 g EPA + 0.8 g DPA/day	Thrombogenic factors, cardiovascular risk factors, plasma lipids and TG plasma glucose	No significant effect on glucose levels or cardiovascular risk factors.
McGrath *et al.*, 1996	NIDDM patients of 46 to 61 years of age	6 weeks, randomized, double-blind, placebo-controlled crossover of 2 treatment periods separated by a 6-week washout	1.2 g DHA and 1.8 g EPA/day	Thiobarituric acid-reactive substances, glycosylated LDL and hemoglobin A1, fasting blood glucose, plasma lipids and TG	Significant decrease in fibrinogen concentration and increase in protein C concentration. No significant changes in any other measured parameter.
Lenzi *et al.*, 1996	8 patients with chronic glomerular disease (1 NIDDM, 2 hypertensive, 5 hypercholesterolemic), 19 to 70 years of age	6 weeks, open study, 4 patients of group A also in group B, 1 patient (NIDDM) duplicated in group B	2.97 g DHA + EPA/day, or 7.65 g DHA + EPA/day	Bleeding time, TxB_2, plasma lipids and TG	Significant increase in bleeding time, and decrease in plasma TxB_2 and plasma TG. No other significant effects were reported.
Turini *et al.*, 1994	Healthy males of 25 to 28 years of age	6 weeks	1.2 g DHA and 3.3 g EPA/day	Platelet aggregation, stimulated neutrophil eicosanoid production	Significant decrease in LTB_4, and 5-HEPE levels.

Table 4.3.2-1 Summary of Clinical Studies Evaluating the Effect of DHA and/or EPA Consumption

Study	Population	Study Design	DHA/EPA Dose	Endpoints	Results
Henderson *et al.*, 1994	Patients with cystic fibrosis (CF), 12.2 ± 5.4 years old, and patients without CF, 13.4 ± 6.3 years old	6 weeks, randomized, double-blind, placebo-controlled	2.2 g DHA and 3.2 g EPA/day, or 1.6 mg EPA/day	Total leukocytes, plasma alkaline phosphatase, ALAT, bilirubin, albumin, platelet count, aggregation, plasma glucose	No increase on bleeding incidence or platelet aggregation. No changes in any other parameters examined. Noted occurrence of diarrhea and eructation in some CF patients.
Lungershausen *et al.*, 1994	Hypertensive patients concurrently treated with diuretics or beta-blockers with a mean age of 61±3	6 weeks, randomized, double-blind, placebo-controlled crossover of consecutive 6-week treatments	1.5 g DHA and 1.9 g EPA/day	Plasma lipids and TG	Significant decrease in plasma TG, no further effects were observed.
McVeigh *et al.*, 1994	NIDDM patients of 45 to 64 years of age	6 weeks, randomized, double-blind, placebo-controlled crossover of 2 treatment periods with a 6-week washout period	1.2 g DHA and 1.8 g EPA/day, or 10 g olive oil/day	Fasting glucose	No significant changes in fasting blood glucose, and no adverse effects observed.
Scheurlen *et al.*, 1993	Healthy males, 25 to 38 years of age	6 weeks	3.35 g DHA + 4.5 g EPA/day or 6.5 g DHA + 3.5 g EPA/day	Bleeding time, platelet aggregation	No significant effect on bleeding time. Significant alterations in platelet response to endoperoxide and prostacyclin analogs.
Almdahl *et al.*, 1993	Patients with stable angina pectoris and accepted for CAB surgery of 48 to 69 years of age	6 weeks, randomized, double-blind, placebo-controlled	2.84 g DHA and 4.72 g EPA/day	Platelet and monocyte counts, thromboplastin	No effect on measured parameters
Hansen *et al.*, 1993a	Patients of 29 to 59 years of age with familial hypercholesterolemia (type IIa) randomized to one of three treatments	Double-blind, placebo-controlled, crossover, 3 treatment periods of 6 weeks separated by 6- to 8-week washout periods	1.8 g DHA and 3.3 g EPA/day (treatment 1) for 1st period, 40 mg HMG-CoA reductase inhibitor/day (treatment 2) for 2nd period, and combination of both (treatment 3) for 3rd period	Bleeding time and hematological analysis, before and after a standardized exercise test, plasma lipids and TG	A significant decrease in exercise-induced shortening of bleeding times was observed, however they were still shorter than resting

Table 4.3.2-1 Summary of Clinical Studies Evaluating the Effect of DHA and/or EPA Consumption

Study	Population	Study Design	DHA/EPA Dose	Endpoints	Results
			Treatment 2, treatment 3, and then treatment 1 Treatment 3, treatment 1, and then treatment 2		bleeding times. Significant decrease in plasma TG levels but no changes in total, LDL-, or HDL-cholesterol levels. No adverse effects were reported.
Higdon *et al.*, 2000	Postmenopausal women aged 50 to 75 years	3 treatment periods of 5 weeks separated by 7-week washout periods	2.0 g EPA/day and 1.4 g DHA/day	Indexes of *in vivo* lipid peroxidation	No evidence of increased lipid peroxidation
Nordøy *et al.*, 1998	Patients aged 38.9 to 56 years, with combined hyperlipidemia, serum TG 2.0-15.0 mmol/L, serum total cholesterol >5.3 mmol/L	5-week randomized, double-blind, placebo-controlled treatment period following 5-10 weeks of treatment with Simvastatin	1.56 g DHA and 1.8 g EPA/day	Plasma lipid and lipoprotein content, plasma glucose, hematological parameters and liver enzyme levels	No effect on glucose or insulin levels. Significant decrease in total cholesterol and TG. No other significant effects and no registered side effects due to treatment were reported.
Badalamenti *et al.*, 1997	Cirrhotic patients with ascites	1 month	2.76 g DHA and 3.24 g EPA/day	Bleeding time, renal function	No significant increase in bleeding time or adverse effects on renal function.
Park and Harris, 2002	Male and female normal volunteers	4 weeks, placebo controlled, parallel design	4 g of EPA, DHA, or safflower oil (placebo)/day	Platelet activation and fatty acid composition	
Nomura *et al.*, 2002	Hyperlipidemic and non-hyperlipidemic patients with and without type 2 diabetes	4 weeks	1,800 mg EPA/day	Microparticles, platelet activation markers, soluble CAMS, and oxidized LDL antibody	Significant decrease in TG, total cholesterol, soluble CAMs, oxidized LDL antibodies, and platelet and monocyte activation was observed.
Otto *et al.*, 2000	Healthy, non-pregnant women of 20 to 45 years of age	4 week	0.266, 0.285, 0.532, or 0.570 g DHA/day	Plasma fatty acids, and erythrocyte phospholipids	Significant, dose-dependent increase in DHA levels, some gastrointestinal side effects

Table 4.3.2-1 Summary of Clinical Studies Evaluating the Effect of DHA and/or EPA Consumption

Study	Population	Study Design	DHA/EPA Dose	Endpoints	Results
Brister and Buchanan, 1998	Patients undergoing elective CABG surgery	4 weeks prior to CABG; in addition, all patients received 325 mg aspirin/day until 7-10 days before CABG	1.26 g DHA and 1.9 g EPA/day, 3.2 g evening primrose oil/day, or 3.2 g primrose oil + 1 g DHA and 1.52 g EPA/day	Platelet TxA_2 and 12-hydroxyeicosatetraenoic acid levels, platelet aggregation and adhesivity	No effect on platelet adhesivity or aggregation, or TXB2 or 12-HETE synthesis.
Freese and Mutanen, 1997a	Healthy Finnish adults or 22 to 44 years of age	4 weeks supplement with 12-week follow-up,	Mean DHA intake of 2.3 g/day (1.8-3.4 g DHA/day), mean EPA intake 2.9 g/day (2.2-4.2 g EPA/day)	Platelet aggregation, TxB_2, platelet fatty acid composition, bleeding time, coagulation factors, PAL-1, plasma lipids and TG	No significant effect on any hemostatic factors, decrease in TG and total cholesterol levels. Sole adverse effect reported was "abnormally long bleeding times".
Freese and Mutanen. 1997b	Healthy volunteers of 22 to 42 years of age	4-week supplementation with 12-week follow-up	2.45 g DHA + 3.04 g EPA/day	FVII:C activity, PAI-1, platelet aggregation, TxB_2, plasma TG, cholesterol, plasma glucose	No significant change in any hemological parameters examined. Significant decrease in TG and cholesterols levels. Treatment was well tolerated with no gastrointestinal side effects.
Luostarinen *et al.*, 1995; Engström *et al.*, 1996	Males with plasma TG concentrations of 1.5-4 mmol/L (normal to mildly high), 39 to 60 years of age	4 week treatment period in randomized, double-blind crossover with a 6 week washout period between treatments	30 mL fish oil/day containing 1.5 IU vitamin E/g; providing 3.2 g DHA and 5.4 g EPA/day 30 mL fish oil/day containing 4.5 IU vitamin E/g; providing 3.2 g DHA and 5.4 g EPA/day	Fibrinogen, PAI-1 activity, PAI-1 antigen, plasma TxB_2, LTB_4 and prostacyclin, plasma glucose, plasma insulin	Significant increase in PAI-1 activity, and decrease in TxB_2 and LTB_4 levels. No change in plasma prostacyclin or fibrinogen. Significant increase in fasting glucose levels.
Balestrieri *et al.*, 1996	Patients with familial hypercholesterolemia on long term treatment with simvastatin with a mean age of 45.2±15.0 years	4 week; randomized, double-blind, placebo-controlled crossover of 2 treatment periods with 4-week washout periods	6g fish oil ethyl ester (Esapent)/day, providing 2.55g DHA and 2.55 g EPA/day	Plasma lipids and TG	No effect on any measured parameter.

Table 4.3.2-1 Summary of Clinical Studies Evaluating the Effect of DHA and/or EPA Consumption

Study	Population	Study Design	DHA/EPA Dose	Endpoints	Results
Misso and Thompson, 1995	Healthy volunteers of 23 to 40 years of age	4 weeks, randomized, double-blind with a washout of 4 weeks	1.44 g DHA and 2.16 g EPA/day or 12 g olive oil	Platelet aggregation, fibrinogen, coagulation tests	No significant effect on platelet aggregation, fibrinogen, or any measures of coagulation.
Chin and Dart, 1994	Hypercholesterolemic patients of 39 to 49 years of age	4 weeks	2.32 g DHA and 3.56 g EPA/day	Plasma lipids and TG	No effects on cholesterol or TG levels.
Mundal *et al.*, 1993	Hypertensive males with hyperlipidemia of 33 to 64 years of age	4 weeks, randomized, double-blind, placebo-controlled crossover with a 4 week washout period	2.8 g DHA + 1.8 g EPA/day	Bleeding time, β-thromboglobulin, platelet count	No effect on bleeding time, β-thromboglobulin, or platelet count.
Harris *et al.*, 1993	Mildly hypertriglyceridemic patients of 34 to 68 years of age	4 weeks, randomized, double-blind, placebo-controlled crossover with a 4 week washout period	1g fish oil providing ~2.0 g DHA and ~3.5 g EPA/day	Bleeding time, plasma lipids and TG, lipoprotein oxidation: thiobarituric acid-reactive substances	No effect on bleeding time. Significant increase in LDL-cholesterol and LDL-apolipoproteins, and decrease in TG levels. No further effects or adverse events reported.
Sanders *et al.*, 1997	Healthy, non-obese males of 18 to 34 years of age	Crossover of 3 weeks of nonrandomized saturated fat diet followed by 3 weeks of n-3 or n-6 diet, with 8-week washout between diets	Saturated fat diet - "trace" amounts of DHA + EPA n-3 diet – 2 g DHA + 3 g EPA/day n-6 diet – 5 g linoleic acid	Fibrinogen, plasma FVIIC, FVIIC antigen, PAI-1, t-PA, plasma F, von Willebrand factor, β-thromboglobulin, plasma lipids and TG	Significant increase in fasting FVIIC activity and von Willebrand factor levels. Significant decrease in β-thromboglobulin, platelet counts, and total and LD-cholesterol levels.
Tsai and Lu, 1997	Healthy normolipidemic males	3 weeks of fish oil dietary supplementation subsequent to 3 weeks of soybean oil dietary supplementation	50 or 250 mg dietary cholesterol/4,187 kJ and 20 g soybean oil/day for 3 weeks followed by 1.6 g DHA and 7.2 g EPA/day	Plasma lipids and TG	Significant decrease in VLDL-cholesterol and total and VLDL-TG.

Table 4.3.2-1 Summary of Clinical Studies Evaluating the Effect of DHA and/or EPA Consumption

Study	Population	Study Design	DHA/EPA Dose	Endpoints	Results
Nordøy *et al.*, 1994	Healthy, non-smoking, normolipemic males of 27 to 58 years of age	3 weeks per diet with 6-week washout between high and low saturated fat diets	High saturated fat supplemented with 1.2 g DHA and 2.1 g EPA/day followed by similar dietary protocol with low saturated fat (5% of energy) diet.	Platelet and vascular eicosanoid formation, and hemostatic parameters associated with arterial thrombosis	No significant changes in bleeding time or haemostatic parameters as compared to controls. No adverse effects were reported.
Burns *et al.*, 1999	Patients aged 34 to 76 years with diagnosis of cancer not amendable to curative treatment	2 weeks per dose	Dose-escalation in groups of 2 starting at 0.0249 g DHA + 0.0378 g EPA/kg bw/day	Maximum tolerated dose using a graded toxicity criteria	Maximum tolerated doses estimated to be 13.2 g DHA + EPA/day, adverse effects were gastrointestinal
Fasching *et al.*, 1996	Hyperlipidemic, NIDDM patients with a mean age of 61.1±6.2 years	2 weeks, randomized, open, crossover of 2 treatment periods with an 8-week washout period	1.784 g DHA and 2.890 g EPA/day	Fasting blood glucose, glycosylated hemoglobin, liver and kidney function, plasma lipids and TG	No significant changes in measured parameters of glycemic control or measures of liver and kidney function. Significant decrease in total and VLDL-cholesterol, and total and VLDL-TG levels.
Krokan *et al.*, 1993	Healthy males of 18 to 55 years of age	2 weeks	1.2 g DHA + 2.2 g EPA/day, 2.4 g DHA + 4.4 g EPA/day, 4.2 g DHA + 7.7 g EPA/day, 1.4 g DHA + 2.2 g EPA/day, or 2.9 g DHA + 4.4 g EPA/day	Fatty acid composition of plasma lipids and phospholipids	No adverse effects or discomfort as a result of treatment.
Yosefy *et al.*, 1996	Hypertensive, mildly obese and dyslipidemic patients of 57 to 61 years of age	Non-randomized crossover of 3, 13-day treatment periods containing 4, 20-h fasting periods, each treatment period separated by a 3-week washout	Period I: 1.8g DHA and 2.7g EPA/day, administered after fasting and followed by refeeding Period II: fasting followed by refeeding without DHA or EPA Period III: 1.8g DHA and 2.7g EPA/day, without fasting and refeeding	Platelet count, adhesion and aggregation, α_2-anti-plasmin, fibrinogen, plasma lipids and TG	Significant reduction in platelet aggregation and adhesion, and α_2-anti-plasmin following period I but not periods II or III. No effect on fibrinogen, platelet counts, or plasma lipids and TG.

Abbreviations: ASAT = Aspartate Amino Transferase; ALAT = alanine amino transferase; CAB = Coronary Artery Bypass; CABG = Coronary artery bypass grafting; CHD = Coronary Heart Disease; FDL = Familial dysbetalipoproteinemia; FHTG = Familial hypertriglyceridemia; FVIIC = Coagulation Factor VII; GFR = Glomerular filtration rate; Hcy = Homocystein; PAI = Plaminogen Activator Inhibitor; SAC = systemic arterial compliance; TAT = Thrombin-antithrombin III; TG= Triglycerides; t-PA= tissue plasminogen activator; TPF = Tissue factor pathway inhibitor

4.3.3 CarDiabeat

4.4 Background Dietary Exposure From the Diet

4.4.1 Phytosterols

Dietary intake of phytosterols has been reported to be approximately 160 to 250 mg/day in non-vegetarian populations, and approximately 500 mg/day for vegetarians (Ling and Jones, 1995; Morton *et al.*, 1995). In the Tarahumara Indians of Mexico, phytosterol intake has been reported to exceed 400 mg/day (Cerqueira *et al.*, 1979). In a study of the British phytosterol intakes it was reported that β-sitosterol, campesterol, and stigmasterol represent approximately 65, 30, and 5% of the total phytosterol consumption (Morton *et al.*, 1995).

4.4.2 EPA/DHA

The dietary intake of DHA and EPA has been reported to vary widely between populations due to the varied prevalence of fish in the diet. The examination of the eating habits of individuals in France for a 2.5-year period revealed that the average DHA intake is 273 and 226 mg/day in men and women, respectively, and the average EPA intake is 150 and 118 mg/day in men and women, respectively (Astorg *et al.*, 2004). In the Australian population, the average daily intakes of DHA and EPA were reported to be 106 and 56 mg/day, respectively (Meyer *et al.*,

2003). In a study of the dietary intake of pregnant Canadian women, it was reported that the average intakes of DHA and EPA were 160 and 78 mg/day, respectively (Innis and Elias, 2003).

4.5 Regulatory Status

4.5.1 Phytosterols

The FDA has determined that vegetable or plant phytosterol esters are GRAS as food ingredients in vegetable oils, spreads, salad dressings, bars and yogurt. Additionally, the FDA has published the IFR (FDA, 2000b), which authorized the use, on food labels and in food labeling, of health claims relating to the relationship between phytosterol esters and a reduced risk of CHD. The IFR authorizes the use of a health claim relating plant sterol esters and reduced risk of CHD on labeling of spreads and dressings for salad containing at least 0.65 g of phytosterol esters per serving, and providing a minimum of 1.3 g/day.

4.5.2 EPA/DHA

The FDA has established a maximum daily intake of 3.0 g of DHA and EPA. This level was initially established in 1997 when the FDA determined that menhaden oil was GRAS up to a level which provided a maximum of 3.0 g DHA + EPA/day. In 2004, the FDA reaffirmed that the intake of DHA and EPA must not exceed 3.0 g/day from all fish oil sources (FDA, 2004).

4.6 Assessment of Safety

4.6.1 Phytosterols

Both *in vitro* and *in vivo* testing has indicated that phytosterols are not mutagenic nor genotoxic. Acute, subchronic, and chronic animal studies have indicated that phytosterols posses little oral toxicity to normal animals. In SHRP rats consumption of very high levels of phytosterols (1,350 mg phytosterols/kg body weight/day) may produce a decrease in life span. Clinical trials examining the effects of the consumption of phytosterol doses as high as 10 g/day have reported that phytosterols are well tolerated and produce no adverse effects. The exposure to phytosterols resulting from the recommended use of carDiabeat is not expected to result in any adverse effects.

The FDA has determined that the consumption of a minimum of 1.3 g of phytosterol esters/day in divided doses of at least 0.65 g/serving provide a reduced risk for CHD. In acute, subchronic, and chronic toxicity studies conducted in a variety of laboratory animals, phytosterols esters were reported to display low oral toxicity. No adverse effect are expected to result from the

consumption of phytosterols according to the recommended daily intake of carDiabeat, and in accordance with the regulations set by the FDA carDiabeat is expected to provide phytosterol esters at levels that whill have a beneficial effect on the risk of developing CHD.

4.6.2 EPA/DHA

Subchronic and chronic animals trials have indicated that DHA and EPA possess low oral toxicity. Clinical trials conducted with DHA and EPA doses at least 4.5 times larger than the amount present in carDiabeat have indicated that DHA and EPA consumption produced no adverse effects on bleeding times, LDL-cholesterol levels, and fasting glucose levels in individuals with NIDDM. The amount of DHA and EPA provided by the recommended daily intake of carDiabeat is not expected pose any safety concerns.

4.6.3 CarDiabeat

Enzymotec has conducted both animal and clinical trials with carDiabeat, and no adverse effects were reported in either of them. Additionally, the safety of carDiabeat is supported by extensive safety data relating to its primary components plant phytosterols and the omega-3 fatty acids DHA and EPA. No adverse effects are expected to result from the recommended daily consumption of carDiabeat.

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January 17, 2004