ORIGINAL SUBMISSION

PULEVA Biotech

Dr. Robert L. Martin
U.S. Food and Drug Administration
5100 Paint Branch Parkway, HFS-255
College Park, MD 20740-3835
United States of America

REC'D FEB 3 2006

Date: January 25, 2006

Re: GRAS notification for Eupoly-EPA and Eupoly-DHA

To whom it may concern:

Pursuant to regulation 21 CFR 170.36, 62 FR 18938 (April 17, 1997), Puleva Biotech hereby notifies that Eupoly-EPA and Eupoly-DHA (both fish oils) are exempt from the premarket approval requirement of the Federal Food, Drug and Cosmetic Act because they have been determined, through scientific procedures, to be GRAS (Generally Recognized As Safe), for addition to food as a nutrient supplement to increase the dietary intake of the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and as an alternative to other food-grade oils. The categories of foods to which Eupoly-EPA and Eupoly-DHA are intended to be added, and the corresponding levels of EPA and DHA in those foods, are identical as those specified for menhaden oil under FDA´s final rule for menhaden oil (21 CFR Part 184, March 23, 2005).

Detailed information about this GRAS notification is enclosed, including contact details where the FDA may review and copy any additional data and information that are the basis for this GRAS determination.

For any additional questions or requirements, please do not hesitate in contacting the undersigned.

Sincerely,

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GRAS NOTIFICATION

Eupoly-EPA and Eupoly-DHA derived from fish oils

GRAS NOTIFICATION FOR EUPOLY-EPA AND EUPOLY-DHA DERIVED FROM FISH OILS

GRAS Exemption Claim

Puleva Biotech hereby notifies the Food and Drug Administration that the use of Eupoly-EPA and Eupoly-DHA described below is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because Puleva Biotech has determined that such use is Generally Recognized As Safe (GRAS).

January 25, 2006

Jesus Jimenez Lopez, Ph.D. Date
General Director, Puleva Biotech

Name and Address of Notifier

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PART A GRAS DETERMINATION

A.1 Identity of GRAS Substance

The two substances that have been determined to be Generally Recognized As Safe (GRAS) and are thus the subject of this GRAS notification are products derived from fish oils, commonly called Eupoly-EPA and Eupoly-DHA. Both products are manufactured by Puleva Biotech, a company that is part of the Ebro-Puleva group, and are principally composed of triglycerides although may contain small amounts of mono- and di-glycerides.

The main differences between Eupoly-EPA and Eupoly-DHA are their contents of the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), being approximately 20% EPA and 10% DHA for Eupoly-EPA and approximately 10% EPA and 20% DHA for Eupoly-DHA. These differences in EPA:DHA ratios are due to the different sources of the crude oils used to produce Eupoly-EPA and Eupoly-DHA. Eupoly-EPA is obtained mainly from the oil of sardine and anchovy and Eupoly-DHA from tuna oil.

Eupoly-EPA and Eupoly-DHA are substantially similar to other fish oils that are already regarded as GRAS for addition to foods, including menhaden oil (21 CFR 184.1472), small planktivorous pelagic fish body oil (GRAS Notice GRN 102), salmon oil (GRAS Notice GRN 146), anchovy-sardine 18/12 TG fish oil (GRAS Notice GRN 138), Marinol Omega-3 fish oil derived from anchovy, sardine and mackerel (GRAS Notice GRN 105), and tuna oil (GRAS Notice GRN 109).

A.2 Intended Use and Consumer Exposure

The intended uses of Eupoly-EPA and Eupoly-DHA are to enrich foods in EPA and DHA and replace saturated unhealthy fats in existing foods (partly) with long chain polyunsaturated fatty acids. For this reason the technical effect that covers the use of both fish oils presented herein can be defined by the purpose of providing a benefit to the consumer of the food, or, in other words, a nutrient supplement (definition #20 of the

Code of Federal Regulations 21 CFR 170.3) not to be confused with a dietary supplement.

The food categories to which Eupoly-EPA or Eupoly-DHA are intended to be added are presented in Table I. The maximum levels are also presented in this Table for each category. The food categories and the maximum EPA+DHA supplementation levels for each category are identical as approved by the FDA for menhaden oil (21 CFR Part 184, final rule as published in the Federal Register at page 14530 on March 23, 2005). Since the EPA and DHA levels in Eupoly-EPA and Eupoly-DHA are higher than in menhaden oil, the maximum proposed use levels of Eupoly-EPA or Eupoly-DHA are corrected for their EPA+DHA content (30%) with respect to EPA+DHA content found in menhaden oil (20%). In other words, Eupoly-EPA and Eupoly-DHA are proposed for addition at 67% of the use levels as permitted for menhaden oil (also reflected in Table I). Because the combined EPA and DHA content of foods to which Eupoly-EPA or Eupoly-DHA will be added is identical to that permitted for menhaden oil under the final rule (21 CFR Part 184), Eupoly-EPA and Eupoly-DHA will merely provide an alternative to menhaden oil or other fish oils as sources of EPA and DHA in the diet. Therefore, no incremental increase in potential intake of combined EPA and DHA combined will result from the proposed uses of Eupoly-EPA and Eupoly-DHA. It is concluded that the estimated mean intake of EPA and DHA combined from the proposed uses of Eupoly-EPA and Eupoly-DHA listed in Table I in the general population, excluding infants under the age of one year, is identical as for menhaden oil and does therefore not exceed 3 grams/person/day (21 CFR Part 184, final rule).

It is important to note that for the enrichment of a specific food only Eupoly-EPA or only Eupoly-DHA will be used, depending on the wishes of the manufacturer. The difference between Eupoly-EPA and Eupoly-DHA is their ratio in EPA:DHA but not in the sum of EPA+DHA. Therefore, a liquid milk product containing 2 grams per liter of Eupoly-EPA will contain approximately 600 mg per liter EPA+DHA (approximately 400 mg EPA + 200 mg DHA), the same as a milk product containing 2 grams per liter Eupoly-DHA (approximately 200 mg EPA + 400 mg DHA).

Furthermore, it is also important to note that when Eupoly-EPA or Eupoly-DHA are added to food they are not used in combination with other added oils that are significant sources of EPA and DHA.

A.3 Basis for the GRAS Determination

The GRAS determination for Eupoly-EPA and Eupoly-DHA under the proposed uses and at the maximum use levels listed in Table I is based on scientific procedures as described under title 21 of the Code of Federal Regulations (21 CFR §170.30(b)).

To demonstrate safety through scientific procedures first, the estimated cumulative intake of EPA and DHA combined resulting from the ingestion of Eupoly-EPA and Eupoly-DHA under their intended conditions of use was shown not to exceed 3 grams per person per day, a safe level of intake established by the FDA, and second, this cumulative intake of EPA and DHA combined was determined GRAS by demonstrating that the safety of this level of intake is generally recognized by experts qualified by both scientific training and experience to evaluate the safety of substances directly or indirectly added to food, and is based on generally available information.

In affirming the GRAS status of menhaden oil under 21 CFR §184.1472, the FDA established that a daily intake of EPA and DHA combined not exceeding 3 grams per person per day is safe. The scientific basis to support the establishment of this accepted daily intake of 3 g/p/d was published in the Federal Register at page 30571 on June 5, 1997, as part of the final rule on menhaden oil. A review of the scientific literature published since the date of the GRAS affirmation of menhaden oil confirms that the conclusion reached by the FDA regarding the safety of ingestion of up to 3 g/p/d of EPA and DHA combined is consistent with current information regarding the safety of EPA and DHA (see part C of this GRAS notification).

As mentioned earlier, the proposed uses and maximum addition levels for Eupoly-EPA and Eupoly-DHA as presented in Table I are identical to those permitted for menhaden oil under 21 CFR, Part 184. Because Eupoly-EPA and Eupoly-DHA will merely provide an alternative to menhaden oil or other fish oils as sources of EPA and DHA in the diet, it is therefore concluded that the total intake of EPA and DHA combined will not exceed 3.0

grams per person per day, as was concluded for menhaden oil (21 CFR Part 184, final rule). Thus, the proposed uses and use levels for Eupoly-EPA and Eupoly-DHA can be considered safe.

Safety of consumption of Eupoly-EPA and Eupoly-DHA for use as an ingredient in food was also determined by evaluating the sources of both products, their production process, the nature and quantity of impurities, product specifications, and fatty acid compositions. Appropriate specifications have been established to ensure that the final products are food grade, and compositional analyses of Eupoly-EPA and Eupoly-DHA support the presumption that there is no toxicological concern from the ingestion of any product impurities (see part B of this notification).

The publicly available data demonstrating the safety of the proposed uses of Eupoly-EPA and Eupoly-DHA was reviewed by an expert panel consisting of Julio Boza Puerta, Ph.D. (director of research, Puleva Biotech), Ángel Gil Hernández, Ph.D. (expert in Omega-3 supplementation, and professor in Nutrition, University of Granada, Spain), and Nicolas Olea, Ph.D. (expert in xenobiotics and human health, and professor in Environmental Science and Radiology, University of Granada, Spain). This panel, which Puleva Biotech regards as qualified by scientific training and experienced to evaluate the safety of substances to be added to food, reviewed all the data and other information presented in this notification regarding source of the substances, manufacturing method, specifications, contaminant levels, dietary exposure, and recent published toxicological and human studies. This panel concluded that:

> *Eupoly-EPA and Eupoly-DHA have been sufficiently characterized to ensure that these are food grade products, are substantially similar to other fish oils already determined to be GRAS, and have no toxicity concerns from impurities. No evidence exists in the available information that suggests that the cumulative intake of EPA and DHA combined from the proposed uses and maximum use levels of Eupoly-EPA and Eupoly-DHA in food are a hazard for the public health. Therefore, Eupoly-EPA and Eupoly-DHA, meeting the food grade specifications described in this notification, are safe and GRAS under their intended conditions of use.*

In the opinion of these experts, other qualified and competent scientists reviewing the same publicly available data would reach the same conclusion. It is therefore concluded that, based on scientific procedures, the intended uses of Eupoly-EPA and Eupoly-DHA, as shown in Table I, are safe and GRAS. Because Eupoly-EPA and Eupoly-DHA are GRAS for their proposed uses, they are excluded from the definition of a food additive, and thus may be lawfully marketed and sold for these uses in the U.S. without the promulgation of a food additive regulation under 21 CFR.

A.4 Availability of Information

The detailed data and information that serve as the basis for this GRAS determination will be sent to the FDA upon request and are available for the FDA´s review and copying at Puleva Biotech, contact person: Arjan Geerlings, Camino de Purchil 66, 18004 Granada, Spain, telephone: +34-958240152, fax: +34-958240160, and email: ageerlings@pulevabiotech.com.

Table I Intended food categories for supplementation with Eupoly-EPA or Eupoly-DHA, their maximum addition levels, and the resulting addition levels of EPA and DHA combined. Food categories were selected from the 43 food categories as defined in the Code of Federal Regulations 21 CFR 170.3. The maximum amount of menhaden oil for each food category as established by the FDA (21 CFR Part 184, final rule) is also presented in this Table.

Food Category	**Maximum level of use in food (as served)**		**Maximum EPA+DHA addition level**
	Menhaden oil	**Eupoly-EPA or Eupoly-DHA**	
Baked goods and baking mixes (1)	5.0%	3.3%	1.0%
Cereals (4)	4.0%	2.6%	0.8%
Cheese products (5)	5.0%	3.3%	1.0%
Chewing gum (6)	3.0%	2.0%	0.6%
Condiments (8)	5.0%	3.3%	1.0%
Confections and frostings (9)	5.0%	3.3%	1.0%
Dairy product analogs (10)	5.0%	3.3%	1.0%
Egg products (11)	5.0%	3.3%	1.0%
Fats and oils (12), not in infant formulas	12.0%	8.0%	2.4%
Fish products (13)	5.0%	3.3%	1.0%
Frozen dairy desserts (20)	5.0%	3.3%	1.0%
Gelatins and puddings (22)	1.0%	0.6%	0.2%
Gravies and sauces (24)	5.0%	3.3%	1.0%
Hard candy (25)	10.0%	6.6%	2.0%
Jams and jellies (28)	7.0%	4.6%	1.4%
Meat products (29)	5.0%	3.3%	1.0%
Milk products (31)	5.0%	3.3%	1.0%
Nonalcoholic beverages (3)	0.5%	0.3%	0.1%
Nut products (32)	5.0%	3.3%	1.0%
Pastas (23)	2.0%	1.3%	0.4%
Plant protein products (33)	5.0%	3.3%	1.0%
Poultry products (34)	3.0%	2.0%	0.6%
Processed fruit juices (35)	1.0%	0.6%	0.2%
Processed vegetable juices (36)	1.0%	0.6%	0.2%
Snack foods (37)	5.0%	3.3%	1.0%
Soft candy (38)	4.0%	2.6%	0.8%
Soup mixes (40)	3.0%	2.0%	0.6%
Sugar substitutes (42)	10.0%	6.6%	2.0%
Sweet sauces, toppings, and syrups (43)	5.0%	3.3%	1.0%
White granulated sugar (41)	4.0%	2.6%	0.8%

PART B CHEMICAL AND TECHNOLOGICAL DATA

B.1 Identity of Substance

The food ingredients subject to this GRAS notification are two refined and deodorized fish oils that are already used for the preparation of food stuffs in several countries (e.g. Spain). They are traded under their commercial names Eupoly-EPA and Eupoly-DHA. The main difference between Eupoly-EPA and Eupoly-DHA is their DHA to EPA ratio, being Eupoly-EPA rich in EPA and Eupoly-DHA containing more DHA than EPA. The manufacturing process and product specifications are identical for both oils.

Eupoly-EPA is a fish oil obtained from sardine and anchovy caught at the open sea near Peru. Eupoly-DHA is derived from tuna fished at the open sea (Atlantic Ocean). Some other edible fish species might also have been used in the preparation of the crude oils used to prepare Eupoly-EPA and Eupoly-DHA. The crude oils are obtained from a provider and are further refined, deodorized, and stabilized in the installations of Puleva Biotech, and sold under the names Eupoly-EPA and Eupoly-DHA. Table II shows the typical composition of both oils rich in the long-chain polyunsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

Table II Composition of Eupoly-EPA and Eupoly-DHA.

	Eupoly-EPA	**Eupoly-DHA**
Marine oil source	sardine and anchovy	tuna
Eicosapentaenoic acid (EPA)	20% average	10% average
Docosahexaenoic acid (DHA)	10% average	20% average
EPA+DHA	around 30%	around 30%
EPA:DHA ratio	close to 2	close to 0.5

Eupoly-EPA and Eupoly-DHA are fish oils suitable for their use in the preparation of (functional) foods because of their high stability and optimal organoleptic characteristics. Both are liquid oils at room temperature and have a clear and yellowish appearance. Table III shows the physical and chemical characteristics of both oils.

Table III Physical and chemical characteristics of Eupoly-EPA and Eupoly-DHA.

	Eupoly-EPA	**Eupoly-DHA**
Appearance at room temperature	yellowish oil	yellowish oil
Acid value	maximum 1.0 mg KOH/g	maximum 1.0 mg KOH/g
Peroxide value	maximum 2.0 meq/kg	maximum 2.0 meq/kg
p-Anisidine	maximum 20	maximum 20
Totox number (2 x peroxide + anisidine)	maximum 24	maximum 24
Humidity	maximum 0.1%	maximum 0.1%
Unsaponifiable	maximum 3.0%	maximum 3.0%
Cholesterol	maximum 0.5%	maximum 1.0%
Stability to oxidation, Rancimat (100ºC)	minimum 8 hours	minimum 8 hours
Lead (Pb)	less than 0.1 mg/kg	less than 0.1 mg/kg
Cadmium (Cd)	less than 0.1 mg/kg	less than 0.1 mg/kg
Mercury (Hg)	less than 0.1 mg/kg	less than 0.1 mg/kg
Arsenic (As)	less than 0.1 mg/kg	less than 0.1 mg/kg
Iron (Fe)	less than 0.5 mg/kg	less than 0.5 mg/kg
Copper (Cu)	less than 0.1 mg/kg	less than 0.1 mg/kg
PCDD/Fs	maximum 1.5 pg WHO-TEQ/g	maximum 1.5 pg WHO-TEQ/g
PCBs similar to dioxins	maximum 3.5 pg WHO-TEQ/g	maximum 3.5 pg WHO-TEQ/g
Benzo(a)pyrene	maximum 2.0 µg/kg	maximum 2.0 µg/kg

Eupoly-EPA and Eupoly-DHA are food ingredients containing refined fish oil, tocopherols, and some other antioxidants. The antioxidants used are food grade and serve to stabilize the oil and prevent it from oxidation. The fatty acid compositions of Eupoly-EPA and Eupoly-DHA are presented in Table IV. In both Eupoly-EPA as well as in Eupoly-DHA, these fatty acids are found esterified as triacylglycerides. The unsaponifiable fraction of both Eupoly-EPA and Eupoly-DHA consist principally in cholesterol and the antioxidants used to stabilize the oil.

Eupoly-EPA and Eupoly-DHA are substantially similar to other fish oils that are already regarded as GRAS for addition to foods, including menhaden oil (21 CFR 184.1472), small planktivorous pelagic fish body oil (GRAS Notice GRN 102), salmon oil (GRAS Notice GRN 146), anchovy-sardine 18/12 TG fish oil (GRAS Notice GRN 138), Marinol Omega-3 fish oil derived from anchovy, sardine and mackerel (GRAS Notice GRN 105), and tuna oil (GRAS Notice GRN 109).

Table IV Typical fatty acid composition of Eupoly-EPA and Eupoly-DHA (in % of total fatty acids, in weight). Fatty acid composition might differ slightly from batch to batch.

Fatty acid	Eupoly-EPA	Eupoly-DHA
C14:0 Myristic acid	7	5
C15:0	< 1	< 1
C16:0 Palmitic acid	16	18
C16:1	8	6
C17:0	< 1	2
C17:1	1	< 1
C18:0 Estearic acid	3	4
C18:1n9 cis-Oleic acid	10	14
C18:1n7	3	3
C18:2n6 cis-Linoleic acid	1	2
C18: 3n6	< 1	< 1
C20:0	< 1	< 1
C18:3n3 Linolenico	< 1	< 1
C22:1n11	< 1	< 1
C20:1n9	2	2
C18:4n3	3	2
C20:4n6 Araquidonic acid	2	2
C20:4n3	1	< 1
C20:5n3 EPA	20	10
C24:1	< 1	< 1
C22:5n3	2	2
C22:6n3 DHA	10	20
Total, identified	93	96
Others less than 1%, unidentified	7	4

B.2 Description of Manufacturing Process

Introduction

The manufacturing plant producing the ingredients object of this petition is located in Granada (Spain) next to one of the milk processing fabrics of Puleva Food. Because of its localization the manufacturing plant receives all industrial utilities (hot and cold water, electricity and steam) from Puleva Food. The manufacturing plant has been operative since June, 2003.

Puleva Biotech´s manufacturing plant has the objective of obtaining and purifying crude fish oils and preparing them for human consumption. Nowadays, foods contain mainly saturated fats increasing the risk of cardiovascular diseases in the human population.

The replacement of these saturated fats by unsaturated and polyunsaturated fats is important for the prevention of these diseases. Fish oils are especially rich in long chain polyunsaturated fatty acids, also called Omega-3 fatty acids of which EPA and DHA are the most important.

The manufacturing plant was designed around the principle steps in obtaining edible oils: refination, decolorization, deodorization, mixing, and stabilizing the final product. Therefore, the process starts with crude fish oils and consists in a series of chemical and physical operations similar to the ones used commonly in refination of edible oils (e.g. olive and sunflower oils). As a consequence, fish oils rich in Omega-3 fatty acids are obtained that are suitable for human consumption and their incorporation into food. The final products are packaged into bag-in-box containers and sold to other companies within the Ebro-Puleva group as well as to external companies.

The manufacturing plant produces both Eupoly-EPA as well as Eupoly-DHA and can be called a semi-industrial plant because of its dimensions. The plant has a total area of 300 square meters, and is divided into an operation room (75m^2), a warehouse of crude oil (33 m^2) and a control room (20 m^2). The remaining space can be used in the future to increase the plant's capacity. An additional warehouse (16 m^2) where waste products are stored is located in another building to avoid cross-contamination. The final products are stored in a cold room located in yet another building.

Capacity

Being a relatively small plant of semi-industrial scale, the production capacity is variable depending on the characteristics of the crude oil to be treated. The quality of the crude oil determines the time required for each of the oils in each stage of the process. Taking into account that the passage time of the oil in the deodorization step is the most crucial step, the standard production capacity of Puleva Biotech´s plant is approximately 200 kg of oil per batch of 2 hours. Therefore, during a normal working day of 8 hours, daily production is between 800 and 1000 kg of processed oil.

Below, a description is given of the production process including the different steps, from reception of the crude oil to the storage of the final products. Schematically, the process that is used for the production of Eupoly-EPA and Eupoly-DHA is presented in scheme I.

Reception of materials

The following raw materials are required for the manufacturing of Eupoly-EPA and Eupoly-DHA; crude fish oils, decolorizing earths, active carbon, nitrogen gas, silica, sodium hydroxide, bag-in-boxes, and carton boxes. All these materials are stored in the raw material warehouse that is well separated from the rest of the plant.

The crude refined fish oils used for the production of Eupoly-EPA and Eupoly-DHA are acquired from a crude fish oil producer. The process applied to obtain these refined fish oils is a standard refination process normally used to obtain edible oils.

The crude fish oil is received in 1000 L containers and stored in the raw material warehouse. The size of the containers is practical because it is the same as the daily capacity of the plant. The crude oils are stored in the warehouse at room temperature for not more than 1 month. Although the crude oils do not suffer any degradation during this time, it is considered convenient not to have large stocks of crude oil.

The decolorizing earths are received in 25 kg bags, silica in 15 kg bags, and the active carbon in 20 kg bags. These materials are stored in the raw material warehouse well separated from each other and the rest of the materials.

Sodium hydroxide is received in solid form or in 50% liquid form in 200 L drums. It is handled according to internal procedures for the manipulation of chemicals until its use in the plant.

The antioxidant used for stabilizing the final product is received in 10 kg polyethylene containers and stored in the raw material warehouse. This antioxidant is a natural product, is food grade, and does not need any special treatments or handling procedures.

Initial steps of manufacturing process

A 1000 L container full of raw fish oil is situated at the beginning of the plant with the help of an electric fork-lift truck. The oil is passed to a 1500 L container using a volumetric pump. At this point it is possible to mix in the container several batches of oils with the

purpose of obtaining an oil with the right fatty acid profile. This mixing of different oils is sometimes necessary since the fatty acid profile and the EPA+DHA content of the raw oil is not always the same (sum of EPA+DHA and their ratio may differ somewhat from batch to batch). By mixing different batches of crude fish oil with different EPA and DHA contents it is possible to obtain final products with the desired EPA-DHA profile and content. All this is possible because from each 1000 L container of raw fish oil a fatty acid analysis is performed in the laboratory.

Neutralization and washing

Once all the oil is in the 1500 L container and mixed a series of chemical-physical procedures are performed on the oil in a fully automatic way. The whole process is controlled from the control room by taking on-line measures (temperature, pH, pressure, flow rate, etc.) at several points in the process and correcting these to the values previously determined.

The first treatment that is performed is the neutralization of the oil's free acids. Therefore, the oil is heated until 60ºC by interchange of heat from hot plates, and a solution sodium hydroxide is added and admixed until the oil is neutralized.

After neutralization, the mix is pumped to a centrifuge where the oil is separated from the paste. The paste, consisting of free fatty acids, is passed automatically to a 50 L container. Once a week this container is emptied and the paste is transported to the by-products warehouse before it is taken away and processed as industrial waste.

Then, the neutralized oil is pumped into a container from where it is submitted to a washing step. This operation consists in the mixing of the neutralized oil with warm water and subsequently the separation of both phases by centrifugation. In this way small paste particles that may be still present at this point are removed from the oil.

Decolorization

After washing, the oil is passed to a container where it is subjected to a vacuum treatment to remove any remaining moisture still left in the oil. After the vacuum treatment, the oil is then retained in another container where silica is added to the oil.

This step is necessary to eliminate any remaining paste and/or moisture. After the silica addition the oil and silica are separated by filtering.

The oil is then pumped to the decolorization reactor. In this reactor a mix of decolorizing earths and active carbon is added to the oil and incubated during 30 min. After the incubation the oil is filtered again to remove the earths and active carbon.

The reason for adding a small amount of active carbon to the decolorization reaction is to improve the ability of the earths to absorb the color of the oil and thereby improving its stability. The active carbon also absorbs contaminants like PCB´s, dioxins and heavy metals that might be present in the crude oil. In this way these contaminants are reduced to a minimum, and are always below the permitted levels.

Deodorization

Deodorization is performed by injecting water vapor into the oil. In this way volatiles that give the fishy smell and taste to a fish oil are removed by evaporation. These volatiles leave the deodorization container through the vacuum system and are then condensated and deposited in a stainless steel container. This deposit that contains mainly fatty acids is emptied periodically and its content stored in the by-products warehouse until it is taken away by a specialized waste company.

Stabilization

After the deodorization step the oil is cooled, and stabilized by the addition of antioxidants. The antioxidant blend that is admixed at this point is a mix of natural products consisting of tocopherols and some other food grade antioxidants.

Packaging

After the addition of the antioxidant, the oil is passed to a stainless steel deposit and from there it is packaged. Packaging is done manually using a filler machine normally used for nutritional oils and fats. The oil is pushed to the filler by overpressure in the deposit. The operator holds the mouth of an aluminum bag in the opening of the filler, and this machine automatically fills the bag with oil, injects nitrogen gas to remove remaining air, and closes the bag. The operator takes the bag away and stores the bag in a carton box.

At the end of the day, all the bag-in-boxes are labeled, and stored in a cold room. The amount of oil in each bag depends on the wishes of the client but is usually 20 kg.

Storage of final product

The finished product (bag-in-boxes) is transported to the cold room using a fork-lift truck. The cold room is located in another building in front of the manufacturing plant. The final product is preferably stored at a temperature below 10ºC and is commonly at 5ºC until it is shipped to its client. Storage time in the cold room is typically about 2 weeks before it is shipped. Therefore, no large stocks are available but production capacity is adjusted to the demand. In any case, the final product is perfectly stable without any organoleptic degradation up to a shelf life of 6 months.

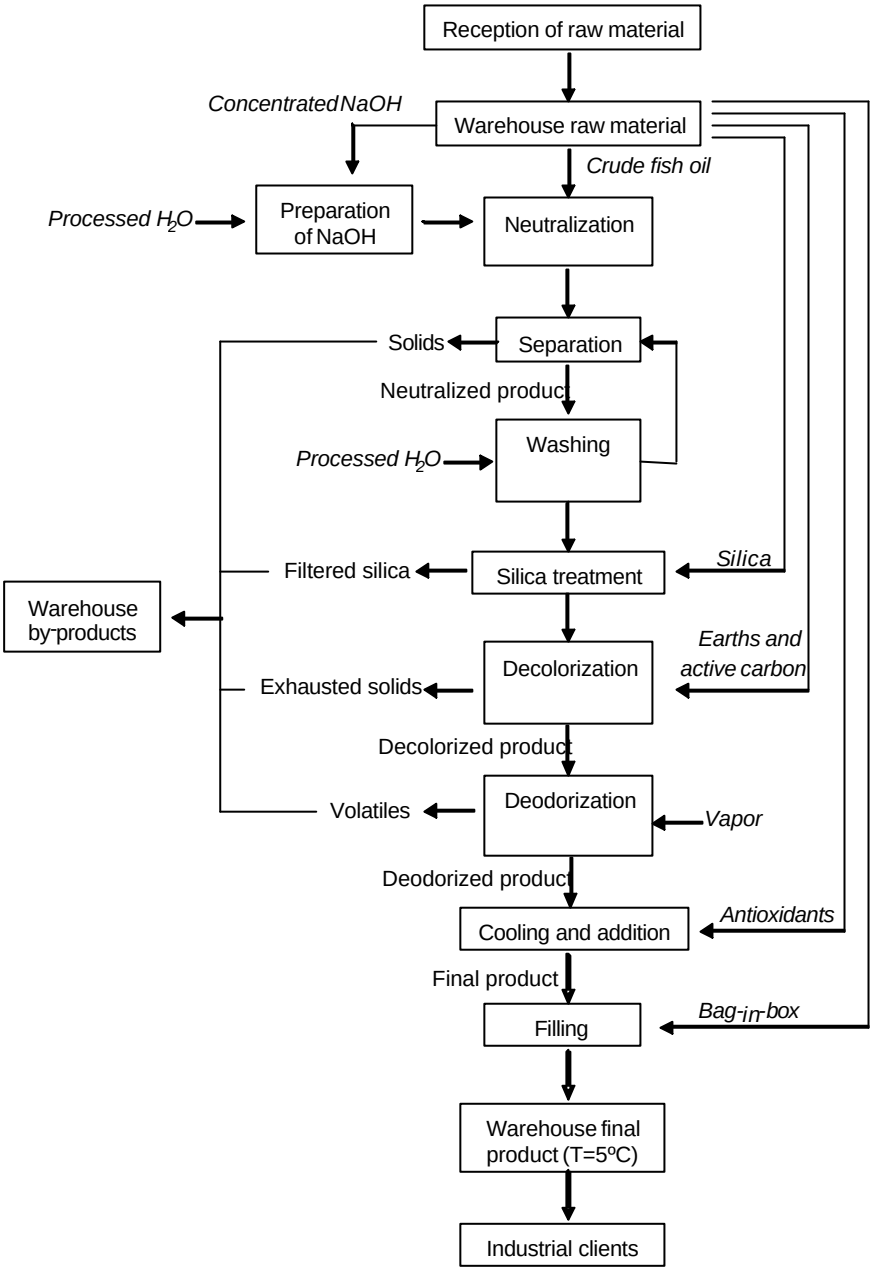
Labeling of final products

Before the final products are transported to the warehouse (cold room), the bag-in-boxes are labeled. On the label the following information is clearly visible: name of product (Eupoly®-EPA or Eupoly®-DHA), lot number, production date, and expiration date. A lot is defined as the oil produced during one day and is typically between 800 and 1000 L of final product.

Standard analysis of final products

Routinely the following parameters are analyzed: acid value, rancimat (stability), peroxide value, *p*-anisidine value, and heavy metals (lead, cadmium, mercury, arsenic, iron and copper). These measurements are normally performed in the analytical department of Puleva Biotech. Twice a year a lot of Eupoly-EPA and Eupoly-DHA is analyzed for dioxins, PAHs, and PCBs. These analyses are performed by a third-party laboratory (see also part C of this document).

Scheme I Schematic representation of the manufacturing process of Eupoly-EPA and Eupoly-DHA.



B.3 Specifications for Food grade Material

Puleva Biotech has developed specifications for Eupoly-EPA and Eupoly-DHA to demonstrate that both products are food grade. These specifications are listed below, together with the standard operating procedures used by Puleva Biotech's for determining compliance with each specification (except for arsenic, benzo(a)pyrene, PCDD/Fs and PCBs similar to dioxins, which are analyzed by a third-party laboratory using accepted validated methods). Specifications are valid for both products (Eupoly-EPA and Eupoly-DHA), except when indicated explicitly.

• Description:	Food grade Eupoly-EPA and Eupoly-DHA are mixtures of fatty acids (in the form of triacylglycerides, and lesser amounts of mono- and diglycerides), extracted from edible fish species (mainly sardine and anchovy for Eupoly-EPA, and tuna in the case of Eupoly-DHA) and refined with standard methods.
• Appearance:	Clear and light-yellowish oil at room temperature
• Odor and taste:	A slightly fishy taste and odor might be noticed
• EPA content:	NLT 15% and NMT 23% for Eupoly-EPA, and NLT 7% and NMT 14% for Eupoly-DHA (ISO 5509:2000)
• DHA content:	NLT 7% and NMT 14% for Eupoly-EPA, and NLT 15% and NMT 23% for Eupoly-DHA (ISO 5509:2000)
• EPA + DHA:	NLT 24% and NMT 34%
• Acid value:	NMT 1.0 mg KOH/g (ISO 660:1996)
• Peroxide value:	NMT 2.0 meq/kg (ISO 3960:2001)
• *p*-Anisidine value:	NMT 20 (ISO 6885:1998)
• Totox number:	NMT 24 (anisidine value + [2 x peroxide value])
• Moisture content:	NMT 0.1% (ISO 662:1998)
• Unsaponifiables:	NMT 3.0% (ISO 3596:2000)
• Lead (Pb):	NMT 0.1 mg/kg (ISO 12193:1994)
• Cadmium (Cd):	NMT 0.1 mg/kg (ISO 15774:2000)
• Mercury (Hg):	NMT 0.1 mg/kg (in-house method)
• Arsenic (As):	NMT 0.1 mg/kg
• Iron (Fe):	NMT 0.5 mg/kg (ISO 8294:1994)
• Copper (Cu):	NMT 0.1 mg/kg (ISO 8294:1994)
• PCDD/Fs	NMT 1.5 pg WHO-TEQ/g
• PCBs similar to dioxins	NMT 3.5 pg WHO-TEQ/g
• Benzo(a)pyrene:	NMT 2.0 µg/kg

B.4 Batch Analysis Results

To demonstrate conformance with the proposed specifications listed above, four different lots of Eupoly-EPA and four lots of Eupoly-DHA were analyzed. The results of these analyses are shown in Tables V and VI, and confirm that all lots of both final products are in full agreement with the established specifications, and thus the production process is under control.

Table V Analyses results of four different lots of Eupoly-EPA.

Parameter	Specification	Lot number			
		EPA-1	**EPA-2**	**EPA-3**	**EPA-4**
Appearance at room temperature	Clear yellowish oil	Clear yellowish oil	Clear yellowish oil	Clear yellowish oil	Clear yellowish oil
Odor and taste	Slightly fishy	Slightly fishy	Slightly fishy	Slightly fishy	Slightly fishy
EPA content	15-23%	18.3%	18.8%	18.5%	18.9%
DHA content	7-14%	10.1%	9.0%	8.7%	8.6%
EPA+DHA	24-34%	28.4%	27.8%	27.2%	27.5%
Acid value	NMT 1.0 mg KOH/g	0.32 mg KOH/g	0.46 mg KOH/g	0.34 mg KOH/g	0.26 mg KOH/g
Peroxide value	NMT 2.0 meq/kg	<2.0 meq/kg	<2.0 meq/kg	<2.0 meq/kg	<2.0 meq/kg
p-Anisidine	NMT 20	8.9	6.6	8.6	7.9
Totox number	NMT 24	12.9	10.6	12.6	11.9
Moisture	NMT 0.1%	0.022%	0.018%	0.025%	0.008%
Rancimat 100ºC	NLT 8h	12.2 h	15.6 h	14.3 h	11.4 h
Unsaponifiables	NMT 3.0%	1.70%	1.65%	1.71%	1.58%
Lead	NMT 0.1 mg/kg	<0.005 mg/kg	<0.005 mg/kg	<0.005 mg/kg	<0.005 mg/kg
Cadmium	NMT 0.1 mg/kg	<0.005 mg/kg	<0.005 mg/kg	<0.005 mg/kg	<0.005 mg/kg
Mercury	NMT 0.1 mg/kg	<0.1 mg/kg	<0.1 mg/kg	<0.1 mg/kg	<0.1 mg/kg
Arsenic	NMT 0.1 mg/kg	<0.05 mg/kg	<0.05 mg/kg	<0.05 mg/kg	<0.05 mg/kg
Iron	NMT 0.5 mg/kg	0.30 mg/kg	0.25 mg/kg	0.22 mg/kg	0.27 mg/kg
Copper	NMT 0.1 mg/kg	<0.01 mg/kg	<0.01 mg/kg	<0.01 mg/kg	<0.01 mg/kg
PCDD/Fs	NMT 1.5 pg WHO-TEQ/g	0.23 pg WHO-TEQ/g	0.26 pg WHO-TEQ/g	0.21 pg WHO-TEQ/g	0.71 pg WHO-TEQ/g
PCBs similar to dioxins	NMT 3.5 pg WHO-TEQ/g	0.78 pg WHO-TEQ/g	0.66 pg WHO-TEQ/g	0.68 pg WHO-TEQ/g	0.71 pg WHO-TEQ/g
Benzo(a)pyrene	NMT 2.0 µg/kg	<0.5 µg/kg	<0.5 µg/kg	<0.5 µg/kg	<0.5 µg/kg

Table VI Analyses results of four different lots of Eupoly-DHA.

Parameter	Specification	Lot number			
		DHA-1	DHA-2	DHA-3	DHA-4
Appearance at room temperature	Clear yellowish oil	Clear oil, yellow to orange	Clear oil, yellow to orange	Clear oil, yellow to orange	Clear oil, yellow to orange
Odor and taste	Slightly fishy	Slightly fishy	Slightly fishy	Slightly fishy	Slightly fishy
EPA content	7-14%	10.7%	11.3%	9.7%	9.6%
DHA content	15-23%	19.0%	18.9%	20.9%	20.4%
EPA+DHA	24-34%	29.7%	30.2%	30.6%	30.0%
Acid value	NMT 1.0 mg KOH/g	0.52 mg KOH/g	0.50 mg KOH/g	0.54 mg KOH/g	0.60 mg KOH/g
Peroxide value	NMT 2.0 meq/kg	<2.0 meq/kg	<2.0 meq/kg	<2.0 meq/kg	<2.0 meq/kg
p-Anisidine	NMT 20	7.6	11.6	14.9	15.6
Totox number	NMT 24	11.6	15.6	18.9	19.6
Moisture	NMT 0.1%	0.046%	0.023%	0.017%	0.025%
Rancimat 100ºC	NLT 8h	13.5 h	13.9 h	13.3 h	18.5 h
Unsaponifiables	NMT 3.0%	2.10%	2.28%	2.01%	2.40%
Lead	NMT 0.1 mg/kg	<0.005 mg/kg	<0.005 mg/kg	<0.005 mg/kg	<0.005 mg/kg
Cadmium	NMT 0.1 mg/kg	<0.005 mg/kg	<0.005 mg/kg	<0.005 mg/kg	<0.005 mg/kg
Mercury	NMT 0.1 mg/kg	<0.1 mg/kg	<0.1 mg/kg	<0.1 mg/kg	<0.1 mg/kg
Arsenic	NMT 0.1 mg/kg	<0.05 mg/kg	<0.05 mg/kg	<0.05 mg/kg	<0.05 mg/kg
Iron	NMT 0.5 mg/kg	0.23 mg/kg	0.49 mg/kg	0.36 mg/kg	0.32 mg/kg
Copper	NMT 0.1 mg/kg	<0.01 mg/kg	<0.01 mg/kg	<0.01 mg/kg	<0.01 mg/kg
PCDD/Fs	NMT 1.5 pg WHO-TEQ/g	0.32 pg WHO-TEQ/g	0.23 pg WHO-TEQ/g	0.20 pg WHO-TEQ/g	0.20 pg WHO-TEQ/g
PCBs similar to dioxins	NMT 3.5 pg WHO-TEQ/g	3.06 pg WHO-TEQ/g	1.64 pg WHO-TEQ/g	1.84 pg WHO-TEQ/g	1.62 pg WHO-TEQ/g
Benzo(a)pyrene	NMT 2.0 µg/kg	<0.5 µg/kg	<0.5 µg/kg	<0.5 µg/kg	<0.5 µg/kg

B.5 Contaminants

The same lots as described above in part B.4 were analyzed for the following contaminants: pesticides, dioxins, furans, PCBs, and PAHs. These contaminants have been related to fish and fish oils and were therefore determined in four different lots in both final products.

Pesticides

Four lots of Eupoly-EPA and four lots of Eupoly-DHA were tested in an external contract laboratory for the presence of a total of 101 pesticides. The following pesticides were analyzed for their presence in Eupoly-EPA and Eupoly-DHA: tecnazene, etridiazol, chloroneb, propachlor, trifluraline, alpha-HCH, HCB, dichloran, vinchlozoline, quintozene, beta-HCH, pentachloroaniline, gamma-HCH, chlorodene, chlorothalonil, delta-HCH,

telodrin, heptachlor, methylpentachlorophenylsulfide, aldrin, dimethyl-chlorthal (DCPA), dicofol, op´-DDE, isodrin, heptachloro-epoxide, t-chlordane, endosulfan-I, c-chlordane, op´-DDD, trans-nonachlor, pp´-DDE, dieldrin, endrin, op´-DDT, pertan, endosulfan-II, tetrasul, chlorobenzilate, cis-nonachlor, pp´-DDD, endrin-aldehyde, tetradifon, endosulfan-sulphate, pp´-DDT, endrin-ketone, methoxychlor, mirex, nitrofen, disulfoton, dichlorofenthion, dichlorvos, mevinphos, demeton S, ethoprophos, tolclofos-me, fenitrothion, sulfotep, monocrotophos, phorate, bromophos-me, dimethoate, demeton O, fenthion, bromophos-et, dioxathion, terbufos, fonofos, diazinon, phosphamidon, parathion-me, fenchlorphos, phosalone, azinphos-me, malathion, chlorpyriphos-et, methacrifos, parathion-et, trichloronate, chlorphenvinphos, methidathion, tetrachlorvinphos, tokuthion, fensulfothion, ethion, sulprofos, chlorpyrifos-me, famphur, carbiophenothion, phosmet, EPN, leptophos, azinphos-et, coumaphos, pirimiphos-me, deltamethrin (isomers), tetramethrin (isomers), cyfluthrin (isomers), cypermethrin (isomers), fenothrin (isomers), permethrin (isomers), and fenvalerate (isomers).

None of the 101 tested pesticides was detected in any of the eight lots analyzed (EPA-1 to EPA-4 and DHA-1 to DHA-4). Therefore, it is concluded that the concentrations of all these pesticides in these lots tested are below their detection limits (40 µg/kg for all pesticides), and are therefore of no concern.

Dioxins and Furans

The presence of polychlorinated dibenzo-para-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) was determined in four lots of each final product. The results of these analyses are presented in Tables VII and VIII.

Polychlorinated Biphenyls (PCBs)

The presence of PCBs was determined in four lots of each final product. The results of these analyses are presented in Tables IX and X.

Polycyclic Aromatic Hydrocarbons (PAHs)

Analyses results of PAHs in four lots of Eupoly-EPA (Table XI) and in four lots of Eupoly-DHA (Table XII) demonstrate their very low levels in these lots.

Table VII Analyses results of dioxins and furans (PCDD/F) in 4 batches of Eupoly-EPA (expressed in pg WHO-TEQ/gram of oil).

PCDD/F	**Lot number**			
	EPA-1	**EPA-2**	**EPA-3**	**EPA-4**
Furan				
2,3,7,8-TCDF	0.01	0.01	<0.003	<0.006
1,2,3,7,8-PeCDF	<0.002	<0.0025	<0.0025	<0.009
2,3,4,7,8-PeCDF	0.04	<0.03	<0.03	<0.15
1,2,3,4,7,8-HxCDF	<0.007	<0.007	<0.006	<0.016
1,2,3,5,7,8-HxCDF	<0.007	<0.007	<0.006	<0.014
2,3,4,6,7,8-HxCDF	<0.008	<0.009	<0.008	<0.017
1,2,3,7,8,9-HxCDF	<0.011	<0.011	<0.011	<0.033
1,2,3,4,6,7,8-HpCDF	<0.0005	<0.0005	<0.0005	<0.0021
1,2,3,4,7,8,9-HpCDF	<0.0008	<0.0008	<0.0008	<0.0039
OCDF	<0.000004	<0.000006	<0.000001	<0.000020
Dioxin				
2,3,7,8-TCDD	<0.03	<0.05	<0.003	<0.07
1,2,3,7,8-PeCDD	<0.08	<0.11	<0.10	<0.31
1,2,3,4,7,8-HxCDD	<0.009	<0.009	<0.011	<0.029
1,2,3,6,7,8-HxCDD	<0.010	<0.009	<0.012	<0.030
1,2,3,7,8,9-HxCDD	<0.009	<0.009	<0.011	<0.029
1,2,3,4,6,7,8-HpCDD	0.001	0.002	<0.00003	<0.0008
OCDD	0.0001	0.0003	0.00004	0.0001
Total 2,3,7,8 (lowerbound)	0.06	0.01	0.00004	0.0001
Total 2,3,7,8 (upperbound)	0.23	0.26	0.21	0.71

Table VIII Analyses results of dioxins and furans (PCDD/F) in 4 batches of Eupoly-DHA (expressed in pg WHO-TEQ/gram of oil).

PCDD/F	**Lot number**			
	DHA-1	**DHA-2**	**DHA-3**	**DHA-4**
Furan				
2,3,7,8-TCDF	0.02	0.01	<0.004	0.01
1,2,3,7,8-PeCDF	<0.0025	<0.002	<0.002	<0.002
2,3,4,7,8-PeCDF	0.10	<0.025	<0.025	<0.025
1,2,3,4,7,8-HxCDF	<0.007	<0.007	<0.006	<0.006
1,2,3,5,7,8-HxCDF	<0.007	<0.007	<0.006	<0.006
2,3,4,6,7,8-HxCDF	<0.008	<0.009	<0.010	<0.008
1,2,3,7,8,9-HxCDF	<0.011	<0.011	<0.012	<0.011
1,2,3,4,6,7,8-HpCDF	<0.0006	<0.0005	<0.0005	<0.0005
1,2,3,4,7,8,9-HpCDF	<0.001	<0.0008	<0.0008	<0.0007
OCDF	<0.000006	<0.000003	<0.000002	<0.0000003
Dioxin				
2,3,7,8-TCDD	<0.02	<0.02	<0.01	<0.01
1,2,3,7,8-PeCDD	<0.11	<0.10	<0.09	<0.09
1,2,3,4,7,8-HxCDD	<0.001	<0.008	<0.009	<0.008
1,2,3,6,7,8-HxCDD	<0.001	<0.009	<0.010	<0.009
1,2,3,7,8,9-HxCDD	<0.001	<0.008	<0.009	<0.009
1,2,3,4,6,7,8-HpCDD	0.004	0.01	0.002	0.002
OCDD	0.0004	0.001	0.0003	0.0002
Total 2,3,7,8 (lowerbound)	0.12	0.02	0.003	0.01
Total 2,3,7,8 (upperbound)	0.32	0.23	0.20	0.20

Table IX Analyses results of PCBs of the coplanar type, similar to dioxins in 4 batches of Eupoly-EPA (expressed in pg WHO-TEQ/gram of oil).

PCB	Lot number			
	EPA-1	**EPA-2**	**EPA-3**	**EPA-4**
PCB 81	0.0001	<0.000039	<0.000052	0.00004
PCB 77	0.009	0.01	0.01	0.007
PCB 123	0.005	0.003	0.004	0.003
PCB 118	0.13	0.12	0.13	0.10
PCB 114	0.01	0.01	0.01	0.01
PCB 105	0.04	0.03	0.04	0.03
PCB 126	0.43	0.36	0.36	0.46
PCB 167	0.002	0.001	0.001	0.001
PCB 156	0.11	0.09	0.10	0.08
PCB 157	0.03	0.02	0.02	0.02
PCB 169	0.01	0.01	0.01	0.01
PCB 189	0.004	0.003	0.003	0.002
Total coplanar PCBs	0.78	0.66	0.68	0.71

Table X Analyses results of PCBs of the coplanar type, similar to dioxins in 4 batches of Eupoly-DHA (expressed in pg WHO-TEQ/gram of oil).

PCB	Lot number			
	DHA-1	**DHA-2**	**DHA-3**	**DHA-4**
PCB 81	0.0001	<0.000048	<0.000058	0.00004
PCB 77	0.004	0.002	0.003	0.002
PCB 123	0.01	0.005	0.006	0.008
PCB 118	0.31	0.18	0.21	0.18
PCB 114	0.02	0.01	0.02	0.01
PCB 105	0.09	0.06	0.06	0.06
PCB 126	2.11	1.03	1.15	1.00
PCB 167	0.005	0.003	0.004	0.003
PCB 156	0.35	0.23	0.25	0.23
PCB 157	0.07	0.05	0.06	0.05
PCB 169	0.08	0.05	0.06	0.06
PCB 189	0.01	0.009	0.01	0.009
Total coplanar PCBs	3.06	1.64	1.84	1.62

Table XI Analyses results of PAHs in 4 batches of Eupoly-EPA (expressed in µg/kg of oil).

PAH	Lot number			
	EPA-1	EPA-2	EPA-3	EPA-4
Fluorene	1	1	1	1
Phenanthrene	1	1	1	1
Anthracene	<0.5	<0.5	<0.5	<0.5
Fluoranthene	<0.5	<0.5	<0.5	<0.5
Pyrene	<0.5	<0.5	<0.5	<0.5
Crysene	<0.5	<0.5	<0.5	<0.5
Benzo (a) anthracene	<0.5	<0.5	<0.5	<0.5
Benzo (b) fluoranthene	<0.5	<0.5	<0.5	<0.5
Benzo (k) fluoranthene	<0.5	<0.5	<0.5	<0.5
Benzo (a) pyrene	<0.5	<0.5	<0.5	<0.5
Indene (1,2,3-cd) pyrene	<0.5	<0.5	<0.5	<0.5
Dibenzo (ah) anthracene	<0.5	<0.5	<0.5	<0.5
Benzo (ghi) perylene	<0.5	<0.5	<0.5	<0.5

Table XII Analyses results of PAHs in 4 batches of Eupoly-DHA (expressed in µg/kg of oil).

PAH	Lot number			
	DHA-1	DHA-2	DHA-3	DHA-4
Fluorene	1	1	1	1
Phenanthrene	2	1	1	1
Anthracene	<0.5	<0.5	<0.5	<0.5
Fluoranthene	1	<0.5	<0.5	<0.5
Pyrene	1	<0.5	<0.5	<0.5
Crysene	<0.5	<0.5	<0.5	<0.5
Benzo (a) anthracene	<0.5	<0.5	<0.5	<0.5
Benzo (b) fluoranthene	<0.5	<0.5	<0.5	<0.5
Benzo (k) fluoranthene	<0.5	<0.5	<0.5	<0.5
Benzo (a) pyrene	<0.5	<0.5	<0.5	<0.5
Indene (1,2,3-cd) pyrene	<0.5	<0.5	<0.5	<0.5
Dibenzo (ah) anthracene	<0.5	<0.5	<0.5	<0.5
Benzo (ghi) perylene	<0.5	<0.5	<0.5	<0.5

PART C SAFETY EVALUATION

Several concerns about the use of fish oils have been extensively discussed. First, the possible contamination of fish and fish oils with heavy metals, dioxins and other toxins is of great concern. Apart from possible contaminants, it was suggested that over consumption of EPA and DHA might lead to prolonged bleeding time, increased levels of low-density lipoprotein cholesterol, and might affect glycemic control in non-insulin dependent diabetics. Furthermore, the possible oxidation of the fatty acids in the fish oil might be a concern. In the following paragraphs, these concerns are separately discussed in the light of Eupoly-EPA and Eupoly-DHA.

C.1 Heavy Metals and Other Toxins

Since fish might sometimes be contaminated with heavy metals, dioxins, PCB´s, and other toxins, these contaminants might appear in the oil extracted from these fish. The US Council for Responsible Nutrition and their Omega-3 working group advised in a voluntary monograph dated October 2002, to check fish oils on a regular basis for the following contaminants (see Table XIII).

Table XIII The US Council for Responsible Nutrition recommends analyzing fish oils for the following contaminants and advised for their maximum levels.

Contaminants	**Maximum levels**
Acid value	maximum 3 mg KOH/g
Peroxide value	maximum 5 meq/kg
Anisidine value	maximum 20
Sum of polychlorinated dibenzo-*para*-dioxins and polychlorinated dibenzofurans	maximum 2 pg WHO-PCDD/F-TEQ/g
Total polychlorinated biphenyls	less than 0.09 mg/kg
Lead (Pb)	less than 0.1 mg/kg
Cadmium (Cd)	less than 0.1 mg/kg
Mercury (Hg)	less than 0.1 mg/kg
Arsenic (As)	less than 0.1 mg/kg

Each of these contaminants mentioned in this monograph is separately discussed in the following paragraphs.

Acid value

The acid content in an alimentary fat or oil is given by the quantity of free fatty acids deriving from the triglycerides developing hydrolytic rancidity. This alteration occurs under unsuitable conditions of treatment and preservation of the fats and thus the acidity represents a basic indicator of the genuineness of the product. It is measured in mg KOH/g which is the amount of KOH needed to neutralize the acidity of the oil. The limit for fish oil is thus set at 3 mg KOH/g. However, the maximum acid value of Eupoly-EPA and Eupoly-DHA is well below that number; 1.0 mg KOH/g.

Peroxide value

One of the most widely used tests for oxidative rancidity, the peroxide value, is a measure of the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation. Milliequivalents of peroxide per kg of fat (meq/kg) are measured by titration with iodide ion. The US Council for Responsible Nutrition recommends a maximum value of 5 meq/kg of fish oil. Specifications for Eupoly-EPA and Eupoly-DHA indicate a maximum of 2.0 meq/kg.

Anisidine value

When hydroperoxides break down, they produce volatile aldehydes like hexanal, leaving behind a non-volatile portion of the fatty acid that remains a part of the glyceride molecule. This non-volatile reaction product can be measured by reaction with anisidine. High anisidine values may be an indication that a fat has been oxidized even when TBA and other aldehyde tests give low results because volatile aldehydes may incidentally or intentionally be removed during processing. Anisidine value is defined as 100 times the absorbance (at 350nm) of a solution resulting from reaction of 1 g of fat in 100 mL of solvent. The maximum recommended value is 20 for fish oils (US Council for Responsible Nutrition), the same as the maximum value for Eupoly-EPA and Eupoly-DHA.

PCDDs, PCDFs and PCBs

Polychlorinated dibenzo-para-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) similar to dioxins are analyzed in an ISO17025 accredited laboratory. Each year two lots of Eupoly-EPA and two lots of Eupoly-DHA are routinely analyzed, and levels of these contaminants (PCDDs, PCDFs and PCBs similar

to dioxins) have never reached the maximum levels specified (maximum 1.5 pg WHO-TEQ/g for the sum of dioxins and furans, and 3.5 pg WHO-TEQ/g for PCBs of the coplanar type, similar to dioxins).

It is important to note that Puleva Biotech declares the maximum amount of dioxin-like PCBs in the specifications of Eupoly-EPA and Eupoly-DHA. The reason for measuring PCBs of the coplanar type is because this family of PCBs is similar to dioxins and is therefore more toxic. This measurement is more specific than measuring total PCBs and satisfies European regulation (466/2001).

Heavy metals (Pb, Cd, Hg, and As)

These heavy metals are analyzed by Puleva Biotech on a regular basis and in each order that is shipped to its client, and should be below the recommended levels (0.1 mg/kg), if not that specific lot will not be used for human consumption.

Other contaminants

Apart from the heavy metals mentioned above, iron and copper levels are routinely measured by Puleva Biotech as well. The maximum levels of these metals are 0.5 mg/kg for iron and 0.1 mg/kg for copper. Finally, Benzo(a)pyrene is measured in Puleva Biotech routinely. According to European regulation (466/2001) benzo(a)pyrene can be used as a marker for the occurrence and effect of carcinogenic polycyclic aromatic hydrocarbons (PAH) in food. Eupoly-EPA and Eupoly-DHA will never contain more than 2.0 µg/kg of benzo(a)pyrene meeting European regulation.

C.2 Stability and Oxidation

Eupoly-EPA and Eupoly-DHA oils are highly stable because of their production process and of the antioxidant blend used. Each production lot is routinely analyzed for different stability parameters (acid value, peroxide value and p-anisidine value, see part C.1). The stability, read the amount of EPA and DHA, of Eupoly-EPA and Eupoly-DHA are guaranteed for the shelf life of any food supplemented with these ingredients.

C.3 Adverse Effects in Human Studies

On June 5, 1997, the FDA issued a final rule (62 FR 30751) affirming menhaden oil as GRAS for use as a direct human food ingredient with limitations on the maximum use levels of menhaden oil in specific food categories. FDA concluded that these limitations are necessary to ensure that daily intakes of EPA and DHA from menhaden oil do not exceed 3.0 grams per person per day (g/p/d). The maximum limit of 3.0 g/p/d is a safeguard against the possible effects of these fatty acids on increased bleeding time (the time taken for bleeding from a standardized skin wound to cease), glycemic control in non-insulin-dependent diabetics, and increased levels of low-density lipoprotein (LDL) cholesterol. In the following paragraphs the literature is reviewed up to date for these possible adverse effects.

Increased bleeding time

As part of FDA's evaluation of uses of menhaden oil in food, FDA examined the scientific literature for evidence that consumption of fish oils may contribute to excessive bleeding. In the June 1997 final rule (62 FR 30751), FDA concluded, based on the examination of more than 50 publications, that when consumption of fish oils is limited to 3.0 g/p/d of EPA and DHA, there is no significant risk for increased bleeding time beyond the normal range. FDA also concluded that amounts of fish oils providing more than 3.0 g/p/d of EPA and DHA have generally been found to produce increases in bleeding time that are statistically significant, but that there are insufficient data to evaluate the clinical significance of this finding. Therefore, because of the potential risk of excessive bleeding in some individuals with intakes at higher levels, FDA concluded that the intake of EPA and DHA should be limited to 3.0 g/p/d.

An up-to-date literature review revealed three publications presenting clinical trials evaluating the effects of fish oil on bleeding time, since the date of publication of the June 1997 final rule (62 FR 30751). In the first trial, 28 healthy volunteers took fish oil supplements containing 3.4 g EPA and DHA daily during 12 weeks. Although bleeding time increased by 30 seconds in these volunteers this increase was not significant (Grundt et al, 1999). In the second clinical trial (Freese and Mutanen, 1997), healthy volunteers took fish oil capsules containing 5.2 g of EPA and DHA each day during 4 weeks. In this study, an 18.5% increase in bleeding time in the fish oil group was reported, however, no statistical analyses were provided in this publication. Finally, Smitz

et al. (2002) did not find any effect on bleeding time in hemodialysis patients taking 2.72 g/p/d of EPA and DHA together for 1 year.

The results of these publications released after FDA´s evaluation of menhaden oil (1997), are in accordance with FDA´s conclusions that EPA and DHA consumption should be limited to 3 g/p/d. Moreover, no publications have appeared after the date of publication of the June 1997 final rule (62 FR 30751) that contradict FDA´s conclusions regarding effects of fish oil on bleeding time.

Reduced glycemic control

FDA also concluded in the June 1997 final rule that 3.0 g/p/d of EPA and DHA is a safe level with respect to glycemic control (62 FR 30751 at 30753). This conclusion was based on FDA's review of a series of studies in non-insulin-dependent diabetics. Based on its evaluation of the available information, FDA concluded in the June 1997 final rule that consumption of EPA and DHA in fish oils at 3.0 g/p/d by diabetics has no clinically significant effect on glycemic control, although higher amounts of EPA and DHA (4.5 g/p/d and above) remain of concern. No studies, since 1997 on type-2 diabetics have reported increased glucose levels in plasma when higher amounts (4.5 to 8 g/p/d) of omega-3 fatty acids were ingested.

Table XIV Clinical trials, reporting on the effects of fish oils in type-2 diabetes and glucose levels, published since 1997.

Publication	Study design	Amount EPA+DHA	Outcome
Sirtori et al. 1998	Trial in patients with glucose intolerance (n=868)	2 g/p/d for 1 year (in form of ethyl esters)	Glycemic parameters unaltered
Luo et al. 1998	Double blind cross-over study in type-2 diabetic men (n=12)	1.8 g/p/d for 2 months	No alterations in glycemic control
Friedberg et al. 1998	Meta analysis of 26 trials in diabetic patients	Varying amounts	No deleterious effects on glycemic control.
Patti et al. 1999	Effect of fish oil in type-2 diabetes	2.5 g/p/d for 6 months	No increase in fasting plasma glucose
Montori et al. 2000	Meta analysis of 12 trials in type-2 diabetic patients	Varying amounts	No statistically or clinically significant increase in fasting glucose
Farmer et al. 2001	Meta analysis of 18 published trials in type-2 diabetes	Ranging from 1.7 to 10 g/p/d	No significant effects on fasting glucose levels in meta analysis
Yam et al. 2001	Study in hyperlipidemic	4.55 g/p/d for 12	No increase in glucose

	volunteers	weeks	or evidence of hyperglycemia
Durrington et al. 2001	Study in hyperlipidemic subjects, some diabetic, taking simvastatin	3.2 g/p/d for 1 year	No effect on glycemic control observed
Kesavulu et al. 2002	Trail in non-insulin dependent diabetics	1.8 g/p/d for 2 months	No change in glycemic control
Chan et al. 2002	Clinical trial in obese men with dyslipidaemia and insulin resistance	3.36 g/p/d for 6 weeks	No change in insulin resistance or in fasting blood glucose
Petersen et al. 2002	Trial in type-2 diabetic patients	2.6 g/p/d for 8 weeks	No effect on blood glucose levels
Pedersen et al. 2003	Trial in type-2 diabetes	4 g/p/d of fish oil with 53.6 mg vitamin E for 8 weeks	No increase in fasting blood glucose or glycemic control

Table XIV presents the publications released after the year 1997 presenting clinical trials on type-2 diabetics consuming fish oils, and their effect on fasting glucose levels and glycemic control. In this literature review no publications were found that describe significant changes in fasting glucose levels or glycemic control in type-2 diabetics consuming omega-3 fatty acids. This, together with FDA´s previous conclusions (62 FR 30751), reveals that there is no concern for type-2 diabetics to consume fish oils up to 3 grams of EPA and DHA combined per person per day.

Increased LDL cholesterol

The June 1997 final rule also considered the reported effects of fish oil on LDL cholesterol levels in healthy persons with normal cholesterol levels, as well as in persons with diabetes mellitus, hypertension, abnormal blood lipid levels, and cardiovascular disease (62 FR 30751 to 30754). As a result of its evaluation, FDA found that there appears to be a trend toward increased LDL cholesterol values with increased fish oil consumption in all population subgroups. Based on its evaluation, FDA concluded that 3.0 g/p/d of EPA and DHA is a safe level with respect to LDL cholesterol.

A literature review of clinical trials published after June 1997, revealed several studies describing the effects of fish oil supplementation on LDL cholesterol. In Table XV, a summery of these clinical trials is given.

Table XV Clinical trials, reporting on the effects of fish oils on serum LDL cholesterol levels, published since 1997.

Publication	Study design	Amount EPA+DHA	Outcome
von Schacky et al. 1999	Trial in patients diagnosed for coronary angiography	3.3 g/p/d for 3 months, followed by 1.65 g/p/d for 21 months	Small but significant increase in LDL cholesterol after 1, 6 and 12 months
Minihane et al. 2000	Men with atherogenic lipoprotein phenotype	3.0 g/p/d for 6 weeks	Non-significant increase in LDL cholesterol
Stark et al. 2000	Trial in postmenopausal women	4.0 g/p/d for 28 days	Significant increase in LDL cholesterol
Hsu et al. 2000	Clinical trial in hyperlipidemic patients	3.0 g/p/d for 4 weeks	LDL cholesterol increased significantly
Farmer et al. 2001	Meta-analysis of 10 clinical trials studying effects in type-2 diabetes	between 1.7 and 10 g/p/d	In one of the ten studies a significant increase in LDL cholesterol was seen. Intake in this study was 6 g/p/d EPA+DHA
Donadio et al. 2001	Randomized trial in IgA nephropathy patients	One group received 3.35 g/p/d and another 6.7 g/p/d during 2 years	No change in LDL cholesterol in low-dosed group, significant decrease in high-dosed group.
Mabile et al. 2001	Trial in hypertriglyceridemic (12) and control (16) subjects	3.0 g/p/d during 8 weeks	No increase in LDL cholesterol
Yam et al. 2001	Study in hyperlipidemic volunteers	4.55 g/p/d for 12 weeks	No effect on total and LDL cholesterol
Durrington et al. 2001	Study in hyperlipidemic subjects taking simvastatin	3.2 g/p/d for 1 year	No increase in LDL cholesterol
Bucher et al. 2002	Meta-analysis of 5 clinical trials studying effects in CHD patients.	between 0.9 to 6.9 g/p/d	Intake of EPA+DHA had little effects on LDL cholesterol.
Nestel et al. 2002	Trial in dyslipidemic volunteers divided into 3 groups	3 g/p/d of EPA (n = 12), 3 g/p/d of DHA (n = 12), or placebo (n = 14), 7 weeks	No effect on total and LDL cholesterol in all groups
Leigh-Firbank et al. 2002	Mildly hypertriacylglycerolaemic men	3 g/p/d for 6 weeks	Significant increase in LDL cholesterol
Puiggros et al. 2002	Trial in hypercholesterolemic patients	2.45 g/p/d for 6 weeks	No increase in LDL cholesterol
Marchioli et al. 2002	Patients surviving a recent (<3 months) myocardial infarction	1.0 g/p/d for 42 months	Initial increase of LDL cholesterol after 6 months, then declined to baseline. No statistical analysis is given.
Chan et al. 2002	Clinical trial in obese men	3.36 g/p/d for 6	No effect seen in LDL

	with dyslipidaemia and insulin resistance	weeks	cholesterol
Petersen et al. 2002	Trial in type-2 diabetic patients	2.6 g/p/d for 8 weeks	No effect on LDL cholesterol
Piolot et al. 2003	Trial in 16 normolipidemic subjects	3.0 g/p/d for 8 weeks	No effect on LDL cholesterol and LDL oxidation.
Laidlaw and Holub, 2003	Clinical trial in healthy women	4.0 g/p/d for 28 days	No effect on LDL cholesterol
Buckley et al. 2004	Study in normolipidaemic adult subjects	One group receiving 4.8 g/p/d EPA, another group receiving 4.9 g/p/d DHA, for 4 weeks	No effect on LDL cholesterol in neither group
Singer et al. 2004	Trial with patients with cardiac arrhythmias	1.0 g/p/d for 6 months	Decrease in LDL cholesterol
Vanschoonbeek et al. 2004	Twenty-five healthy males with borderline overweight	3.0 g/p/d for 4 weeks	Small but significant increase in LDL cholesterol
Dyerberg et al. 2004	Trial in healthy males	4 g/p/d for 8 weeks	Non-significant increase

As is presented in Table XV, several clinical trials found significant increases in serum LDL cholesterol after consumption of fish oils (von Schacky et al. 1999, Stark et al. 2000, Hsu et al. 2000, Leigh-Firbank et al. 2002, and Vanschoonbeek et al. 2004). However, in all these trials the daily intake of EPA and DHA was 3.0 grams or more. On the other hand, most of the clinical trials published after 1997 did not find significant changes in LDL cholesterol levels after consumption of EPA and DHA, and one study showed a significant decrease in LDL cholesterol levels after two years of consumption of 6.7 g/p/d of EPA and DHA (Donadio et al. 2001).

In conclusion, no publications have appeared recently of clinical trials, performed in human volunteers consuming fish oils with a daily intake of less than 3 grams per person of EPA and DHA combined, that found increases in LDL cholesterol. Therefore, this review of clinical trials published after 1997 continues to be consistent with FDA´s conclusions (62 FR 30751) that a daily intake of DHA and EPA combined should be limited to 3 grams per person per day.

C.4 Conclusions

In affirming the GRAS status of menhaden oil under 21 CFR §184.1472, the FDA established that a daily intake of EPA and DHA combined not exceeding 3 grams per

person per day is safe. The scientific basis to support the establishment of this accepted daily intake of 3 g/p/d was published in the Federal Register at page 30571 on June 5, 1997, as part of the final rule on menhaden oil. A review of the scientific literature published since the date of the GRAS affirmation of menhaden oil confirm that the conclusion reached by the FDA regarding the safety of ingestion of up to 3 g/p/d of EPA and DHA combined is consistent with current information regarding the safety of EPA and DHA.

End of submission.

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SUBMISSION END

AM

Glew, Jeanette G

From: Geerlings, Arjan [bg113291@pulevabiotech.es]
Sent: Thursday, July 13, 2006 10:40 AM
To: Glew, Jeanette G
Subject: RE: GRAS Notice 193

Dear Mrs. Glew,
Thank you very much for your email. It will be my pleausure working with you on our GRAS notice.
I agree with you that the paragraph on page 12 where we describe the capacity of our plant is not completely clear. I appologize for this. What I meant to describe in this paragraph is the total capacity of our plant taking into account the time needed to complete the production of one batch of oil. The step that takes most of the time is the deodorization step. In this step vapour is injected into the oil in order to remove volatiles that give the bad taste to fish oils (see page 15 for a description of this step). Depending on the quality of the crude oil, more or less vapour is injected into the oil to remove all volatiles and therefore optimize organoleptic charateristics of the final product. What I meant with crucial step is that this deodorization step is the most important step in the whole process for optimal organoleptic characteristics of the final product, and that the time needed to complete the whole process (and therefore the capacity of the plant) depends on the passage time of the oil in this step.

To answer your question, we consider crucial quality parameters for the deodorization step the organoleptic quality of the final product.

I hope this clarifies the mentioned paragraph. Please contact me for any additional questions or comments.
Could you please give me an estimation of when we can expect an official statement from the FDA on our notice?
Thank you very much,
Sincerely yours,

-----Mensaje original-----
De: Glew, Jeanette G [mailto:jeanette.glew@fda.hhs.gov]
Enviado el: mié 12/07/2006 21:54
Para: Geerlings, Arjan
CC:
Asunto: FW: GRAS Notice 193

Dear Dr. Geerlings;

I am writing to you in regard to the GRAS Notice you submitted on behalf of Puleva Biotech for EPA-rich fish oil and DHA-rich tuna oil (GRN 193). I am communicating on behalf of Dr. Carrie Hendrickson who is temporarily working on another project.

I have a question of clarification regarding a statement in your notice. On page 12 of the notice you state that "the passage of time of the oil in the deodorization step is the most crucial step." Could you identify what you consider "crucial" quality parameters for the deodorization step?

Please let me know if you have any questions.

Sincerely,

Jeanette Glover Glew

Supervisory Consumer Safety Officer

Center for Food Safety and Applied Nutrition