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# WRITTEN SUBMISSION TO HEARING NOTICE

Our Ref.: PO/14/1396/RA/SP/15
December 07, 2021

The Controller of Patents,
The Patent Office,
New Delhi.

**(Kind Attn: Dr. Rajesh Patel, Assistant Controller of P. & D.)**

**Re: Société des Produits Nestlé SA,**

**Application No.: 201817040811**

**Date of filing: October 29, 2018**

**Your Ref.: POD/Application No /201817040811**

Dear Sir,

With reference to your letter no. **POD/Application No /201817040811** and subsequently discussed with you on **November 23, 2021** the below mentioned documents are returned herewith with the following observations for the objections in the hearing notice.

Before replying to the objections raised in the Hearing Notice, we would like to place an amended claim set to replace the claims presently on file.

## CLAIM AMENDMENTS:

Claim 1 has been amended to introduce weight percentage from specification page 16 and 17, and features related to method of treatment are deleted without prejudice by way of correction/explanation.

Claim 3 has been amended to introduce weight percentage from specification page 17 by way of correction/explanation.

Claims 4 and 5 have been deleted without prejudice.

A marked-up as well as clean copy of amended claims is enclosed herewith.

The claims have been amended by way of disclaimer/explanation and the amendments carried out fall within the scope of the original specification. No new matter has been added. The claim amendments meet the requirements of section 57

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and 59 of the Patents Act, 1970 and The Patents Rules, 2003 (as amended in 2016). The Applicant reserves the opportunity to further amend the claims at a later point of time.

We now present our reply to the objections raised in the hearing notice *in seriatim*:

**CLARITY AND CONCISENESS:**

*The Terms "preferably", "greater than" and "at least" to define ranges in the claims introduces uncertainty and thus results in lack of clarity of the claims. Thus the respective claims of the present application are not allowable u/s 10(4)(C) of The Patents (Amendment) Act,*

**Reply:** The applicant submits to the Ld. Controller that the objected terms have been deleted without prejudice in the interests of expediency of prosecution. A marked-up as well as clean copy of amended claims is submitted with the response.

The Ld. Controller is requested to take the same on record and withdraw the present objection.

**FORMAL REQUIREMENT(S):**

*1. As per Rule 20(3)b, verified English translation of the Priority documents and the International application shall be filed. It should be verified by applicant or authorized patent agent.*

*2. A copy of signatory assignment (signed by both applicant) should be given for the change in the applicant.*

**Reply:**

**1.** The applicant submits to the Ld. Controller that priority document and PCT application are filed in English language. Copy of the same is attached for quick reference of the Ld. Controller. The Ld. Controller is requested to take the same on record and withdraw the objection.

**2.** The Applicant respectfully submits that the original notorized assignment from Nestec S.A. to Societe des Produits Nestle S.A. and its verified translation has been submitted with the patent office with application number 5574/DELNP/2012. Further, agent of the applicant and person authorized by the applicant has attested the assignment document. A copy of the same is uploaded along with the response for quick reference of the Ld. Controller. The Ld. Controller is requested to take the same on record and withdraw the present objection.

**INVENTION U/S 2(1)(J):**

*Objection raised U/S 2(1)(j) as mentioned in this office communication letter dated on 29/01/2021 is still maintained and applicant reply to objections on 28/07/2021 have been fully considered but they are not persuasive in view of the following cited documents.*

*D1: US 5591446 A*

*D2: US 6150411 A*

*Applicant argued that D1 nowhere teaches and is silent about composition with omega-3 polyunsaturated fatty acids and DGLA. But d1 disclose other omega-polyunsaturated fatty acids.But is obvious to a person skilled in the art to change the polyunsaturated fatty acids and amount of it.D2 discloses n-6 essential fatty acids (EFAs) selected from the group consisting of linoleic, gamma-linolenic, dihomogamma-linolenic and arachidonic acids and/or one or more n-3 EFAs selected from the group consisting of alpha-linolenic, stearidonic and eicosapentaenoic acids is used in addition to the DHA. A person skilled in the art can use these conbinations of the components with the omega-polyunsaturated fatty acids to arrive at the present invention. Hence the subject matter of the claims 1-5 does not involve any inventive step over D1-D2.*

**Reply:** The Ld. Controller has objected to pending claims lacking inventive step in view of cited document **D1** and **D2**.

*D1: US 5591446 A*

*D2: US 6150411 A*

**Present Invention**

The applicant submits to the Ld. Controller that the present invention relates to a composition comprising DGLA wherein the composition is enriched in DGLA and contains an omega-3 polyunsaturated fatty acid, selected from the group consisting of DHA and EPA or a combination of DHA and EPA, wherein said DGLA is comprised in said composition in a concentration of at least 35wt%, relative to the total fatty acid content of the composition; and wherein the concentration of DHA is 20 to 26wt% and concentration of EPA is 7wt%.

The problem at the hand is to identify a composition for therapy, especially prophylactic therapy for allergic diseases. In particular, it would be desirable to prevent or reduce the risk of development of allergies.

The solution for the above problem is provided by a composition of present invention comprising composition enriched in DGLA and an omega-3 polyunsaturated fatty acid, selected from the group consisting of DHA and EPA or a combination of DHA and EPA, wherein said DGLA is comprised in said composition in a concentration of at least 35wt%, relative to the total fatty acid content of the composition; and wherein the concentration of DHA is 20 to 26wt% and concentration of EPA is 7wt%.

The technical effects of the present invention are shown in examples 1-3 as below:

1. **Example 1** shows that the composition as claimed in the present invention results in: (a) total lgE and specific lgG1 to be significantly lower (figures 1 and 2); (b) skin symptoms were significantly milder (figure 3); and (c) significant lower number of mast cells in the jejunum.

2. **Example 2** shows that the composition as claimed in the present invention when DGLA and NIF (DHA and EPA) were given together, a synergistic reduction of IL4 production was observed.

3. **Example 3** shows that the composition as claimed in the present invention where IL-10 was significantly increased in pups from fish oil+DGLA.

Now, the Applicant will discuss the difference between the cited prior arts D1 and D2 along with claims of the present invention.

**D1: US 5591446 A:**

The applicant submits to the Ld. Controller that cited document D1 discloses atopy-prophylaxis dietary supplement comprising at least one substance selected from the group consisting of γ-linolenic acid, dihomo-γ-linolinic acid. Cited document D1 discloses two compositions under table 2 and 3 (for pregnant or nursing mothers a composition comprising GLA or DGLA or GLA+DGLA).

However, cited document D1 nowhere teaches and is silent about composition with omega-3 polyunsaturated fatty acids and DGLA. D1 also does not teach DGLA with omega-3 polyunsaturated fatty acids selected from the group consisting of DHA

and EPA or a combination of DHA and EPA, wherein said DGLA is comprised in said composition in a concentration of at least 35wt%, relative to the total fatty acid content of the composition; and wherein the concentration of DHA is 20 to 26wt% and concentration of EPA is 7wt%. Thus, it is evident that the presently amended claims are novel and inventive over cited document D1.

**D2: US 6150411A:**

The applicant submits to the Ld. Controller that cited document D2 is of entirely different field relating to combating dyslexia or inadequate night vision or dark adaptation in dyslexics or normal individuals, by administering OHA or a precursor n-3 EFA. Cited document D2 discloses one formulation of granules or powder for use as above, made with gum acacia, gelatin, starch or other appropriate material containing by weight in each gram, 50 mg DHA, optionally with 50 mg of DGLA, 50 mg AA and/or 50 mg SA.

Cited document D2 does not add to the teachings of cited document D1. Thus, a person skilled in art would not combing D1 with D2 as it would not result in a composition comprising DGLA and an omega-3 polyunsaturated fatty acid, selected from the group consisting of DHA and EPA or a combination of DHA and EPA. Further, it would also not lead to specific amounts of the each components of composition as claimed in present invention and wherein the concentration of DGLA is greater than the concentration of DHA or EPA. Further, D2 also does not teach or indicate about a composition comprising DGLA with omega-3 polyunsaturated fatty acids selected from the group consisting of DHA and EPA or a combination of DHA and EPA, wherein said DGLA is comprised in said composition in a concentration of at least 35wt%, relative to the total fatty acid content of the composition; and wherein the concentration of DHA is 20 to 26wt% and concentration of EPA is 7wt%.

The applicant thus finally submits that presently amended claims are not disclosed or suggested by D1 and D2 alone or in combination nor does it motivate a person skilled in art to arrive at the present invention. Therefore, the subject-matter of the amended claims is inventive over the cited prior arts.

In view of the detailed submission, the Applicant requests the Ld. Controller to withdraw the present objection.

**NON-PATENTABILITY U/S 3:**

*1. The subject matter as claimed in claims falls under section 3(i) of the Patents Act, 1970 as amended by the Patents (Amendment) Act 2005, therefore not allowable.*

*2. The composition of Claims 1-5 attract Sec 3(e) of the Patents Act 1970 as amended by Patents (Amendment) Act 2005 as it is a case of compositions whose components are already known in prior art and in the case of instant application no synergistic effect exemplified by the specification with the same composition. It is case of mere admixture. Therefore amended claims 1-5 are objected.*

**Reply:**

**1. Section 3(i):**

The applicant submits to the Ld. Controller that the claims 4 and 5 have been deleted without prejudice. Further, features related to method of treatment are deleted from claim 1. In view of deletion the objection stands moot. The Ld. Controller is requested to take the same on record and withdraw the present objection.

**2. Section 3 (e):**

The applicant submits to the Ld. Controller that the presently amended claims relate to a compositions comprising DGLA with omega-3 polyunsaturated fatty acids selected from the group consisting of DHA and EPA or a combination of DHA and EPA, wherein said DGLA is comprised in said composition in a concentration of at least 35wt%, relative to the total fatty acid content of the composition; and wherein the concentration of DHA is 20 to 26wt% and concentration of EPA is 7wt%. The technical effect is clearly shown through examples in specification. The technical effect of the present invention are shown in examples 1-3 is as below:

1. **Example 1** shows that the composition as claimed in the present invention results in: (a) total lgE and specific lgG1 to be significantly lower (figures 1 and 2); (b) skin symptoms were significantly milder (figure 3); and (c) significant lower number of mast cells in the jejunum.

2. **Example 2** shows that the composition as claimed in the present invention when DGLA and NIF (DHA and EPA) were given together, a synergistic reduction of IL4 production was observed.

3. **Example 3** shows that the composition as claimed in the present invention where IL-10 was significantly increased in pups from fish oil+DGLA.

Thus, based on the examples it is clear that the presently amended claims are not directed towards mere admixture but synergistic composition. Thus, the presently amended claims does not fall under the purview of section 3 (e).

In view of the detailed submission, the Applicant requests the Ld. Controller to withdraw the present objection.

**OTHER REQUIREMENT(S):**

*Dependent claims should be prefaced with the term "The" in the respective claims.*

**Reply:** The applicant submits to the Ld. Controller that the dependent claims have been suitably amended for antecedent basis. A marked-up as well as clean copy of amended claims has been submitted with the response. The Ld. Controller is requested to take the same on record and withdraw the present objection.

In the view of detailed submission above, the Ld. Controller is requested to take the same on record and withdraw the present objection.

We request the learned Controller to kindly favorably consider present submission and allow the application. If the Controller requires any further clarification or information in this matter, the applicant may please be granted an opportunity of being heard in the matter before passing any adverse order.

Thanking you,

**(RAHUL ADEY)**
IN/PA-3343
Agent for applicant

***Encl.:***

1. Amended Claims Marked Up and Clean Copy
2. Notarized assignment from Nestec S.A. to Societe des Produits Nestle S.A. as filed
3. Priority document and PCT application as filed

**We claim:**

1. A composition comprising DGLA ~~for use in the prophylaxis of allergic disease in an offspring of a mammalian subject, comprising administration of the composition to said subject pre-pregnancy and/or during pregnancy and/or during lactation and preferably~~ wherein ~~said~~ the composition is ~~a composition~~ enriched in DGLA ~~wherein said compositionand also~~ contains an omega-3 polyunsaturated fatty acid, selected from the group consisting of DHA and EPA or a combination of DHA and EPA, wherein said DGLA is comprised in said composition in a concentration of ~~at least 3wt% relative to the total fatty acid content of the composition and more preferably in a concentration of at least 5wt%, at least 10wt%, at least 20wt%, at least 30wt%,~~ at least 35wt%, ~~or at least 40wt%~~ relative to the total fatty acid content of the composition; and wherein the concentration of ~~DGLA is greater than the concentration of~~ DHA is 20 to 26wt% ~~or~~ and concentration of EPA is 7wt%.

2. ~~A~~ The composition comprising DGLA ~~for use according to~~as claimed in claim 1, wherein ~~said~~ the composition further comprises omega-6 polyunsaturated fatty acid, ~~preferably~~ selected from the group consisting of LA and GLA or a combination of LA and GLA.

3. ~~A~~ The composition comprising DGLA ~~for use as~~ as claimed in claim 2, wherein ~~said~~ the ~~composition comprises DGLA, GLA and LA and wherein the concentration of DGLA is greater than GLA and~~ the concentration of GLA is 2.6wt% ~~is greater than~~and the concentration of LA is 6.4wt%.

~~4. A composition comprising DGLA for use as claimed in anyone of claims 1 to 3, wherein said composition is a maternal nutritional composition and preferably selected from the group consisting of:~~

~~pre-pregnancy supplement, pregnancy supplement or lactation supplement.~~

~~5. A composition comprising DGLA for use as claimed in anyone of claims 1 to 4, wherein the allergic disease is selected from the group consisting of: an atopic disorder including hereditary atopic disorder and a Type 1 allergic disease including IgE mediated allergic disease, preferably wherein the allergic disease is selected from the group consisting of: asthma, allergic arthritis, allergic asthma, allergic bronchitis, allergic conjunctivitis, allergic keratitis, allergic rhinitis, allergic sinusitis, alimentary allergy, allergic respiratory disease, animal dander allergy, atopic dermatitis, atopic eczema, atopy, bronchial asthma, contact dermatitis, dermatitis, drug allergy, eczema, food allergy (particularly selected from the group consisting of egg allergy, fish allergy, milk allergy, nut allergy, shellfish allergy, soya allergy, and wheat allergy), food hypersensitivity, eosinophilic esophagitis, hayfever, house dust mite allergy, hypersensitivity pneumonitis, hypertrophic rhinitis, insect allergy, latex allergy, mould allergy, pruritus, seasonal allergic rhinitis, and vasomotor rhinitis.~~

Dated this 29th day of October 2018

**(RAHUL ADEY)**
IN/PA-3343
Agent for applicant

**We claim:**

1. A composition comprising DGLA wherein the composition is enriched in DGLA and contains an omega-3 polyunsaturated fatty acid, selected from the group consisting of DHA and EPA or a combination of DHA and EPA, wherein said DGLA is comprised in said composition in a concentration of at least 35wt%, relative to the total fatty acid content of the composition; and wherein the concentration of DHA is 20 to 26wt% and concentration of EPA is 7wt%.

2. The composition comprising DGLA as claimed in claim 1, wherein the composition further comprises omega-6 polyunsaturated fatty acid, selected from the group consisting of LA and GLA or a combination of LA and GLA.

3. The composition comprising DGLA as claimed in claim 2, wherein the the concentration of GLA is 2.6wt% and the concentration of LA is 6.4wt%.

Dated this 29th day of October 2018

Rahul Adey.

**(RAHUL ADEY)**
IN/PA-3343
Agent for applicant

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PO/14:1396/RA/07
August 2, 2019

The Controller of Patents,
The Patent Office,
New Delhi.

**Sub.: SUBMISSION OF DULY EXECUTED POWER OF ATTORNEY**

**Indian Patent Application No.: 201817040811**

**Date of filing: 29.10.2018**

**International Application No.: PCT/EP2017/055680**

**International filing date: 10.03.2017**

**Title of the invention: "COMPOSITION FOR USE IN THE PROPHYLAXIS OF ALLERGIC DISEASE"**

**Applicant: SOCIÉTÉ DES PRODUITS NESTLÉ SA.**

Dear Sir,

Please find enclosed duly executed Power of Attorney along with prescribed stamp duty pertaining to the above mentioned Indian patent application.

Please note that a copy of the same was filed with your good office on July 19, 2019 along with Form 6 online.

Please also note that the Original duly executed and notarized assignment has been submitted with Indian Patent Application No. 5574/DELNP/2012 and Original General Power of Attorney has been submitted with Indian Patent Application No. 201917025452.

Kindly put the above mentioned documents on record and update your files accordingly.

Thanking you,

Rahul Adey.

**(RAHUL ADEY)**
IN/PA-3343
Agent for applicant

Encl.:

1. ***Duly executed POA along with prescribed stamp duty.***

**AFFIDAVIT**

I the undersigned, TAUNYA FERGUSON, an employee of CPA Global North America LLC ("**my Company**"), a Delaware limited liability company, having offices at 2318 Mill Road, Alexandria 22314, Commonwealth of Virginia, United States of America, make OATH and ATTEST that to the best of my knowledge and belief, the information contained herein is true, correct and complete:

1. In my capacity as an employee of my Company, I confirm that I am legally authorised to act for and on behalf of **Société des Produits Nestlé S.A., a Swiss corporation.**
2. I confirm that I have been provided by my client and personally sighted, a original Certificate of Company Merger document, together with a Certified English translation thereof (where applicable), showing the merger of **Nestec S.A., a Swiss corporation**, into and wholly absorbed by my Client, where, as a result of this merger, all intellectual property rights owned by **Société des Produits Nestlé S.A.** are now wholly owned by my Client.
3. Now shown to me and marked **Exhibit A** is a true and complete copy of those documents referred to in paragraph [2] above.

**IN WITNESS WHEREOF**, I have signed this affidavit, to be duly attested and notarized herein, as of this 7th day of June 2019.

Name: TAUNYA FERGUSON

Signature: Taunya L Ferguson

City of Alexandria

Commonwealth of Virginia

The foregoing instrument was acknowledged before me this 7th day of June 2019 by Taunya Ferguson.

[Notary Seal] TESA D GARRETT NOTARY PUBLIC REG # 7231074 MY COMMISSION EXPIRES 2/29/2020 COMMONWEALTH OF VIRGINIA

Notary Public: Tesa D. Garrett
Notary's Registration Number: 7231074
My Commission expires: 29 February 2020

technical translation agency

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# CONFIRMATION OF TRANSLATION

COMMERCIAL REGISTRY OF THE CANTON OF VAUD

It is herewith confirmed that the French-English translation has been carried out by Technical Translation Agency GmbH, Fasangarten 8, 2136 Laa/Thaya, Austria to the best of their knowledge and belief.

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www.translation.at
www.patenttranslation.at

i.A. Bauer

06.06.2019



Technical Translation Agency GmbH
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Firmenbuchgericht Korneuburg

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COMMERCIAL REGISTRY OF THE CANTON OF VAUD

[stamp: *COMMERCIAL REGISTRY / VAUD / LIBERTÉ ET PATRIE*]

**Extract without deletions**

EXTRACT FROM THE REGISTER
Transfer dated 5 June 2000
File no. R972/00424
Ref. no. CH-550-0059098-4
**IDE/UID CHE-109.815.753**

**Société des Produits Nestlé S.A.**
registered on 15 December 1936
Company limited by shares

| Ref. | | | | |
|---|---|---|---|---|
| | **Commercial name** | | | |
| 51 | Société des Produits Nestlé S.A.<br>(Prodotti Nestlé S.A.)<br>(Nestlé Produkte AG)<br>(Nestlé Products Co. Ltd) | | | |
| | **Headquarters** | | | |
| 1 | Vevey | | | |
| | **Domicile** | | | |
| 34 | Entre-deux-Villes, 1800 Vevey | | | |
| | **Other addresses** | | | |
| 1 | Office: La Tour-de-Peilz, Entre-Deux-Villes | | | |
| | **Dates of articles of association** | | | |
| 1 | 23.11.1936 | 13.06.1997 (last amended) | 57 | 04.09.2017 |
| 50 | 04.11.2015 | | 65 | 10.12.2018 |
| 51 | 06.01.2016 | | 67 | 15.03.2019 |
| 53 | 28.06.2016 | | | |
| | **Objects, observations** | | | |
| 43 | Identification under number CH-550-0059098-4 is replaced by Unique Business Identification Number (IDE/UID) CHE-109.815.753. | | | |
| 57 | Objects:<br>the company's objects are the manufacture, sale and distribution in Switzerland and abroad of a range of products, especially food, dietetic, pharmaceutical, medical, cosmetic and sanitary products. The company may, under whatsoever form, provide services and engage in any activities, in particular in the field of food for humans and animal feed, dietetics, infant care, education, advertising, catering, pharmaceutical, medical, cosmetic and sanitary products (for a full description of objects, please see articles of association). | | | |
| | **Mergers (Mergers Act)** | | | |
| 36 | Merger:<br>takeover of assets and liabilities of NESTLE SUPER PREMIUM SA, in Lausanne (CH-550-0117242-8), in accordance with the merger agreement of 14 June 2011 and the balance sheet as at 31 December 2010, with assets amounting to CHF 30,246,094.54 and liabilities vis-à-vis third parties amounting to CHF 26,796,644.53, i.e. net assets of CHF 3,449,450.01. Given that all the capital-shares in the two companies are held by the same shareholder, the merger does not result in an increase in capital or in an allocation of shares. | | | |
| 40 | Merger:<br>takeover of assets and liabilities of Emaro S.A., in Romanel-sur Lausanne (CH-550-0070794-6), in accordance with the merger agreement of 10 June 2013 and the balance sheet as at 31 December 2012, with assets amounting to CHF 30,388,673.36 and liabilities vis-à-vis third parties amounting to CHF 7,507,882.10, i.e. net assets of CHF 22,880,791.26. Given that the transferee company holds all the shares in the transferring company, the merger does not result in an increase in capital or in an allocation of shares. | | | |
| 62 | Merger:<br>takeover of assets and liabilities of Materna-Nestlé GmbH, in Zoug (CH-115.216.555), in accordance with the merger agreement of 22 May 2018 and the balance sheet as at 31 December 2017, with assets amounting to CHF 199,945 and liabilities vis-à-vis third parties amounting to CHF 5,833, i.e. net assets of CHF 194,112. Given that all the capital-shares and the share capital respectively in the two companies are held by the same shareholder, respectively partner, the merger does not result in an increase in capital or in an allocation of shares. | | | |

| | |
|---|---|
| 68 | Merger:<br>takeover of assets and liabilities of Nestec S.A., in Vevey (CH-105.996.874), in accordance with the merger agreement of 27 May 2019 and the balance sheet as at 31 December 2018, with assets amounting to CHF 2,643,245,000 and liabilities vis-à-vis third parties amounting to CHF 1,094,148,000, i.e. net assets of CHF 1,549,097,000. Given that all the capital-shares in the two companies are held by the same shareholder, the merger does not result in an increase in capital or in an allocation of shares. |
| | **Transfers of assets (Mergers Act)** |
| 54 | In accordance with the agreement of 21 September 2016, the company transferred assets amounting to CHF 77,993,502 and liabilities vis-à-vis third parties amounting to CHF 46,771,657 to Nestlé Suisse S.A., in Vevey (CHE-101.237.732). Consideration: none.<br>In accordance with the agreement of 21 September 2016, the company transferred assets amounting to CHF 20,781,489 and no liabilities vis-à-vis third parties to Froneri Switzerland SA (formerly ICFF SA) in Goldach (CHE-447.285.993). Consideration: none. |
| 68 | In accordance with the agreement of 27 May 2019, the company transferred assets amounting to CHF 2,604,148,000 and liabilities vis-à-vis third parties amounting to CHF 1,739,554,000 to Nestlé Enterprises SA, in Vevey (CHE-108.731.444). Consideration: none. |
| | **Medium of publication** |
| 1 | Swiss Official Gazette of Commerce |
| 50 | Communications to shareholders: by means of publication of communiqués by other press publications or in writing, provided their addresses are known. |

| Ref. | Capital-shares | | |
|---|---|---|---|
| | **Nominal** | **Paid-up** | **Shares** |
| 67 | CHF 8,746,750 | CHF 8,746,750 | 87,467,500 registered shares each worth CHF 0.10, with restrictions regarding their transferability as set out in the articles of association. |

| Ref. | | | Directors, auditors and authorised signatories | | |
|---|---|---|---|---|---|
| Registered | Amended | Deleted | **First name and surname, origin, domicile** | **Post** | **Type of signing authority** |
| 55 | | | **Settembri** Marco, from Italy, in Lausanne | director, chairman | sole signatory |
| | 59 | | **Humbert** Harold, de France, in Pully | director | sole signatory |
| 63 | | | **Felgines (usual name Lienau)** Muriel Aline Nicole, from France, in Vevey | director | sole signatory |
| 41 | | | **Thiébaud** Jean Christophe, de Brot-Dessous, à Lutry | secretary (not board member) | special signing arrangements (2) |
| | 23 | | **KPMG SA**, in Lausanne | auditor | |
| | 7 | | **De Blic-Hamon** Isabelle, from France, in Montreux | | sole signatory |
| 8 | | | **Bome** Patrice, from France, in Publier (France) | | sole signatory |
| | 10 | | **Menoud** Anne-Lyse, from Cerniat (FR), in Bossonnens | | |
| | 10 | | **Royer** Sandrine, from Salvenach, in La Tour-de- Peilz | | sole signatory |
| 16 | | | **Venetz** Laurent, from Oberems, in Morges | | sole signatory |
| 26 | | | **Kemball** Alexander John, from the United Kingdom, in Bionay | | sole signatory |
| 30 | | | **Delamere** Rachel, from the United Kingdom, in Vevey | | sole signatory |
| 30 | | | **Flury** Olivier, from Kleinlützel, in Botterens | | sole signatory |
| | 37 | | **De Koster Arkesteijn** Annemieke, from the Netherlands, in Jongny | | sole signatory |
| | 37 | | **Schüller** Cornelis, from the Netherlands, in Morges | | sole signatory |
| 38 | | | **Maradan** Claudia, from Cerniat (FR), in Pully | | sole signatory |
| 38 | | | **Wood** Régula, from Saint-Gall, in Lausanne | | sole signatory |
| 39 | | | **Bonaccorso** Ezio, from Italy, in Chardonne | | sole signatory |
| | 39 | | **Byland Mc Connell** Ursula, from Veltheim (AG), in La Tour-de-Peilz | | sole signatory |
| 41 | | | **Jorge** Celine, from France, in Nyon | | sole signatory |
| 41 | | | **Marquardt** Ulf, from Germany, in Epalinges | | sole signatory |
| 41 | | | **Schratti** Malena, from Germany, in Lausanne | | sole signatory |
| 55 | | | **Checa Cortès** José, from Spain, in Saint-Prex | | sole signatory |
| 55 | | | **Lucet** Philippe, from France, in Prangins | | sole signatory |
| 58 | | | **Kessler** Dirk, from Germany, in Lausanne | | sole signatory |

| Ref. | | | Directors, auditors and authorised signatories | | |
|---|---|---|---|---|---|
| Registered | Amended | Deleted | First name and surname, origin, domicile | Post | Type of signing authority |
| 59 | | | **Boulet** Aurélien, from Vionnaz, in La Tour-de Peilz | | sole signatory |
| 59 | | | **Chaudry** Muhammad Arshad, from Pakistan, in Saint-Légier-La Chiésaz | | sole signatory |
| 59 | | | **De Carvalho** Renata, from Portugal, in La Tour-de-Peilz | | sole signatory |
| 59 | | | **Philardeau** Thierry, from France, in Lutry | | sole signatory |
| 59 | | | **Pignatari (usual name Aboutboul)** Serena, from Italy, in La Tour-de-Peilz | | sole signatory |
| 59 | | | **Ribeiro Gonçalves Silva Costa** Alexandre, from Brazil, in Vevey | | sole signatory |
| 60 | | | **Lavoie** Nicholas, from France, in La Tour-de Peilz | | sole signatory |
| 61 | | | **Pop** Viorica-Zorita, from Rumania, in Vevey | | sole signatory |
| 63 | | | **Assis Sporques** Fernando, from Brasil, in Pully | | sole signatory |
| 63 | | | **Fuentes Ruelas** Alejandra, from Mexico, in Montreux | | sole signatory |
| 63 | | | **Onyiego** Daniel Ochieng, from Kenya, in Vevey | | sole signatory |
| 63 | | | **Pleines** Anke, from Germany, in Pully | | sole signatory |
| 63 | | | **Uribe de Luna** Martha Delia, from Mexico, in Corsier-sur-Vevey | | sole signatory |
| 64 | | | **Krampulz** Manuela, from Lugano, in Corseaux | | sole signatory |
| | 27 | | **Steiner** Myriam, from Grossaffoltern, in Semsales | | joint signature of 2 persons |
| 32 | | | **Python** Frank, from Arconciel, in Fribourg | | joint signature of 2 persons |
| 37 | | | **Sudan** Yves, from Broc, in Bulle | | joint signature of 2 persons |
| | 41 | | **Tschiemer Goumaz** Susanne, from Matten bei Interlaken, in Bussigny-sur-Lausanne | | joint signature of 2 persons |
| 46 | | | **Johan** Fabrice, from France, in Bionay | | joint signature of 2 persons |
| 49 | | | **Pons** Giovanni, from Capriasca, in Lutry | | joint signature of 2 persons |
| | 55 | | **Hotin Benkirane** Sefkat, from Belmong-sur-Lausanne, in Belmont-sur-Lausanne | | joint signature of 2 persons |
| 61 | | | **Baumann** Gérard, from Corseaux, in Anniviers | | joint signature of 2 persons |
| 61 | | | **Chiala** Gian Paolo, from Neuchatel, in Anniviers | | joint signature of 2 persons |
| | 66 | | **Bonvin** Jérôme, from Arbaz, in La Tour-de-Peilz | | joint signature of 2 persons |

(2) sole signatory in the capacity of non-board-member secretary, otherwise joint signature of 2 persons

| Ref. | JOURNAL | | PUBLICATION SOGC* | | Ref. | JOURNAL | | PUBLICATION SOGC* | |
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| 4 | 32 | 03.01.2001 | 09.01.2001 | 178 | 5 | 4564 | 23.04.2001 | 27.04.2001 | 3138 |
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| 30 | 14006 | 18.08.2009 | 24.08.2009 | 19/5211348 | 31 | 14427 | Correction | 01.09.2009 | 21/5225576 |

* Swiss Official Gazette of Commerce

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|---|---|---|---|---|---|---|---|---|---|
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| 34 | 14410 | 31.08.2010 | 06.09.2010 | 17/5799880 | 35 | 763 | 12.01.2011 | 18.01.2011 | 19/5991510 |
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| 40 | 9694 | 18.06.2013 | 21.06.2013 | 931867 | 41 | 12669 | 19.08.2013 | 22.08.2013 | 1039993 |
| 42 | 15277 | 10.10.2013 | 15.10.2013 | 1128887 | 43 | | Supplementary | 19.12.2013 | 7225834 |
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| 58 | 16429 | 03.10.2017 | 06.10.2017 | 3795357 | 59 | 4405 | 07.03.2018 | 12.03.2018 | 4106185 |
| 60 | 6258 | 05.04.2018 | 10.04.2018 | 4162081 | 61 | 7669 | 26.04.2018 | 01.05.2018 | 4205191 |
| 62 | 11107 | 20.06.2018 | 25.06.2018 | 4312129 | 63 | 13817 | 30.07.2018 | 03.08.2018 | 4396667 |
| 64 | 21219 | 28.11.2018 | 03.12.2108 | 1004511070 | 65 | 22142 | 11.12.2018 | 14.12.2018 | 1004521715 |
| 66 | 22713 | Correction | 21.12.2018 | 1004528415 | 67 | 9341 | 22.05.2019 | 24.05.2019 | 1004638171 |
| 68 | 9740 | 28.05.2019 | | | | | | | |

***Entry not yet published but approved by the federal commercial registry office (art. 32. para. 1 CRO***

Moudon, 29 May 2019

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| The registry clerk<br>[signature] |

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# REGISTRE DU COMMERCE DU CANTON DE VAUD

**Extrait sans radiations**

EXTRAIT DU REGISTRE
Report du 05 juin 2000
N° doss R972/00424
N° réf. CH-550-0059098-4
**IDE\UID CHE-109.815.753**

## Société des Produits Nestlé S.A.

inscrite le 15 décembre 1936
Société anonyme

| Réf. | **Raison de commerce** |
|---|---|
| 51 | Société des Produits Nestlé S.A.<br>(Prodotti Nestlé S.A.)<br>(Nestlé Produkte AG)<br>(Nestlé Products Co. Ltd) |

| | **Siège** |
|---|---|
| 1 | Vevey |

| | **Domicile** |
|---|---|
| 34 | Entre-deux-Villes, 1800 Vevey |

| | **Autres adresses** |
|---|---|
| 1 | Bureau : La Tour-de-Peilz, Entre-Deux-Villes |

**Dates des statuts**

| | | | |
|---|---|---|---|
| 11 | 23.11.1936 13.06.1997 (dern. modif.) | 57 | 04.09.2017 |
| 50 | 04.11.2015 | 65 | 10.12.2018 |
| 51 | 06.01.2016 | 67 | 15.03.2019 |
| 53 | 28.06.2016 | | |

**But, observations**

43 L'identification sous le numéro CH-550-0059098-4 est remplacée par le numéro d'identification des entreprises (IDE/UID) CHE-109.815.753.

57 But:
la société a pour but la fabrication, la vente et la distribution en Suisse et à l'étranger de tous produits, notamment alimentaires, diététiques, pharmaceutiques, médicaux, cosmétiques et hygiéniques; elle peut, sous n'importe quelle forme, fournir tous services et déployer toute activité, en particulier dans le domaine de l'alimentation humaine et animale, de la diététique, des soins aux nourrissons, de l'éducation, de la publicité, de la restauration, des produits pharmaceutiques, médicaux, cosmétiques et hygiéniques (pour but complet cf. statuts).

**Fusions (LFus)**

36 Fusion:
reprise des actifs et passifs de NESTLE SUPER PREMIUM SA, à Lausanne (CH-550-0117242-8), selon contrat de fusion du 14 juin 2011 et bilan au 31 décembre 2010, présentant des actifs de CHF 30'246'094.54, des passifs envers les tiers de CHF 26'796'644.53, soit un actif net de CHF 3'449'450.01. La totalité du capital-actions des deux sociétés étant détenue par le même actionnaire, la fusion ne donne pas lieu à une augmentation du capital, ni à une attribution d'actions.

40 Fusion:
reprise des actifs et passifs de Emaro S.A., à Romanel-sur-Lausanne (CH-550-0070794-6), selon contrat de fusion du 10 juin 2013 et bilan au 31 décembre 2012, présentant des actifs de CHF 30'388'673.36, des passifs envers les tiers de CHF 7'507'882.10, soit un actif net de CHF 22'880'791.26. La société reprenante détenant l'ensemble des actions de la société transférante, la fusion ne donne pas lieu à une augmentation du capital, ni à une attribution d'actions.

62 Fusion:
reprise des actifs et passifs de Materna-Nestlé GmbH, à Zoug (CHE-115.216.555), selon contrat de fusion du 22 mai 2018 et bilan au 31 décembre 2017, présentant des actifs de CHF 199'945, des passifs envers les tiers de CHF 5'833, soit un actif net de CHF 194'112. La totalité du capital-actions, respectivement du capital social des deux sociétés étant détenue par la même actionnaire, respectivement associée, la fusion ne donne pas lieu à une augmentation du capital, ni à une attribution d'actions.

68 Fusion:
reprise des actifs et passifs de Nestec S.A., à Vevey (CHE-105.996.874), selon contrat de fusion du 27 mai 2019 et bilan au 31 décembre 2018, présentant des actifs de CHF 2'643'245'000, des passifs envers les tiers de CHF

| | Fusions (LFus) |
|---|---|
| | 1'094'148'000, soit un actif net de CHF 1'549'097'000. La totalité du capital-actions des deux sociétés étant détenu par le même actionnaire, la fusion ne donne pas lieu à une augmentation du capital, ni à une attribution d'actions. |
| | **Transferts de patrimoine (LFus)** |
| 54 | Selon contrat du 21 septembre 2016, la société a transféré des actifs pour CHF 77'993'502 et des passifs envers les tiers pour CHF 46'771'657, à Nestlé Suisse S.A. à Vevey (CHE-101.237.723). Contre-prestation: aucune.<br>Selon contrat du 21 septembre 2016, la société a transféré des actifs pour CHF 20'781'489 et aucun passif envers les tiers, à Froneri Switzerland SA (anciennement ICFF SA) à Goldach (CHE-447.285.993). Contre-prestation: aucune. |
| 68 | Selon contrat du 27 mai 2019, la société a transféré des actifs pour CHF 2'604'148'000 et des passifs envers les tiers pour CHF 1'739'554'000, à Nestlé Enterprises SA à Vevey (CHE-108.731.444). Contre-prestation: aucune. |
| | **Organe de publication** |
| 1 | Feuille officielle suisse du commerce |
| 50 | Communications aux actionnaires: par publication de communiqués par la voie d'autres organes de presse ou par écrit, pour autant que leurs adresses soient connues |

| Réf. | Capital-actions | | |
|---|---|---|---|
| | **Nominal** | **Libéré** | **Actions** |
| 67 | CHF 8'746'750 | CHF 8'746'750 | 87'467'500 actions nominatives de CHF 0.10, avec restrictions quant à la transmissibilité selon statuts. |

| Réf. | | | Administrateurs, organe de révision et personnes ayant qualité pour signer | | |
|---|---|---|---|---|---|
| Inscr | Mod. | Rad. | **Nom et prénom, origine, domicile** | **Fonctions** | **Mode de signature** |
| 55 | | | **Settembri** Marco, d'Italie, à Lausanne | adm. président | signature individuelle |
| | 59 | | **Humbert** Harold, de France, à Pully | adm. | signature individuelle |
| 63 | | | **Felgines (nom d'usage Lienau)** Muriel Aline Nicole, de France, à Vevey | adm. | signature individuelle |
| 41 | | | **Thiébaud** Jean Christophe, de Brot-Dessous, à Lutry | secrétaire hors conseil | mode particulier (2) |
| | 23 | | **KPMG SA** , à Lausanne | organe de révision | |
| | 7 | | **De Blic-Hamon** Isabelle, de France, à Montreux | | signature individuelle |
| 8 | | | **Borne** Patrice, de France, à Publier (France) | | signature individuelle |
| | 10 | | **Menoud** Anne-Lyse, de Cerniat (FR), à Bossonnens | | signature individuelle |
| | 10 | | **Royer** Sandrine, de Salvenach, à La Tour-de-Peilz | | signature individuelle |
| 16 | | | **Venetz** Laurent, d'Oberems, à Morges | | signature individuelle |
| 26 | | | **Kemball** Alexander John, du Royaume-Uni, à Blonay | | signature individuelle |
| 30 | | | **Delamere** Rachel, du Royaume-Uni, à Vevey | | signature individuelle |
| 30 | | | **Flury** Olivier, de Kleinlützel, à Botterens | | signature individuelle |
| | 37 | | **De Koster Arkesteijn** Annemieke, des Pays-Bas, à Jongny | | signature individuelle |
| | 37 | | **Schüller** Cornelis, des Pays-Bas, à Morges | | signature individuelle |
| 38 | | | **Maradan** Claudia, de Cerniat (FR), à Pully | | signature individuelle |
| 38 | | | **Wood** Regula, de Saint-Gall, à Lausanne | | signature individuelle |
| 39 | | | **Bonaccorso** Ezio, d'Italie, à Chardonne | | signature individuelle |
| | 39 | | **Byland Mc Connell** Ursula, de Veltheim (AG), à La Tour-de-Peilz | | signature individuelle |
| 41 | | | **Jorge** Celine, de France, à Nyon | | signature individuelle |
| 41 | | | **Marquardt** Ulf, d'Allemagne, à Epalinges | | signature individuelle |
| 41 | | | **Schrettl** Malena, d'Allemagne, à Lausanne | | signature individuelle |
| 55 | | | **Checa Cortés** José, d'Espagne, à Saint-Prex | | signature individuelle |
| 55 | | | **Lucet** Philippe, de France, à Prangins | | signature individuelle |
| 58 | | | **Kessler** Dirk, d'Allemagne, à Lausanne | | signature individuelle |

| Réf. Inscr. | Réf. Mod. | Réf. Rad. | Administrateurs, organe de révision et personnes ayant qualité pour signer<br>Nom et prénom, origine, domicile | Fonctions | Mode de signature |
|---|---|---|---|---|---|
| 59 | | | **Boulet** Aurélien, de Vionnaz, à La Tour-de-Peilz | | signature individuelle |
| 59 | | | **Chaudry** Muhammad Arshad, du Pakistan, à Saint-Légier-La Chiésaz | | signature individuelle |
| 59 | | | **De Carvalho** Renata, du Portugal, à La Tour-de-Peilz | | signature individuelle |
| 59 | | | **Philardeau** Thierry, de France, à Lutry | | signature individuelle |
| 59 | | | **Pignatari (nom d'usage Aboutboul)** Serena, d'Italie, à La Tour-de-Peilz | | signature individuelle |
| 59 | | | **Ribeiro Gonçalves Silva Costa** Alexandre, du Brésil, à Vevey | | signature individuelle |
| 60 | | | **Lavoie** Nicholas, de France, à La Tour-de-Peilz | | signature individuelle |
| 61 | | | **Pop** Viorica-Zorita, de Roumanie, à Vevey | | signature individuelle |
| 63 | | | **Assis Sporques** Fernando, du Brésil, à Pully | | signature individuelle |
| 63 | | | **Fuentes Ruelas** Alejandra, du Mexique, à Montreux | | signature individuelle |
| 63 | | | **Onyiego** Daniel Ochieng, du Kenya, à Vevey | | signature individuelle |
| 63 | | | **Pleines** Anke, d'Allemagne, à Pully | | signature individuelle |
| 63 | | | **Uribe de Luna** Martha Delia, du Mexique, à Corsier-sur-Vevey | | signature individuelle |
| 64 | | | **Krampulz** Manuela, de Lugano, à Corseaux | | signature collective à 2 |
| | 27 | | **Steiner** Myriam, de Grossaffoltern, à Semsales | | signature collective à 2 |
| | 32 | | **Python** Frank, d'Arconciel, à Fribourg | | signature collective à 2 |
| | 37 | | **Sudan** Yves, de Broc, à Bulle | | signature collective à 2 |
| | 41 | | **Tschiemer Goumaz** Susanne, de Matten bei Interlaken, à Bussigny-sur-Lausanne | | signature collective à 2 |
| 46 | | | **Johan** Fabrice, de France, à Blonay | | signature collective à 2 |
| 49 | | | **Pons** Giovanni, de Capriasca, à Lutry | | signature collective à 2 |
| | 55 | | **Hotin Benkirane Sefkat**, de Belmont-sur-Lausanne, à Belmont-sur-Lausanne | | signature collective à 2 |
| 61 | | | **Baumann** Gérard, de Corseaux, à Anniviers | | signature collective à 2 |
| 61 | | | **Chiaia** Gian Paolo, de Neuchâtel, à Montreux | | signature collective à 2 |
| | 66 | | **Bonvin** Jérôme, d'Arbaz, à La Tour-de-Peilz | | signature collective à 2 |

(2) signature individuelle en qualité de secrétaire hors conseil, sinon signature collective à deux

| Réf. | JOURNAL | | PUBLICATION FOSC | | Réf. | JOURNAL | | PUBLICATION FOSC | |
|---|---|---|---|---|---|---|---|---|---|
| | Numéro | Date | Date | Page/Id | | Numéro | Date | Date | Page/Id |
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| 56 | 10911 | 30.06.2017 | 05.07.2017 | 3625459 |
| 58 | 16429 | 03.10.2017 | 06.10.2017 | 3795357 |
| 60 | 6258 | 05.04.2018 | 10.04.2018 | 4162081 |
| 62 | 11107 | 20.06.2018 | 25.06.2018 | 4312129 |
| 64 | 21219 | 28.11.2018 | 03.12.2018 | 1004511070 |
| 66 | 22713 | Rectification | 21.12.2018 | 1004528415 |
| 68 | 9740 | 28.05.2019 | | |

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| | Numéro | Date | Date | Page/Id |
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| 39 | 10471 | 17.07.2012 | 20.07.2012 | 6779238 |
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| 65 | 22142 | 11.12.2018 | 14.12.2018 | 1004521715 |
| 67 | 9341 | 22.05.2019 | 24.05.2019 | 1004638171 |

***Inscription non encore publiée mais approuvée par l'office fédéral du registre du commerce (art. 32, al. 1 ORC)***

Moudon, le 29 mai 2019

REGISTRE DU COMMERCE VAUD

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| Date of mailing *(day/month/year)*<br>30 March 2017 (30.03.2017) | |
| Applicant's or agent's file reference<br>13825-WO-PCT | **IMPORTANT NOTIFICATION** |
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| Applicant<br>NESTEC S.A. | |

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| Priority date | Priority application No. | Country or regional Office or PCT receiving Office | Date of receipt of priority document |
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| 01 June 2016 (01.06.2016) | 16172431.5 | EP | 25 March 2017 (25.03.2017) |

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| **Patentanmeldung Nr.** | **Patent application No.** | **Demande de brevet n°** |
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16172431.5 / EP16172431

The organization code and number of your priority application, to be used for filing abroad under the Paris Convention, is EP16172431.

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le President de l'Office européen des brevets
p.o.

V. Joseph

Anmeldung Nr:
Application no.: 16172431.5
Demande no :

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Anmelder / Applicant(s) / Demandeur(s):

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Bezeichnung der Erfindung / Title of the invention / Titre de l'invention:
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Composition for use in the prophylaxis of allergic disease

In Anspruch genommene Priorität(en) / Priority(Priorities) claimed / Priorité(s) revendiquée(s)
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**Composition for use in the prophylaxis of allergic disease**

## FIELD OF INVENTION

The present invention relates to a composition for use in the prophylaxis of allergy and allergic diseases. In particular the present invention relates to the prophylaxis of allergy and allergic diseases in the offspring of a subject through administration of dihomo-gamma-linolenic acid (hereinafter DGLA), or composition comprising dihomo-gamma-linolenic acid, to said subject pre-pregnancy, during pregnancy and/or during lactation.

## BACKGROUND

Allergies and allergic diseases may be best described as hypersensitivity of the body and in particular the immune system, against normally benign foreign materials. Over the last few decades there has been an increasing number of children and adults suffering from allergies and allergic diseases e.g. eczema, atopic dermatitis, allergic rhinitis and asthma; this ever increasing numbers of patients poses a huge burden on healthcare systems worldwide.

Despite extensive research treatment options for sufferers of allergies or allergic diseases are limited. Widely-used therapies often only provide symptomatic relief (e.g. antihistamines, decongestants, and steroids) and said treatments may suffer from draw backs e.g. secondary effects such as drowsiness. Known preventative treatments e.g. immunotherapy or desensitisation therapy, may be expensive and are only of varying success or not applicable e.g. for those allergies caused by food.

Accordingly, there exists a need to provide a therapy, especially a prophylactic therapy for allergic diseases or conditions. In particular, it would be desirable to provide a therapy that can prevent or reduce the risk of development of allergies.

The inventors have now surprisingly found that dihomo-gamma-linolenic acid (DGLA), or a composition comprising DGLA, may prevent or reduce the severity of allergic disease in an offspring of a subject when administered to said subject during pregnancy and/or lactation.

## SUMMARY OF THE INVENTION

The invention is set out in the claims. The invention provides DGLA or a composition comprising DGLA for use in the prophylaxis of allergic disease in an offspring of a subject, wherein said DGLA or composition comprising DGLA is adminisered to said subject pre-pregnancy, during pregnancy and/or during lactation. The Concentration of DGLA in the composition may be at least 3wt% relative to the total fatty acid content of the composition e.g. in a concentration of at least 5wt%, at least 10wt%, at least 20wt%, at least 30wt%, at least 35wt%, or at least 40wt% relative to the total fatty acid content of the composition and said composition may be an enriched composition.

The prophylactic effect of the DGLA or composition comprising DGLA may persist after administration has ceased and said effect in the offspring may be long term and may extend through infancy, childhood and/or into adulthood e.g. 3months, 6months, 12months, 1 year, 5 years, 10 years, 20 years or more after administration to the subject has ceased.

The DGLA may be more effective if it is further administered with at least one other omega-6 polyunsaturated fatty acid and/ or one omega-3 polyunsaturated fatty acid. Said one other omega-6 polyunsaturated fatty acid and/ or one omega-3 polyunsaturated fatty acid may be administered separately, simultaneously or sequentially to said DGLA. The one other omega-6 polyunsaturated fatty acid and/ or one omega-3 polyunsaturated fatty acid may be comprised in the composition comprising DGLA. It may be particularly beneficial if the one other omega-6 polyunsaturated fatty acid is selected from the group consisting of: linoleic acid (LA) and gamma-linolenic acid (GLA) or a combination thereof. It may be particularly beneficial if the omega-3 polyunsaturated fatty acid is selected from the group consisting of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), or a combination thereof.

If the composition comprising DGLA further comprises GLA, LA, EPA, and /or DHA, the concentration of DGLA may be greater than that of GLA, LA, EPA, and/or DHA.

The DGLA or composition comprising DGLA may be for maternal administration and in the case of the composition may be a maternal nutritional composition and in particular may be a pre-pregnancy supplement, a pregnancy supplement or a lactation supplement.

The DGLA or composition comprising DGLA may be particularly effective for use in the prophylaxis of allergy and allergic diseases selected from the group consisting of: an atopic disorder including hereditary atopic disorder and Type 1 allergic disease including IgE mediated allergic disease (e.g. due to eosinophil infiltration and/or mast cell sensitization or activation) and asthma, allergic arthritis, allergic asthma, allergic bronchitis, allergic conjunctivitis, allergic keratitis, allergic rhinitis, allergic sinusitis, alimentary allergy, allergic respiratory disease, animal dander allergy, atopic dermatitis, atopic eczema, atopy, bronchial asthma, contact dermatitis, dermatitis, drug allergy, eczema, food allergy (particularly selected from the group consisting of egg allergy, fish allergy, milk allergy, nut allergy, shellfish allergy, soya allergy, and wheat allergy), food hypersensitivity, hayfever, house dust mite allergy, hypersensitivity pneumonitis, hypertrophic rhinitis, insect allergy, latex allergy, mould allergy, pruritus, seasonal allergic rhinitis, and vasomotor rhinitis.

The DGLA or composition comprising DGLA may be used in a method for the prophylaxis of allergic disease in an offspring of a subject comprising administering a said DGLA or composition to a subject pre-pregnancy, during pregnancy and/or during lactation. The DGLA or composition comprising DGLA may be administered in a therapeutically active amount.
The DGLA or composition comprising DGLA may also be used in the manufacture of a composition for use in the prophylaxis of allergic disease in an offspring of a subject wherein said DGLA or composition is administered to said subject pre-pregnancy and/or during

pregnancy and/or during lactation. The DGLA or composition comprising DGLA may be administered in a therapeutically active amount.

The present invention also provides a kit comprising DGLA or a composition comprising DGLA and at least one other omega-6 polyunsaturated fatty acid and/or at least one omega-3 polyunsaturated fatty acid wherein said other omega-6 polyunsaturated fatty acid may be selected from the group consisting of LA and GLA or a combination thereof, and said omega-3 polyunsaturated fatty acid may be selected from the group consisting of EPA and DHA, or a combination thereof.

The kit may be for use in the prophylaxis of allergic disease in the offspring of a subject.

**DESCRIPTION OF THE DRAWINGS**

Figure 1: Total serum IgE content in three experimental groups (control, fish oil, and fish oil plus DGLA oil).

Figure 2: Aspergillus-specific serum IgG1 content in three experimental groups (control, fish oil, and fish oil plus DGLA oil).

Figure 3: Atopic dermatitis symptoms score in three experimental groups (control, fish oil, and fish oil plus DGLA oil).

Figure 4: Jejunum mast cell numbers in three experimental groups (control, fish oil, and fish oil plus DGLA oil).

Figure 5: Provoked IL4 production by cultured basophils pre-treated with fatty acids in ratios as found in fish oil, DGLA oil, or fish oil plus DGLA oil.

**DETAILED DESCRIPTION OF THE INVENTION**

Unless otherwise indicated, references to % relate to weight %.

As used herein, unless indicated otherwise, references to weight % of a particular polyunsaturated fatty acid, e.g. DGLA, EPA, DHA, LA, GLA, etc. in a composition is a weight % relative to the total fatty acid content of the composition.

As used herein, references to specific polyunsaturated fatty acids, including DGLA, LA, GLA, EPA, DHA, etc. include physiologically acceptable derivatives thereof. Examples of physiologically acceptable derivatives of the polyunsaturated fatty acids include esters of e.g. glycerides, including triglycerides, diglycerides and monoglycerides), alkyl esters (including methyl and ethyl esters), phospholipids and glycolipids. Preferably the physiologically acceptable derivatives of the polyunsaturated fatty acids are glycerides.

**DGLA and DGLA compositions**

In an aspect of the present invention there is provided Dihomo-gamma-linolenic acid (DGLA) for use in the prophylaxis of allergic disease in the offspring of a subject wherein said DGLA is administered to said subject pre-pregnancy and/or during pregnancy and/or during lactation.

The term subject as used herein refers to a mammalian subject such as cat, dog or human. In particular the term refers to a pregnant mammal, a lactating mammal or a mammal trying to conceive/become pregnant. More particularly the term refers to a pregnant human, a lactating human or a human trying to conceive/become pregnant.

As used herein, unless otherwise indicated, the term offspring encompasses the offspring of the subject at any stage of development including fetus, neonate, infant, child and adult stages.

Preferably, in any embodiment of the present invention, the term offspring refers to the neonate, infant, child and adult stages, and more preferably the infant, child and adult stages.

Preferably, in humans, the neonate stage refers to the first 28 days after birth. Preferably, in humans, the infant stage refers to period from 1 month to 24 months. Preferably, in humans, the child stage refers to the period from 2 years to 16 years. Preferably, in humans, the adult stage refers to period beyond 16 years.

The DGLA may be comprised in a composition as an active agent.

In another aspect there is provided a composition comprising DGLA. Said composition may be for use in the prophylaxis of allergic disease in the offspring of a subject wherein said

composition is administered to said subject pre-pregnancy and/or during pregnancy and/or during lactation.

As used herein, unless otherwise indicated, the term DGLA includes DGLA as a free fatty acid, or in the form of physiologically acceptable fatty acid derivatives such as fatty acid esters, including monoglycerides, triglycerides, diglycerides, phospholipids, cholesterol esters. In Particular, in any aspect or embodiment of the present invention, the DGLA is in the form of its triglyceride.

The DGLA employed in the invention or comprised in the composition may stem from any source for example from fish products, meat products, eggs, and microorganisms. For example, EP0399494A discloses a process for the production of a DGLA-containing oil by the fermentation of a microorganism such as a fungus selected form the group consisting of fungi belong to the genus Conidiobolus or Mortierella on a culture medium containing a compound which is an inhibitor of Δ5 desaturase inhibitor such as curcumin, anisole, methoxyphenol, dimethyoxybenzene, and eugenol.

As another example, EP0535940A discloses a process for the production of a composition containing DGLA by culturing a microorganism (e.g. fungi such as Mortierella, Pythium or Entomorphyhora, preferably Mortierella, e.g. Mortierella alpina), having the ability to produce arachidonic acid (ARA) and having reduced or lost Δ5 desaturase activity (e.g. by the addition of a Δ5 desaturase inhibitor). The composition extracted from the fermentation broth may contain a high content of DGLA. A particularly suitable composition for use according to the present invention can be prepared from Mortierella alpina following the processes disclosed in EP0535940A and Kawashima, H., et al, J. Amer. Oil Chem. Soc. (2000), 77(11), 1135-1139. Such compositions comprise a triglyceride in which about 40% of the constituent fatty acids are DGLA. Compositions prepared by the processes disclosed in e.g. EP0535940A may be particularly suitable for the DGLA composition according to the present invention.

The composition of the invention may comprise DGLA in any concentration. However, the composition of the invention may be more effective if it comprises DGLA in a concentration of at least 2wt%, at least 3wt%, at least 5wt%, at least 10wt%, at least 20wt%, at least 25wt%, at

least 30wt%, at least 35wt%, or at least 40wt%, at least 50wt%, at least 60wt%, at least 70wt% at least 80wt%, at least 95wt% relative to the total fatty acid content of the composition. In particular the composition may comprise DGLA in a concentration of at least 30wt%, more particularly at least 35wt% relative to the total fatty acid content of the composition.

In particular the composition of the invention will be a composition enriched in DGLA.

The term enriched as used herein refers to a composition to which DGLA has been added and thereby to a composition wherein the concentration of DGLA is greater than that normally or naturally occurring in said composition.

In particular the composition may be a fish oil composition enriched in DGLA.

The composition comprising DGLA may take any form suitable for ingestion by the subject e.g. it may be a powdered nutritional composition, a food product, a functional food product, a drink (beverage), a dairy product, a pharmaceutical formulation, a pet food product, a nutraceutical, a nutritional supplement e.g. pre-pregnancy, pregnancy and/or lactation supplement, a food product (e.g. a powder, liquid or oil for addition to food or a food/nutritional supplement), or may be included in a food – i.e. the food product can be a food to which DGLA) or the composition comprising DGLA as described herein has been added. The composition may also be included in a pharmaceutical product (e.g. a tablet, capsule or liquid). In Particular the composition will be a pre-pregnancy, pregnancy and/or lactation supplement.

The composition may comprise one or more physiologically or pharmaceutically acceptable additives or excipients, or an ingredient commonly comprised in a particular type/form of composition e.g. pre-pregnancy, pregnancy and/or lactation supplement. Non limiting examples include: preservatives e.g. antioxidants (e.g. tocopherol, ascorbic acid) or flavourings, lipids, carbohydrates, protein, micronutrients, pharmaceutically active agents, conventional food additives such as anti-oxidants, stabilizers, emulsifiers, acidulants, thickeners, buffers or agents for pH adjustment, chelating agents, colorants, excipients, osmotic agents, pharmaceutically acceptable carriers, preservatives, sugars, sweeteners, texturizers, emulsifiers, water, and

vitamins and minerals, for example, vitamins and minerals recommended by a governmental body, such as USRDA, for supplementation in pregnancy e.g. calcium, magnesium, phosphorus, iron, zinc, copper, iodine, selenium, vitamin A or retinol activity equivalent (RAE) e.g. beta carotene or a mix of carotenoids, vitamin C, vitamin B1, niacin, folic acid, biotin, vitamin E.

The DGLA or composition comprising DGLA may be administered in a therapeutically active amount.

A therapeutically effective dose may be any dose that has a prophylactic effect with respect to allergic disease in the offspring of a subject to whom the DGLA or DGLA comprising composition has been administered pre-pregnancy and/or during pregnancy and/or during lactation.

It is well within the purview of the skilled person to determine a therapeutically effective dose. Said dose may depend on age, size and health status of the subject, on the subject's lifestyle, as well as on its genetic heritage and whether there is a history of allergy.

An effective dose may, for example, be determined by measuring the effect of a dose on a subject's offspring's risk of developing an allergenic disorder. An effective dose should preferably result in a statistically significant decrease in said risk in comparison to the risk calculated for that of an offspring of a subject to whom no DGLA or composition comprising DGLA has been administered. Allergenic marker scores such as antibody scores e.g. IgE and IgG scores, and/or skin allergy symptom scores may be used to determine and compare risks.

Particularly beneficial concentrations for humans may be those equating to a dose of DGLA of about 5-1000 mg, about 5-800 mg, about 5-500 mg, about 5-250 mg, about 5-150 mg about 5-100 mg per subject per day, in particular a dose of DGLA of about 10-1000 mg, about 10-800 mg, about 10-500 mg, about 10-250 mg, about 10-150 mg about 10-100 mg per subject per day.

More particularly concentrations equating to a dose of DGLA of about 25-1000 mg, about 25-800 mg, about 25-500 mg, about 25-250 mg, about 25-150 mg about 25-100 mg per adult per

day. The composition may alternatively provide a dose of DGLA of about 50-1000 mg, about 50-800 mg, about 50-500 mg, about 50-250 mg, about 50-150 mg about 50-100 mg per subject per day.

The DGLA or composition comprising DGLA may be simultaneously, sequentially or separately administered with other omega-6 PUFAs in addition to DGLA. In particular, the composition comprising DGLA, may further comprise at least one other omega-6-PUFA, the DGLA is thereby administered simultaneously to the other omega-6 PUFA.

Particular omega-6 PUFAs include linoleic acid (LA) (18:2 n-6) and gamma-linolenic acid (GLA).

It may be particularly beneficial if the composition further comprises LA and/or GLA.

If the composition comprising DGLA also comprise LA and/or GLA it may be particularly beneficial if the concentration of DGLA in said composition is greater than the concentration of GLA or LA, or GLA and LA.

The DGLA or composition comprising DGLA may be simultaneously, sequentially or separately administered with omega-3 PUFAs, such as those present in fish oils – for example eicosapentaenoic acid (EPA) (20:5 n-3) and/or docosahexaenoic acid (DHA) (22:6 n-3). In particular, the composition comprising DGLA, may further comprise at least one omega-3-PUFA, the DGLA is thereby administered simultaneously to this omega-3 PUFA.

If the composition comprising DGLA also comprise DHA and/or EPA it may be particularly beneficial if the concentration of DGLA in said composition is greater than the concentration of DHA or EPA, or DHA and EPA.

The DHA and EPA, if present in the composition of the invention, may be present in a ratio of 1:5 to 5:1.

In an embodiment the composition comprises DGLA and one or both of (i) and/or (ii):

(i) at least one other omega-6 polyunsaturated fatty acid, preferably selected from the group consisting of LA and GLA or a combination of LA and GLA

(ii) at least one omega-3 polyunsaturated fatty acid preferably wherein the omega-3 polyunsaturated fatty acid is selected from the group consisting of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), preferably DHA, or a combination of EPA and DHA.

In another aspect of the invention there is provided a method for the prophylaxis of allergic disease in an offspring comprising administering a therapeutically active amount of DGLA or a composition comprising DGLA as disclosed herein, to a subject pre-pregnancy, during pregnancy and/or during lactation, and preferably during pregnancy and/or during lactation.

In another aspect of the invention there is provided DGLA or a composition comprising DGLA as disclosed herein, for use in the manufacture of a composition for use in the prophylaxis of allergic disease in an offspring of a subject wherein said composition is administered to said subject pre-pregnancy and/or during pregnancy and/or during lactation.

In another aspect there is provided a kit containing a composition comprising DGLA or a physiologically acceptable derivative of DGLA and one or both of (i) and/or (ii):

(i) at least one other omega-6 polyunsaturated fatty acid, preferably selected from the group consisting of LA and GLA or a combination of LA and GLA

(ii) at least one omega-3 polyunsaturated fatty acid preferably wherein the omega-3 polyunsaturated fatty acid is selected from the group consisting of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), preferably DHA, or a combination of EPA and DHA.

wherein (i) and/or (ii) are for sequential, separate or simultaneous administration with the DGLA or composition comprising DGLA, preferably wherein the kit is for use as described in any aspect or embodiment of the invention as disclosed herein.

**Allergy and Allergic Conditions**

As used herein, unless otherwise indicated, prophylaxis refers to preventing a disorder, as well as reducing the risk of development or preventing the onset of a disorder. For example, prophylaxis of allergic disease includes preventing an allergic disease in an offspring, as well as reducing the risk of development of an allergic disease in an offspring.

The prophylactic effect of the invention may persist after administration of the composition has ceased and said effect in the offspring may be long term and may extend through infancy, childhood and/or into adulthood e.g. 3months, 6months, 12months, 1 year, 5 years, 10 years, 20 years or more after administration to the subject has ceased.

The invention is particularly suitable for the prophylaxis of allergic conditions in an offspring due to atopy (e.g. offspring at high risk of developing atopic conditions due to maternal and/or paternal atopy); Type 1 allergic diseases including those mediated by IgE (e.g. by eosinophil infiltration and/or mast cell sensitisation or activation and/or basophil sensitization or activation).

Thus, the compositions of the invention are especially suitable for the prophylaxis of an allergic condition in an offspring, wherein the allergic condition may be selected from the group consisting of: asthma, allergic arthritis, allergic asthma, allergic bronchitis, allergic conjunctivitis, allergic keratitis, allergic rhinitis, allergic sinusitis, alimentary allergy, allergic respiratory disease, animal dander allergy, atopic dermatitis, atopic eczema, atopy, bronchial asthma, contact dermatitis, dermatitis, drug allergy, eczema, food allergy (particularly selected from the group consisting of egg allergy, fish allergy, milk allergy, nut allergy, shellfish allergy, soya allergy, and wheat allergy), food hypersensitivity, eosinophilic esophagitis, hayfever, house dust mite allergy, hypersensitivity pneumonitis, hypertrophic rhinitis, insect allergy, latex allergy, mould allergy, pruritus, seasonal allergic rhinitis, and vasomotor rhinitis.

In any embodiment of the present invention, the composition may be particularly useful for the prophylaxis of an allergic disease selected from the group consisting of: asthma, allergic asthma,

allergic bronchitis, allergic conjunctivitis, allergic keratitis, allergic rhinitis, allergic respiratory disease, animal dander allergy, atopic dermatitis, atopic eczema, atopy, contact dermatitis, dermatitis, eczema, food allergy (particularly selected from the group consisting of egg allergy, fish allergy, milk allergy, nut allergy, shellfish allergy, soya allergy, and wheat allergy), hayfever, house dust mite allergy, latex allergy, mould allergy, pruritus, and/or seasonal allergic rhinitis.

Of these, the allergic disease may especially be selected from the group consisting of: asthma, allergic asthma, allergic conjunctivitis, allergic rhinitis, atopic dermatitis, atopic eczema, dermatitis, eczema, food allergy (particularly selected from the group consisting of egg allergy, fish allergy, milk allergy, nut allergy, shellfish allergy, soya allergy, and wheat allergy), hayfever, house dust mite allergy, pruritus, and/or seasonal allergic rhinitis..

More especially, allergic disease or atopic disorder is selected from the group consisting of: asthma, allergic asthma, allergic conjunctivitis, allergic rhinitis, atopic dermatitis, atopic eczema, dermatitis, eczema, hayfever and/or seasonal allergic rhinitis. Even more particularly, the allergic disease may be selected from the group consisting of: atopic dermatitis and atopic eczema, eosinophilic esophagitis, and IgE mediated asthma.

**Administration**

The DGLA and or composition comprising DGLA according to any aspect or embodiment of the invention, may be administered to the subject pre-pregnancy i.e. prior to conception and/or during pregnancy and/or during lactation. In particular the DGLA or composition comprising DGLA may be administered to the subject during pregnancy and/or lactation and more particularly during pregnancy and lactation.

As used herein, unless otherwise indicated, a reference to administration during pregnancy (i.e. perinatal administration), particularly refers to administration of the DGLA or composition comprising DGLA as defined herein , during any part of, or the whole of, the gestation period wherein a subject is pregnant with an offspring to which the prophylactic treatment is aimed e.g. The first week, the first two weeks, the first month, the first trimester, the second trimester or the third trimester of pregnancy. Particularly, the administration may be continued until at least the birth of the offspring. Thus, for example, in any embodiment of the present invention, administration during pregnancy refers to administration as soon as possible from conception (as defined above) until birth, i.e. during the full gestation period. In humans, the administration may be for a period of from: about 1 week to birth, about 2 weeks to birth, about 4 weeks to birth, about 8 weeks to birth, about 12 weeks to birth, about 18 weeks to birth.

As used herein, unless otherwise indicated, a reference to administration during lactation, particularly includes administration of the DGLA or composition comprising DGLA postnatally at any time during which the offspring to which the prophylactic treatment is aimed is exclusively or partially ingesting the subject's maternal milk. For example administration during lactation may be for the period starting from onset of lactation until the end of the weaning process, i.e. when the offspring has ceased to ingest the maternal milk. During this period, the offspring may be exclusively or partially ingesting the maternal milk. More particularly, administration during lactation may refer to administration: for two weeks following the onset of lactation when the offspring is exclusively or partially ingesting the maternal milk. The administration during lactation may also include administration for a period of 1-24 months, 2-20 months, 3-18 months, 4-12 months or 4-8 months following the onset of lactation during which the offspring is exclusively or partially ingesting the maternal milk of the subject.

In any embodiment of the present invention the administration may be both prenatal for any period as defined above in relation to prenatal administration, as well as postnatal for any

period as defined above in relation to the lactation period, and any combination of these periods as described above.

It may be particularly beneficial if administration is from 4 weeks of gestation or earlier to at least 6 months following the onset of lactation.

In any embodiment of the present invention the administration may be prenatal, i.e. at any period from conception to birth, as well as prenatal and/or postnatal during lactation, i.e. at any period from birth until the end of the weaning process, i.e. when the offspring has ceased to ingest the maternal milk, and any combination of these periods as described above.

In any aspect or any embodiment of the present invention, pre-pregnancy supplementation or administration preferably refers to administration from about 1-24 months, 1-18 months, 1-12 months, 1-6 months, or 1-3 months prior to pregnancy. Administration of the DGLA or composition comprising DGLA pre-pregnancy may be particularly beneficial as it may enable the subject to build up an optimal amount of DGLA in the body from which the offspring of said subject may benefit in terms of prophylactic effect against allergic diseases.

Preferably, in any embodiment of the present invention, the offspring is not directly administered the DGLA. Thus, the DGLA or composition comprising DGLA of the present invention, when administered prenatally to the subject is indirectly transmitted to the developing embryo or fetus, e.g., via the placenta or amniotic fluid. In other words, the exposure of the offspring to the DGLA is *in utero* when the DGLA is administered to the subject (mother) during pregnancy.

Similarly, the DGLA or composition comprising DGLA of the invention, when administered postnatally to a lactating subject, is indirectly transmitted to the neonate or infant via the ingestion of maternal milk, i.e. the exposure of the offspring to the DGLA or composition

containing DGLA that is administered to the subject (mother), is solely via the subject's (mother's) milk.

Those skilled in the art will understand that they can freely combine all features of the present invention disclosed herein. In particular, features described for different embodiments of the present invention may be combined. Where known equivalents exist to specific features, such equivalents are incorporated as if specifically referred to in this specification. Further advantages and features of the present invention are apparent from the figure and non-limiting example.

The present invention will now be described in further details by the way of the following examples.

The following examples serve to illustrate various features and embodiments of the present invention. It will be appreciated that the examples are non-limiting and that those skilled in the art will recognize that various modifications may be made to the foregoing description and the following examples without departing from the spirit and scope of the invention.

## EXAMPLES

### Example 1

MATERIALS AND METHODS

Soybean oil – was obtained from Florin AG (Switzerland; lot number 280813). The oil's fatty acids consisted mainly of the saturated fatty acids palmitic acid (14%) and stearic acid (4%), of the n-6 PUFA LA (47%) and GLA (0.2%), oleic acid (27%), and the n-3 PUFA ALA (5%). DGLA, EPA and DHA were undetectable.

High DHA fish oil (containing 20-26% docosahexaenoic acid (DHA; 22:6 n3), and 7% eicosapentaenoic acid (EPA; 20:5 n3) was obtained from Sofinol SA (a subsidiary of Nestlé corporation). Other major fatty acids consisted of palmitic acid (21%), palmitoleic acid (3%),

stearic acid (5%), and oleic acid (19%). GLA and DGLA levels were below 0.2%, ARA content was ~1%.

DGLA oil, derived from the fungus Mortierella alpina, was obtained from Nippon Suisan Kaisha Ltd. The DGLA content was 35.6%. Other major fatty acids consisted of palmitic acid (16.3%), stearic acid (7.1%), oleic acid (8.2%), LA (6.4%), GLA (2.6%), behenic acid (2.5%) and lignoceric acid (8.6%). The n-3 PUFA content was very low, with only ALA being detectable (0.5%) and EPA and DHA being absent.

Pregnant mice were divided into 3 groups (3-7 pregnant mice per group) – (i) control, (ii) fish oil, and (iii) fish oil + DGLA. The mice were fed a low-fat based diet (standard rodent diet), to which was added (per 95 gram of that diet):

(i) 5 g soybean oil (control group);

(ii) 5 g high DHA fish oil (FO group); or

(iii) 2.5 g fish oil + 2.5 g DGLA oil (DGLA group).

These diets were being fed to pregnant mice, from days 3-5 of pregnancy (a pregnancy lasts 21 days) until weaning of the pups (weaning period was 3 weeks).

From week two, the pups were able to nibble on food in the cages, so to avoid direct exposure of the pups to the diet, the mothers and their pups were fed the control diet, but the mothers were additionally fed each day via intragastric gavage: (i) 0.1 ml of the soy bean oil, (ii) 0.1 ml of high DHA fish oil, or (iii) 0.05 ml high DHA fish oil + 0.05 ml of DGLA oil. Hence, the pups never had direct access to the diets.

After weaning, all pups were put on the control diet. Two weeks after weaning, the pups (13 pups per group) received a skin patch with an allergen *Aspergillus fumigatus* extract, on a shaved part of their back, to induce allergy. The patch was removed after one week, and new patch was put on two weeks after removal of the first patch. The patch was removed and skin symptoms (signs of atopic dermatitis) were observed for the next 4 days (Figure 3). One day after the patch was removed, the mice also received an intranasal challenge with *Aspergillus* extract to see if an allergic response in the lung occurred.

At the last day of skin assessment, the mice were humanely killed and tissue samples analyzed for antibodies in the serum – total IgE (Figure 1) and specific IgG1 (Figure 2), and for the presence of mast cells in the jejunum (Figure 4).

As shown in Figures 1 and 2, total IgE and specific IgG1 are significantly lower in the fish oil + DGLA group compared to the control and the fish oil only group.

Moreover, as shown in Figure 3, skin symptoms were significantly milder in the fish oil + DGLA group than in the control, and moreover, the symptoms remained mild for a longer period in the fish oil + DGLA group compared with the fish oil only group.

Further, the fish oil + DGLA group were found to have a significant lower number of mast cells in the jejunum compared with the fish oil only group and the control group.

**Example 2**

MATERIALS AND METHODS

RBL-2H3 cells; rat basophilic leukemia cells, were cultured under standard conditions with 15% serum in their culture medium. Once sufficient numbers of cells were present in the dishes, sodium salts of various fatty acids were added directly added to the medium, and the cells were incubated with these fatty acids for 24h. During the last 18h of this incubation period, the cells also received phorbol 12-myristate 13-acetate ("PMA"; final concentration 50 ng/mL) and ionomycin ("IM"; final concentration 0.125 μM) to stimulate IL4 secretion by the cells.

RESULTS

Figure 5 shows that cells incubated with DGLA (final concentration 60 μM) produce less IL4 upon stimulation with PMA and IM than cells not treated with additional fatty acids (control). The same was observed for cells incubated with 2.14 μM of a fatty acid mix ("NIF") consisting of DHA and EPA mixed in a 3:1 ratio. When DGLA and NIF were given together, a synergistic reduction of IL4 production was observed.

**CLAIMS**

1. Dihomo-gamma-linolenic acid (DGLA) for use in the prophylaxis of allergic disease in the offspring of mammalian subject wherein said DGLA is administered to said subject pre-pregnancy and/or during pregnancy and/or during lactation.

2. DGLA for use according to claim 1 wherein said DGLA is administered sequentially, simultaneously or separately to at least one other omega-6 polyunsaturated fatty acid and/or at least one omega-3 polyunsaturated fatty acid wherein, preferably said other omega-6 polyunsaturated fatty acid is selected from the group consisting of LA and GLA or a combination of LA and GLA, and/or wherein preferably said omega-3 polyunsaturated fatty acid is selected from the group consisting of DHA and EPA or a combination of DHA and EPA.

3. DGLA for use according to claims 1 or 2 wherein said prophylaxis of allergic disease is long term.

4. A composition comprising DGLA for use in the prophylaxis of allergic disease in an offspring of a mammalian subject, comprising administration of the composition to said subject pre-pregnancy and/or during pregnancy and/or during lactation and preferably wherein said composition is a composition enriched in DGLA.

5. A composition comprising DGLA for use according to claim 4 wherein said composition further comprises omega-6 polyunsaturated fatty acid, preferably selected from the group consisting of LA and GLA or a combination of LA and GLA and/or an omega-3 polyunsaturated fatty acid, preferably selected from the group consisting of DHA and EPA or a combination of DHA and EPA.

6. A composition comprising DGLA for use according to claims 4 or 5 wherein said prophylaxis is long term.

7. A composition comprising DGLA for use according to anyone of claims 4 to 6 wherein said DGLA is comprised in said composition in a concentration of at least 3wt% relative to the total fatty acid content of the composition and more preferably in a concentration of at least 5wt%, at least 10wt%, at least 20wt%, at least 30wt%, at least 35wt%, or at least 40wt% relative to the total fatty acid content of the composition.

8. A composition according to anyone of claims 5 to 7 wherein said composition comprises DGLA, GLA and LA and wherein the concentration of DGLA is greater than GLA and the concentration of GLA is greater than the concentration of LA.

9. A composition according to anyone of claims 5 to 8 wherein said composition comprising DGLA, DHA and EPA and wherein the concentration of DGLA is greater than the concentration of DHA or EPA.

10. A composition comprising DGLA for use according to anyone of claims 4 to 9 wherein said composition is a maternal nutritional composition and preferably selected from the group consisting of: pre-pregnancy supplement, pregnancy supplement or lactation supplement.

11. DGLA for use according to anyone of claims 1 to 3, or a composition comprising DGLA for use according to any of claims 4 to 10 wherein the allergic disease is selected from the group consisting of: an atopic disorder including hereditary atopic disorder and a Type 1 allergic disease including IgE mediated allergic disease, preferably wherein the allergic disease is selected from the group consisting of: asthma, allergic arthritis, allergic asthma, allergic bronchitis, allergic conjunctivitis, allergic keratitis, allergic rhinitis, allergic sinusitis, alimentary allergy, allergic respiratory disease, animal dander allergy, atopic dermatitis, atopic eczema, atopy, bronchial asthma, contact dermatitis, dermatitis, drug allergy, eczema, food allergy (particularly selected from the group consisting of egg allergy, fish allergy, milk allergy, nut allergy, shellfish allergy, soya allergy, and wheat allergy), food hypersensitivity, eosinophilic esophagitis, hayfever, house dust mite allergy,

hypersensitivity pneumonitis, hypertrophic rhinitis, insect allergy, latex allergy, mould allergy, pruritus, seasonal allergic rhinitis, and vasomotor rhinitis.

12. A method for the prophylaxis of allergic disease in an offspring comprising administering DGLA or a composition comprising DGLA to a subject pre-pregnancy, during pregnancy and/or during lactation, and preferably during pregnancy and/or during lactation.

13. DGLA or a composition comprising DGLA for use in the manufacture of a composition for use in the prophylaxis of allergic disease in an offspring of a subject wherein said composition is administered to said subject pre-pregnancy and/or during pregnancy and/or during lactation.

14. A kit containing DGLA or a composition comprising DGLA, and (i) and/or (ii):

(i) at least one other omega-6 polyunsaturated fatty acid, preferably selected from the group consisting of LA and GLA or a combination of LA and GLA

(ii) at least one omega-3 polyunsaturated fatty acid preferably wherein the omega-3 polyunsaturated fatty acid is selected from the group consisting of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), or a combination of EPA and DHA,

wherein (i) and/or (ii) are for sequential, separate or simultaneous administration with the DGLA or composition comprising DGLA, and preferably wherein the kit is for use in the prophylaxis of allergic disease in the offspring of a mammalian subject.

**ABSTRACT**

Dihomo-gamma-linolenic acid (DGLA) or a composition comprising DGLA for use in the prophylaxis of allergic disease in the offspring of mammalian subject wherein said DGLA is administered to said subject pre-pregnancy and/or during pregnancy and/or during lactation.

**FIGURE 1**

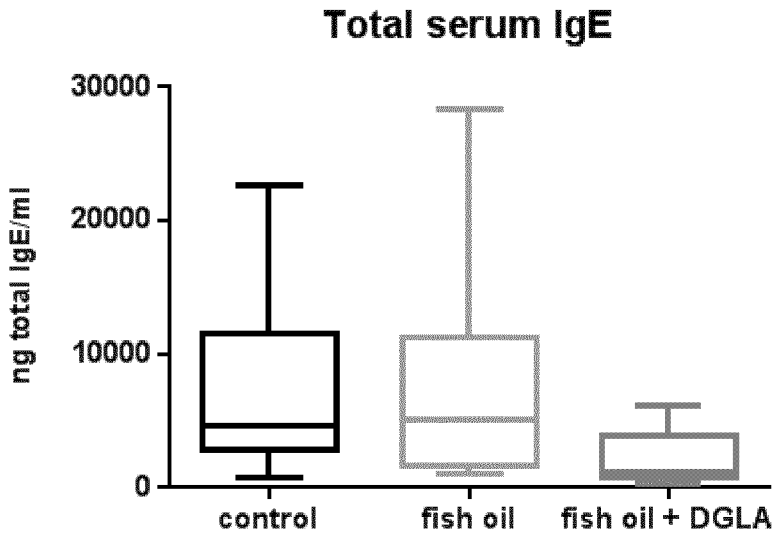

Total serum IgE
ng total IgE/ml
30000
20000
10000
0
control
fish oil
fish oil + DGLA

**FIGURE 2**

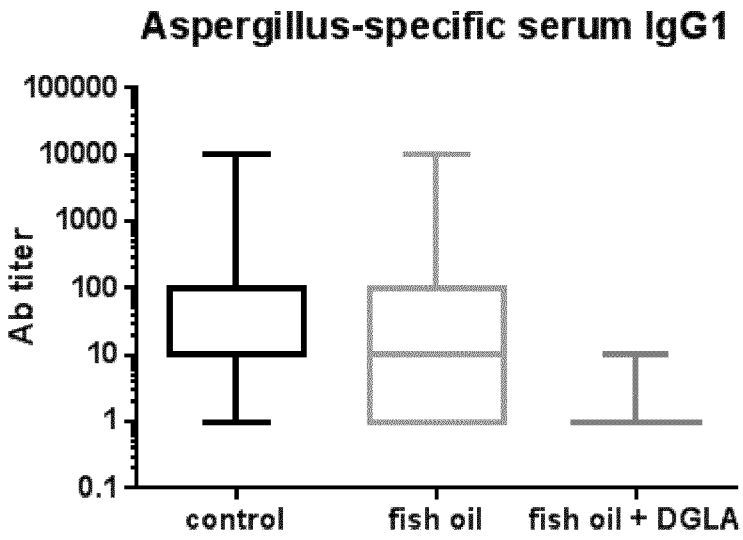

Aspergillus-specific serum IgG1
Ab titer
100000
10000
1000
100
10
1
0.1
control
fish oil
fish oil + DGLA

**FIGURE 3**

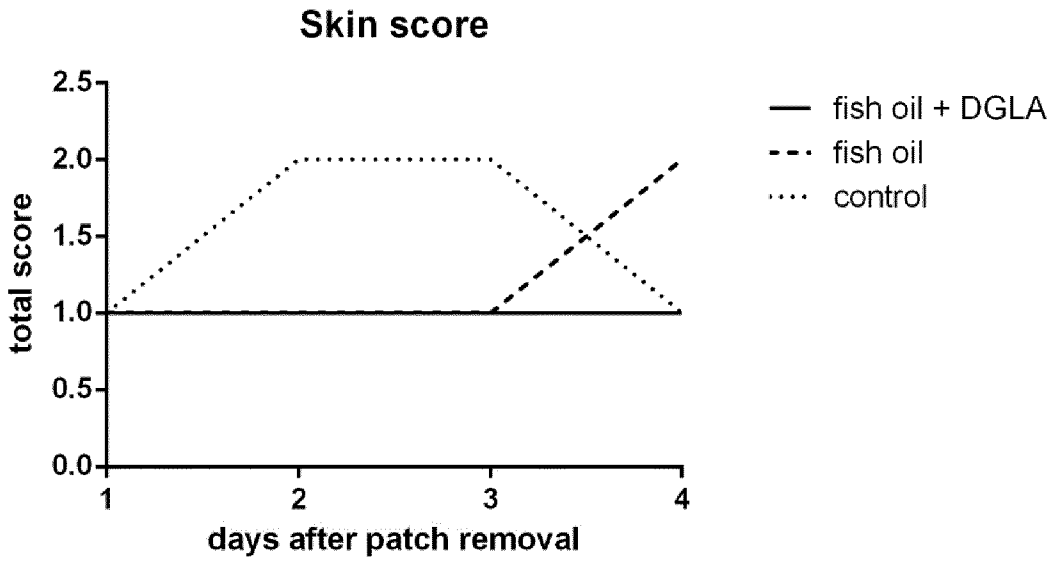

Skin score
total score
2.5
2.0
1.5
1.0
0.5
0.0
1
2
3
4
days after patch removal
fish oil + DGLA
fish oil
control

FIGURE 4

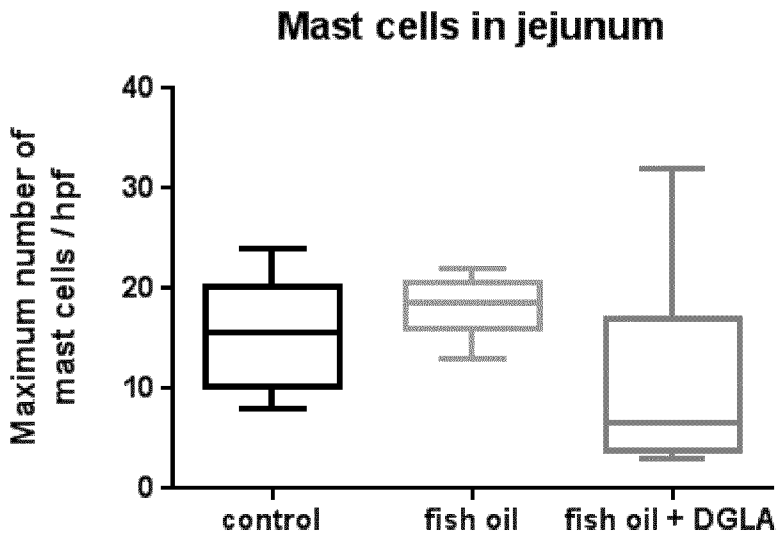
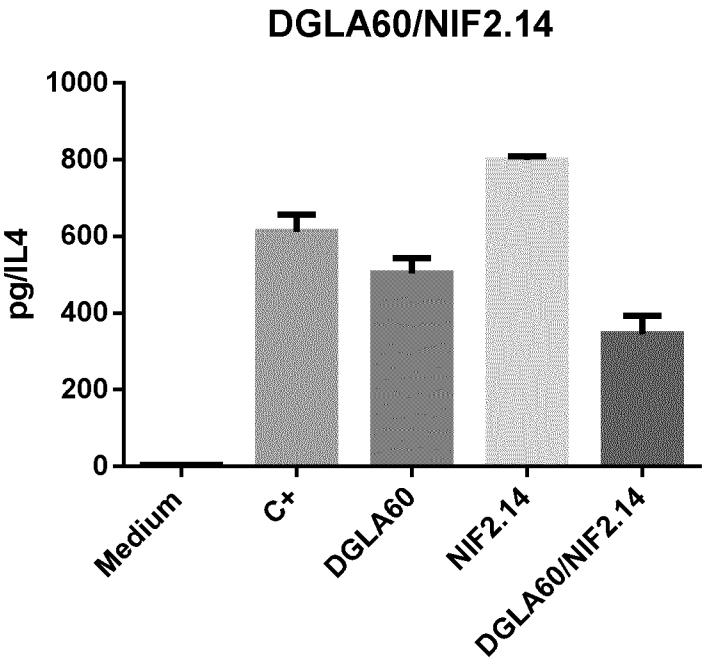

Mast cells in jejunum
Maximum number of mast cells / hpf
40
30
20
10
0
control
fish oil
fish oil + DGLA

**FIGURE 5**


DGLA60/NIF2.14
pg/IL4
1000
800
600
400
200
0
Medium
C+
DGLA60
NIF2.14
DGLA60/NIF2.14

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**Declarations under Rule 4.17:**
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*
— *of inventorship (Rule 4.17(iv))*

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(54) Title: COMPOSITION FOR USE IN THE PROPHYLAXIS OF ALLERGIC DISEASE

(57) Abstract: Dihomo-gamma-linolenic acid (DGLA) or a composition comprising DGLA for use in the prophylaxis of allergic disease in the offspring of mammalian subject wherein said DGLA is administered to said subject pre-pregnancy and/or during pregnancy and/or during lactation.

WO 2017/207124 A1

# Composition for use in the prophylaxis of allergic disease

## FIELD OF INVENTION

The present invention relates to a composition for use in the prophylaxis of allergy and allergic diseases. In particular the present invention relates to the prophylaxis of allergy and allergic diseases in the offspring of a subject through administration of dihomo-gamma-linolenic acid (hereinafter DGLA), or composition comprising dihomo-gamma-linolenic acid, to said subject pre-pregnancy, during pregnancy and/or during lactation.

## BACKGROUND

Allergies and allergic diseases may be best described as hypersensitivity of the body and in particular the immune system, against normally benign foreign materials. Over the last few decades there has been an increasing number of children and adults suffering from allergies and allergic diseases e.g. eczema, atopic dermatitis, allergic rhinitis and asthma; this ever increasing numbers of patients poses a huge burden on healthcare systems worldwide.

Despite extensive research treatment options for sufferers of allergies or allergic diseases are limited. Widely-used therapies often only provide symptomatic relief (e.g. antihistamines, decongestants, and steroids) and said treatments may suffer from draw backs e.g. secondary effects such as drowsiness. Known preventative treatments e.g. immunotherapy or desensitisation therapy, may be expensive and are only of varying success or not applicable e.g. for those allergies caused by food.

Accordingly, there exists a need to provide a therapy, especially a prophylactic therapy for allergic diseases or conditions. In particular, it would be desirable to provide a therapy that can prevent or reduce the risk of development of allergies.

The inventors have now surprisingly found that dihomo-gamma-linolenic acid (DGLA), or a composition comprising DGLA, may prevent or reduce the severity of allergic disease in an offspring of a subject when administered to said subject during pregnancy and/or lactation.

## SUMMARY OF THE INVENTION

The invention is set out in the claims. The invention provides DGLA or a composition comprising DGLA for use in the prophylaxis of allergic disease in an offspring of a subject, wherein said DGLA or composition comprising DGLA is adminisered to said subject pre-pregnancy, during pregnancy and/or during lactation. The Concentration of DGLA in the composition may be at least 3wt% relative to the total fatty acid content of the composition e.g. in a concentration of at least 5wt%, at least 10wt%, at least 20wt%, at least 30wt%, at least 35wt%, or at least 40wt% relative to the total fatty acid content of the composition and said composition may be an enriched composition.

The prophylactic effect of the DGLA or composition comprising DGLA may persist after administration has ceased and said effect in the offspring may be long term and may extend through infancy, childhood and/or into adulthood e.g. 3months, 6months, 12months, 1 year, 5 years, 10 years, 20 years or more after administration to the subject has ceased.

The DGLA may be more effective if it is further administered with at least one other omega-6 polyunsaturated fatty acid and/ or one omega-3 polyunsaturated fatty acid. Said one other omega-6 polyunsaturated fatty acid and/ or one omega-3 polyunsaturated fatty acid may be administered separately, simultaneously or sequentially to said DGLA. The one other omega-6 polyunsaturated fatty acid and/ or one omega-3 polyunsaturated fatty acid may be comprised in the composition comprising DGLA. It may be particularly beneficial if the one other omega-6 polyunsaturated fatty acid is selected from the group consisting of: linoleic acid (LA) and gamma-linolenic acid (GLA) or a combination thereof. It may be particularly beneficial if the omega-3 polyunsaturated fatty acid is selected from the group consisting of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), or a combination thereof.

If the composition comprising DGLA further comprises GLA, LA, EPA, and /or DHA, the concentration of DGLA may be greater than that of GLA, LA, EPA, and/or DHA.

The DGLA or composition comprising DGLA may be for maternal administration and in the case of the composition may be a maternal nutritional composition and in particular may be a pre-pregnancy supplement, a pregnancy supplement or a lactation supplement.

The DGLA or composition comprising DGLA may be particularly effective for use in the prophylaxis of allergy and allergic diseases selected from the group consisting of: an atopic disorder including hereditary atopic disorder and Type 1 allergic disease including IgE mediated allergic disease (e.g. due to eosinophil infiltration and/or mast cell sensitization or activation) and asthma, allergic arthritis, allergic asthma, allergic bronchitis, allergic conjunctivitis, allergic keratitis, allergic rhinitis, allergic sinusitis, alimentary allergy, allergic respiratory disease, animal dander allergy, atopic dermatitis, atopic eczema, atopy, bronchial asthma, contact dermatitis, dermatitis, drug allergy, eczema, food allergy (particularly selected from the group consisting of egg allergy, fish allergy, milk allergy, nut allergy, shellfish allergy, soya allergy, and wheat allergy), food hypersensitivity, hayfever, house dust mite allergy, hypersensitivity pneumonitis, hypertrophic rhinitis, insect allergy, latex allergy, mould allergy, pruritus, seasonal allergic rhinitis, and vasomotor rhinitis.

The DGLA or composition comprising DGLA may be used in a method for the prophylaxis of allergic disease in an offspring of a subject comprising administering a said DGLA or composition to a subject pre-pregnancy, during pregnancy and/or during lactation. The DGLA or composition comprising DGLA may be administered in a therapeutically active amount.
The DGLA or composition comprising DGLA may also be used in the manufacture of a composition for use in the prophylaxis of allergic disease in an offspring of a subject wherein said DGLA or composition is administered to said subject pre-pregnancy and/or during

pregnancy and/or during lactation. The DGLA or composition comprising DGLA may be administered in a therapeutically active amount.

The present invention also provides a kit comprising DGLA or a composition comprising DGLA and at least one other omega-6 polyunsaturated fatty acid and/or at least one omega-3 polyunsaturated fatty acid wherein said other omega-6 polyunsaturated fatty acid may be selected from the group consisting of LA and GLA or a combination thereof, and said omega-3 polyunsaturated fatty acid may be selected from the group consisting of EPA and DHA, or a combination thereof.

The kit may be for use in the prophylaxis of allergic disease in the offspring of a subject.

**DESCRIPTION OF THE DRAWINGS**

Figure 1: Total serum IgE content in three experimental groups (control, fish oil, and fish oil plus DGLA oil).

Figure 2: Aspergillus-specific serum IgG1 content in three experimental groups (control, fish oil, and fish oil plus DGLA oil).

Figure 3: Atopic dermatitis symptoms score in three experimental groups (control, fish oil, and fish oil plus DGLA oil).

Figure 4: Jejunum mast cell numbers in three experimental groups (control, fish oil, and fish oil plus DGLA oil).

Figure 5: Provoked IL4 production by cultured basophils pre-treated with fatty acids in ratios as found in fish oil, DGLA oil, or fish oil plus DGLA oil.

Figure 6: IL10 secretion in brachial lymph nodes

**DETAILED DESCRIPTION OF THE INVENTION**

Unless otherwise indicated, references to % relate to weight %.

As used herein, unless indicated otherwise, references to weight % of a particular polyunsaturated fatty acid, e.g. DGLA, EPA, DHA, LA, GLA, etc. in a composition is a weight % relative to the total fatty acid content of the composition.

As used herein, references to specific polyunsaturated fatty acids, including DGLA, LA, GLA, EPA, DHA, etc. include physiologically acceptable derivatives thereof. Examples of physiologically acceptable derivatives of the polyunsaturated fatty acids include esters of e.g. glycerides, including triglycerides, diglycerides and monoglycerides), alkyl esters (including methyl and ethyl esters), phospholipids and glycolipids. Preferably the physiologically acceptable derivatives of the polyunsaturated fatty acids are glycerides.

**DGLA and DGLA compositions**

In an aspect of the present invention there is provided Dihomo-gamma-linolenic acid (DGLA) for use in the prophylaxis of allergic disease in the offspring of a subject wherein said DGLA is administered to said subject pre-pregnancy and/or during pregnancy and/or during lactation.

The term subject as used herein refers to a mammalian subject such as cat, dog or human. In particular the term refers to a pregnant mammal, a lactating mammal or a mammal trying to conceive/become pregnant. More particularly the term refers to a pregnant human, a lactating human or a human trying to conceive/become pregnant.

As used herein, unless otherwise indicated, the term offspring encompasses the offspring of the subject at any stage of development including fetus, neonate, infant, child and adult stages.

Preferably, in any embodiment of the present invention, the term offspring refers to the neonate, infant, child and adult stages, and more preferably the infant, child and adult stages.

Preferably, in humans, the neonate stage refers to the first 28 days after birth. Preferably, in humans, the infant stage refers to period from 1 month to 24 months. Preferably, in humans, the child stage refers to the period from 2 years to 16 years. Preferably, in humans, the adult stage refers to period beyond 16 years.

The DGLA may be comprised in a composition as an active agent.

In another aspect there is provided a composition comprising DGLA. Said composition may be for use in the prophylaxis of allergic disease in the offspring of a subject wherein said composition is administered to said subject pre-pregnancy and/or during pregnancy and/or during lactation.

As used herein, unless otherwise indicated, the term DGLA includes DGLA as a free fatty acid, or in the form of physiologically acceptable fatty acid derivatives such as fatty acid esters, including monoglycerides, triglycerides, diglycerides, phospholipids, cholesterol esters. In Particular, in any aspect or embodiment of the present invention, the DGLA is in the form of its triglyceride.

The DGLA employed in the invention or comprised in the composition may stem from any source for example from fish products, meat products, eggs, and microorganisms. For example, EP0399494A discloses a process for the production of a DGLA-containing oil by the fermentation of a microorganism such as a fungus selected form the group consisting of fungi belong to the genus Conidiobolus or Mortierella on a culture medium containing a compound which is an inhibitor of Δ5 desaturase inhibitor such as curcumin, anisole, methoxyphenol, dimethyoxybenzene, and eugenol.

As another example, EP0535940A discloses a process for the production of a composition containing DGLA by culturing a microorganism (e.g. fungi such as Mortierella, Pythium or Entomorphyhora, preferably Mortierella, e.g. Mortierella alpina), having the ability to produce arachidonic acid (ARA) and having reduced or lost Δ5 desaturase activity (e.g. by the addition of a Δ5 desaturase inhibitor). The composition extracted from the fermentation broth may contain a high content of DGLA. A particularly suitable composition for use according to the present invention can be prepared from Mortierella alpina following the processes disclosed in EP0535940A and Kawashima, H., et al, J. Amer. Oil Chem. Soc. (2000), 77(11), 1135-1139. Such compositions comprise a triglyceride in which about 40% of the constituent fatty acids are DGLA. Compositions prepared by the processes disclosed in e.g. EP0535940A may be particularly suitable for the DGLA composition according to the present invention.

The composition of the invention may comprise DGLA in any concentration. However, the composition of the invention may be more effective if it comprises DGLA in a concentration of at least 2wt%, at least 3wt%, at least 5wt%, at least 10wt%, at least 20wt%, at least 25wt%, at least 30wt%, at least 35wt%, or at least 40wt%, at least 50wt%, at least 60wt%, at least 70wt% at least 80wt%, at least 95wt% relative to the total fatty acid content of the composition. In particular the composition may comprise DGLA in a concentration of at least 30wt%, more particularly at least 35wt% relative to the total fatty acid content of the composition.

In particular the composition of the invention will be a composition enriched in DGLA.

The term enriched as used herein refers to a composition to which DGLA has been added and thereby to a composition wherein the concentration of DGLA is greater than that normally or naturally occurring in said composition.

In particular the composition may be a fish oil composition enriched in DGLA.

The composition comprising DGLA may take any form suitable for ingestion by the subject e.g. it may be a powdered nutritional composition, a food product, a functional food product, a drink (beverage), a dairy product, a pharmaceutical formulation, a pet food product, a nutraceutical, a nutritional supplement e.g. pre-pregnancy, pregnancy and/or lactation supplement, a food product (e.g. a powder, liquid or oil for addition to food or a food/nutritional supplement), or may be included in a food – i.e. the food product can be a food to which DGLA) or the composition comprising DGLA as described herein has been added. The composition may also be included in a pharmaceutical product (e.g. a tablet, capsule or liquid). In Particular the composition will be a pre-pregnancy, pregnancy and/or lactation supplement.

The composition may comprise one or more physiologically or pharmaceutically acceptable additives or excipients, or an ingredient commonly comprised in a particular type/form of composition e.g. pre-pregnancy, pregnancy and/or lactation supplement. Non limiting examples include: preservatives e.g. antioxidants (e.g. tocopherol, ascorbic acid) or flavourings, lipids, carbohydrates, protein, micronutrients, pharmaceutically active agents, conventional food

additives such as anti-oxidants, stabilizers, emulsifiers, acidulants, thickeners, buffers or agents for pH adjustment, chelating agents, colorants, excipients, osmotic agents, pharmaceutically acceptable carriers, preservatives, sugars, sweeteners, texturizers, emulsifiers, water, and vitamins and minerals, for example, vitamins and minerals recommended by a governmental body, such as USRDA, for supplementation in pregnancy e.g. calcium, magnesium, phosphorus, iron, zinc, copper, iodine, selenium, vitamin A or retinol activity equivalent (RAE) e.g. beta carotene or a mix of carotenoids, vitamin C, vitamin B1, niacin, folic acid, biotin, vitamin E.

The DGLA or composition comprising DGLA may be administered in a therapeutically active amount.

A therapeutically effective dose may be any dose that has a prophylactic effect with respect to allergic disease in the offspring of a subject to whom the DGLA or DGLA comprising composition has been administered pre-pregnancy and/or during pregnancy and/or during lactation.

It is well within the purview of the skilled person to determine a therapeutically effective dose. Said dose may depend on age, size and health status of the subject, on the subject's lifestyle, as well as on its genetic heritage and whether there is a history of allergy.

An effective dose may, for example, be determined by measuring the effect of a dose on a subject's offspring's risk of developing an allergenic disorder. An effective dose should preferably result in a statistically significant decrease in said risk in comparison to the risk calculated for that of an offspring of a subject to whom no DGLA or composition comprising DGLA has been administered. Allergenic marker scores such as antibody scores e.g. IgE and IgG scores, and/or skin allergy symptom scores may be used to determine and compare risks.

Particularly beneficial concentrations for humans may be those equating to a dose of DGLA of about 5-1000 mg, about 5-800 mg, about 5-500 mg, about 5-250 mg, about 5-150 mg about 5-100 mg per subject per day, in particular a dose of DGLA of about 10-1000 mg, about 10-800 mg, about 10-500 mg, about 10-250 mg, about 10-150 mg about 10-100 mg per subject per day.

More particularly concentrations equating to a dose of DGLA of about 25-1000 mg, about 25-800 mg, about 25-500 mg, about 25-250 mg, about 25-150 mg about 25-100 mg per adult per day. The composition may alternatively provide a dose of DGLA of about 50-1000 mg, about 50-800 mg, about 50-500 mg, about 50-250 mg, about 50-150 mg about 50-100 mg per subject per day.

The DGLA or composition comprising DGLA may be simultaneously, sequentially or separately administered with other omega-6 PUFAs in addition to DGLA. In particular, the composition comprising DGLA, may further comprise at least one other omega-6-PUFA, the DGLA is thereby administered simultaneously to the other omega-6 PUFA.

Particular omega-6 PUFAs include linoleic acid (LA) (18:2 n-6) and gamma-linolenic acid (GLA).

It may be particularly beneficial if the composition further comprises LA and/or GLA.

If the composition comprising DGLA also comprise LA and/or GLA it may be particularly beneficial if the concentration of DGLA in said composition is greater than the concentration of GLA or LA, or GLA and LA.

The DGLA or composition comprising DGLA may be simultaneously, sequentially or separately administered with omega-3 PUFAs, such as those present in fish oils – for example eicosapentaenoic acid (EPA) (20:5 n-3) and/or docosahexaenoic acid (DHA) (22:6 n-3). In particular, the composition comprising DGLA, may further comprise at least one omega-3-PUFA, the DGLA is thereby administered simultaneously to this omega-3 PUFA.

If the composition comprising DGLA also comprise DHA and/or EPA it may be particularly beneficial if the concentration of DGLA in said composition is greater than the concentration of DHA or EPA, or DHA and EPA.

The DHA and EPA, if present in the composition of the invention, may be present in a ratio of 1:5 to 5:1.

In an embodiment the composition comprises DGLA and one or both of (i) and/or (ii):

(i) at least one other omega-6 polyunsaturated fatty acid, preferably selected from the group consisting of LA and GLA or a combination of LA and GLA

(ii) at least one omega-3 polyunsaturated fatty acid preferably wherein the omega-3 polyunsaturated fatty acid is selected from the group consisting of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), preferably DHA, or a combination of EPA and DHA.

In another aspect of the invention there is provided a method for the prophylaxis of allergic disease in an offspring comprising administering a therapeutically active amount of DGLA or a composition comprising DGLA as disclosed herein, to a subject pre-pregnancy, during pregnancy and/or during lactation, and preferably during pregnancy and/or during lactation.

In another aspect of the invention there is provided DGLA or a composition comprising DGLA as disclosed herein, for use in the manufacture of a composition for use in the prophylaxis of allergic disease in an offspring of a subject wherein said composition is administered to said subject pre-pregnancy and/or during pregnancy and/or during lactation.

In another aspect there is provided a kit containing a composition comprising DGLA or a physiologically acceptable derivative of DGLA and one or both of (i) and/or (ii):

(i) at least one other omega-6 polyunsaturated fatty acid, preferably selected from the group consisting of LA and GLA or a combination of LA and GLA

(ii) at least one omega-3 polyunsaturated fatty acid preferably wherein the omega-3 polyunsaturated fatty acid is selected from the group consisting of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), preferably DHA, or a combination of EPA and DHA.

wherein (i) and/or (ii) are for sequential, separate or simultaneous administration with the DGLA or composition comprising DGLA, preferably wherein the kit is for use as described in any aspect or embodiment of the invention as disclosed herein.

**Allergy and Allergic Conditions**

As used herein, unless otherwise indicated, prophylaxis refers to preventing a disorder, as well as reducing the risk of development or preventing the onset of a disorder. For example, prophylaxis of allergic disease includes preventing an allergic disease in an offspring, as well as reducing the risk of development of an allergic disease in an offspring.

The prophylactic effect of the invention may persist after administration of the composition has ceased and said effect in the offspring may be long term and may extend through infancy, childhood and/or into adulthood e.g. 3months, 6months, 12months, 1 year, 5 years, 10 years, 20 years or more after administration to the subject has ceased.

The invention is particularly suitable for the prophylaxis of allergic conditions in an offspring due to atopy (e.g. offspring at high risk of developing atopic conditions due to maternal and/or paternal atopy); Type 1 allergic diseases including those mediated by IgE (e.g. by eosinophil infiltration and/or mast cell sensitisation or activation and/or basophil sensitization or activation).

Thus, the compositions of the invention are especially suitable for the prophylaxis of an allergic condition in an offspring, wherein the allergic condition may be selected from the group consisting of: asthma, allergic arthritis, allergic asthma, allergic bronchitis, allergic conjunctivitis, allergic keratitis, allergic rhinitis, allergic sinusitis, alimentary allergy, allergic respiratory disease, animal dander allergy, atopic dermatitis, atopic eczema, atopy, bronchial asthma, contact dermatitis, dermatitis, drug allergy, eczema, food allergy (particularly selected from the group consisting of egg allergy, fish allergy, milk allergy, nut allergy, shellfish allergy, soya allergy, and wheat allergy), food hypersensitivity, eosinophilic esophagitis, hayfever, house dust mite allergy, hypersensitivity pneumonitis, hypertrophic rhinitis, insect allergy, latex allergy, mould allergy, pruritus, seasonal allergic rhinitis, and vasomotor rhinitis.

In any embodiment of the present invention, the composition may be particularly useful for the prophylaxis of an allergic disease selected from the group consisting of: asthma, allergic asthma, allergic bronchitis, allergic conjunctivitis, allergic keratitis, allergic rhinitis, allergic respiratory disease, animal dander allergy, atopic dermatitis, atopic eczema, atopy, contact dermatitis, dermatitis, eczema, food allergy (particularly selected from the group consisting of egg allergy, fish allergy, milk allergy, nut allergy, shellfish allergy, soya allergy, and wheat allergy), hayfever, house dust mite allergy, latex allergy, mould allergy, pruritus, and/or seasonal allergic rhinitis.

Of these, the allergic disease may especially be selected from the group consisting of: asthma, allergic asthma, allergic conjunctivitis, allergic rhinitis, atopic dermatitis, atopic eczema, dermatitis, eczema, food allergy (particularly selected from the group consisting of egg allergy, fish allergy, milk allergy, nut allergy, shellfish allergy, soya allergy, and wheat allergy), hayfever, house dust mite allergy, pruritus, and/or seasonal allergic rhinitis..

More especially, allergic disease or atopic disorder is selected from the group consisting of: asthma, allergic asthma, allergic conjunctivitis, allergic rhinitis, atopic dermatitis, atopic eczema, dermatitis, eczema, hayfever and/or seasonal allergic rhinitis. Even more particularly, the allergic disease may be selected from the group consisting of: atopic dermatitis and atopic eczema, eosinophilic esophagitis, and IgE mediated asthma.

**Administration**

The DGLA and or composition comprising DGLA according to any aspect or embodiment of the invention, may be administered to the subject pre-pregnancy i.e. prior to conception and/or during pregnancy and/or during lactation. In particular the DGLA or composition comprising DGLA may be administered to the subject during pregnancy and/or lactation and more particularly during pregnancy and lactation.

As used herein, unless otherwise indicated, a reference to administration during pregnancy (i.e. perinatal administration), particularly refers to administration of the DGLA or composition comprising DGLA as defined herein , during any part of, or the whole of, the gestation period wherein a subject is pregnant with an offspring to which the prophylactic treatment is aimed e.g. The first week, the first two weeks, the first month, the first trimester, the second trimester or the third trimester of pregnancy. Particularly, the administration may be continued until at least the birth of the offspring. Thus, for example, in any embodiment of the present invention, administration during pregnancy refers to administration as soon as possible from conception (as defined above) until birth, i.e. during the full gestation period. In humans, the administration may be for a period of from: about 1 week to birth, about 2 weeks to birth, about 4 weeks to birth, about 8 weeks to birth, about 12 weeks to birth, about 18 weeks to birth.

As used herein, unless otherwise indicated, a reference to administration during lactation, particularly includes administration of the DGLA or composition comprising DGLA postnatally at any time during which the offspring to which the prophylactic treatment is aimed is exclusively or partially ingesting the subject's maternal milk. For example administration during lactation may be for the period starting from onset of lactation until the end of the weaning process, i.e. when the offspring has ceased to ingest the maternal milk. During this period, the offspring may be exclusively or partially ingesting the maternal milk. More particularly, administration during lactation may refer to administration: for two weeks following the onset of lactation when the offspring is exclusively or partially ingesting the maternal milk. The administration during lactation may also include administration for a period of 1-24 months, 2-20 months, 3-18 months, 4-12 months or 4-8 months following the onset of lactation during which the offspring is exclusively or partially ingesting the maternal milk of the subject.

In any embodiment of the present invention the administration may be both prenatal for any period as defined above in relation to prenatal administration, as well as postnatal for any

period as defined above in relation to the lactation period, and any combination of these periods as described above.

It may be particularly beneficial if administration is from 4 weeks of gestation or earlier to at least 6 months following the onset of lactation.

In any embodiment of the present invention the administration may be prenatal, i.e. at any period from conception to birth, as well as prenatal and/or postnatal during lactation, i.e. at any period from birth until the end of the weaning process, i.e. when the offspring has ceased to ingest the maternal milk, and any combination of these periods as described above.

In any aspect or any embodiment of the present invention, pre-pregnancy supplementation or administration preferably refers to administration from about 1-24 months, 1-18 months, 1-12 months, 1-6 months, or 1-3 months prior to pregnancy. Administration of the DGLA or composition comprising DGLA pre-pregnancy may be particularly beneficial as it may enable the subject to build up an optimal amount of DGLA in the body from which the offspring of said subject may benefit in terms of prophylactic effect against allergic diseases.

Preferably, in any embodiment of the present invention, the offspring is not directly administered the DGLA. Thus, the DGLA or composition comprising DGLA of the present invention, when administered prenatally to the subject is indirectly transmitted to the developing embryo or fetus, e.g., via the placenta or amniotic fluid. In other words, the exposure of the offspring to the DGLA is *in utero* when the DGLA is administered to the subject (mother) during pregnancy.

Similarly, the DGLA or composition comprising DGLA of the invention, when administered postnatally to a lactating subject, is indirectly transmitted to the neonate or infant via the ingestion of maternal milk, i.e. the exposure of the offspring to the DGLA or composition

containing DGLA that is administered to the subject (mother), is solely via the subject's (mother's) milk.

Those skilled in the art will understand that they can freely combine all features of the present invention disclosed herein. In particular, features described for different embodiments of the present invention may be combined. Where known equivalents exist to specific features, such equivalents are incorporated as if specifically referred to in this specification. Further advantages and features of the present invention are apparent from the figure and non-limiting example.

The present invention will now be described in further details by the way of the following examples.

The following examples serve to illustrate various features and embodiments of the present invention. It will be appreciated that the examples are non-limiting and that those skilled in the art will recognize that various modifications may be made to the foregoing description and the following examples without departing from the spirit and scope of the invention.

## EXAMPLES

### Example 1

MATERIALS AND METHODS

Soybean oil – was obtained from Florin AG (Switzerland; lot number 280813). The oil's fatty acids consisted mainly of the saturated fatty acids palmitic acid (14%) and stearic acid (4%), of the n-6 PUFA LA (47%) and GLA (0.2%), oleic acid (27%), and the n-3 PUFA ALA (5%). DGLA, EPA and DHA were undetectable.

High DHA fish oil (containing 20-26% docosahexaenoic acid (DHA; 22:6 n3), and 7% eicosapentaenoic acid (EPA; 20:5 n3) was obtained from Sofinol SA (a subsidiary of Nestlé corporation). Other major fatty acids consisted of palmitic acid (21%), palmitoleic acid (3%),

stearic acid (5%), and oleic acid (19%). GLA and DGLA levels were below 0.2%, ARA content was ~1%.

DGLA oil, derived from the fungus Mortierella alpina, was obtained from Nippon Suisan Kaisha Ltd. The DGLA content was 35.6%. Other major fatty acids consisted of palmitic acid (16.3%), stearic acid (7.1%), oleic acid (8.2%), LA (6.4%), GLA (2.6%), behenic acid (2.5%) and lignoceric acid (8.6%). The n-3 PUFA content was very low, with only ALA being detectable (0.5%) and EPA and DHA being absent.

Pregnant mice were divided into 3 groups (3-7 pregnant mice per group) – (i) control, (ii) fish oil, and (iii) fish oil + DGLA. The mice were fed a low-fat based diet (standard rodent diet), to which was added (per 95 gram of that diet):
(i) 5 g soybean oil (control group);
(ii) 5 g high DHA fish oil (FO group); or
(iii) 2.5 g fish oil + 2.5 g DGLA oil (DGLA group).

These diets were being fed to pregnant mice, from days 3-5 of pregnancy (a pregnancy lasts 21 days) until weaning of the pups (weaning period was 3 weeks).

From week two, the pups were able to nibble on food in the cages, so to avoid direct exposure of the pups to the diet, the mothers and their pups were fed the control diet, but the mothers were additionally fed each day via intragastric gavage: (i) 0.1 ml of the soy bean oil, (ii) 0.1 ml of high DHA fish oil, or (iii) 0.05 ml high DHA fish oil + 0.05 ml of DGLA oil. Hence, the pups never had direct access to the diets.

After weaning, all pups were put on the control diet. Two weeks after weaning, the pups (13 pups per group) received a skin patch with an allergen *Aspergillus fumigatus* extract, on a shaved part of their back, to induce allergy. The patch was removed after one week, and new patch was put on two weeks after removal of the first patch. The patch was removed and skin symptoms (signs of atopic dermatitis) were observed for the next 4 days (Figure 3). One day after the patch was removed, the mice also received an intranasal challenge with *Aspergillus* extract to see if an allergic response in the lung occurred.

At the last day of skin assessment, the mice were humanely killed and tissue samples analyzed for antibodies in the serum – total IgE (Figure 1) and specific IgG1 (Figure 2), and for the presence of mast cells in the jejunum (Figure 4).

As shown in Figures 1 and 2, total IgE and specific IgG1 are significantly lower in the fish oil + DGLA group compared to the control and the fish oil only group.

Moreover, as shown in Figure 3, skin symptoms were significantly milder in the fish oil + DGLA group than in the control, and moreover, the symptoms remained mild for a longer period in the fish oil + DGLA group compared with the fish oil only group.

Further, the fish oil + DGLA group were found to have a significant lower number of mast cells in the jejunum compared with the fish oil only group and the control group.

**Example 2**

MATERIALS AND METHODS

RBL-2H3 cells; rat basophilic leukemia cells, were cultured under standard conditions with 15% serum in their culture medium. Once sufficient numbers of cells were present in the dishes, sodium salts of various fatty acids were added directly added to the medium, and the cells were incubated with these fatty acids for 24h. During the last 18h of this incubation period, the cells also received phorbol 12-myristate 13-acetate ("PMA"; final concentration 50 ng/mL) and ionomycin ("IM"; final concentration 0.125 μM) to stimulate IL4 secretion by the cells.

RESULTS

Figure 5 shows that cells incubated with DGLA (final concentration 60 μM) produce less IL4 upon stimulation with PMA and IM than cells not treated with additional fatty acids (control). The same was observed for cells incubated with 2.14 μM of a fatty acid mix ("NIF") consisting of DHA and EPA mixed in a 3:1 ratio. When DGLA and NIF were given together, a synergistic reduction of IL4 production was observed.

**Example 3**

Pregnant mice were divided into 2 groups and fed, from day 5 of pregnancy until weaning of the pups, a low-fat based diet (standard rodent diet), to which was added (per 95 gram of that diet): 5 g soybean oil (control group) or 2.5 g fish oil + 2.5 g DGLA oil (fish oil + DGLA group). Oil compositions are described in Example 1.

From week two after birth, the pups were able to nibble on food in the cages, so to avoid direct exposure of the pups to the diet, the mothers and their pups were fed the control diet, but the mothers were additionally fed each day via intragastric gavage: 0.1 ml of the soy bean oil or 0.05 ml high DHA fish oil + 0.05 ml of DGLA oil. Hence, the pups never had direct access to the diets.

After weaning, all pups were put on the control diet. Two weeks after weaning, the pups (10 pups per group) received a skin patch as described in Example 1. At the last day of skin assessment, the mice were humanely killed and skin-draining, brachial lymph node collected. Brachial and mediastinal lymph nodes were homogenized using a syringe plunger in a cell strainer. The cells were centrifuged and washed two times with Roswell Park Memorial Institute (RPMI) medium (Sigma) supplemented with 10% fetal bovine serum, 1% L-glutamine, 1% penicillin/streptomycin and 0.1% gentamicin, and 50mM b-mercaptoethanol. The cells (3E5 cells/well) were cultured in a ninety-six-well flat-bottom plate in the absence or presence of *Af*. After 72 h of culture, the plates (including the supernatant and cells) were frozen until analysis of the supernatants.

The concentrations of IL-10 in cell-culture supernatants were determined with an electro-chemiluminescence-based multiplex assay (Mesoscale) according to the manufacturer's instructions.

As shown in Figure 6, the immune-regulatory, anti-inflammatory cytokine IL-10 was significantly increased in pups from the fish oil + DGLA group as compared to pups from mothers fed a control diet. The increased levels of IL-10 might be might have contributed to the decreased skin score observed after patch removal (Figure 3). Alternatively, IL-10 could be an indicator of an anti-inflammatory mechanism induced in the pups by fish oil + DGLA during pregnancy and lactation to dampen the allergic response to *Aspergillus* later in life.

**CLAIMS**

1. Dihomo-gamma-linolenic acid (DGLA) for use in the prophylaxis of allergic disease in the offspring of mammalian subject wherein said DGLA is administered to said subject pre-pregnancy and/or during pregnancy and/or during lactation.

2. DGLA for use according to claim 1 wherein said DGLA is administered sequentially, simultaneously or separately to at least one other omega-6 polyunsaturated fatty acid and/or at least one omega-3 polyunsaturated fatty acid wherein, preferably said other omega-6 polyunsaturated fatty acid is selected from the group consisting of LA and GLA or a combination of LA and GLA, and/or wherein preferably said omega-3 polyunsaturated fatty acid is selected from the group consisting of DHA and EPA or a combination of DHA and EPA.

3. DGLA for use according to claims 1 or 2 wherein said prophylaxis of allergic disease is long term.

4. A composition comprising DGLA for use in the prophylaxis of allergic disease in an offspring of a mammalian subject, comprising administration of the composition to said subject pre-pregnancy and/or during pregnancy and/or during lactation and preferably wherein said composition is a composition enriched in DGLA.

5. A composition comprising DGLA for use according to claim 4 wherein said composition further comprises omega-6 polyunsaturated fatty acid, preferably selected from the group consisting of LA and GLA or a combination of LA and GLA and/or an omega-3 polyunsaturated fatty acid, preferably selected from the group consisting of DHA and EPA or a combination of DHA and EPA.

6. A composition comprising DGLA for use according to claims 4 or 5 wherein said prophylaxis is long term.

7. A composition comprising DGLA for use according to anyone of claims 4 to 6 wherein said DGLA is comprised in said composition in a concentration of at least 3wt% relative to the total fatty acid content of the composition and more preferably in a concentration of at least 5wt%, at least 10wt%, at least 20wt%, at least 30wt%, at least 35wt%, or at least 40wt% relative to the total fatty acid content of the composition.

8. A composition according to anyone of claims 5 to 7 wherein said composition comprises DGLA, GLA and LA and wherein the concentration of DGLA is greater than GLA and the concentration of GLA is greater than the concentration of LA.

9. A composition according to anyone of claims 5 to 8 wherein said composition comprising DGLA, DHA and EPA and wherein the concentration of DGLA is greater than the concentration of DHA or EPA.

10. A composition comprising DGLA for use according to anyone of claims 4 to 9 wherein said composition is a maternal nutritional composition and preferably selected from the group consisting of: pre-pregnancy supplement, pregnancy supplement or lactation supplement.

11. DGLA for use according to anyone of claims 1 to 3, or a composition comprising DGLA for use according to any of claims 4 to 10 wherein the allergic disease is selected from the group consisting of: an atopic disorder including hereditary atopic disorder and a Type 1 allergic disease including IgE mediated allergic disease, preferably wherein the allergic disease is selected from the group consisting of: asthma, allergic arthritis, allergic asthma, allergic bronchitis, allergic conjunctivitis, allergic keratitis, allergic rhinitis, allergic sinusitis, alimentary allergy, allergic respiratory disease, animal dander allergy, atopic dermatitis, atopic eczema, atopy, bronchial asthma, contact dermatitis, dermatitis, drug allergy, eczema, food allergy (particularly selected from the group consisting of egg allergy, fish allergy, milk allergy, nut allergy, shellfish allergy, soya allergy, and wheat allergy), food hypersensitivity, eosinophilic esophagitis, hayfever, house dust mite allergy,

hypersensitivity pneumonitis, hypertrophic rhinitis, insect allergy, latex allergy, mould allergy, pruritus, seasonal allergic rhinitis, and vasomotor rhinitis.

12. A method for the prophylaxis of allergic disease in an offspring comprising administering DGLA or a composition comprising DGLA to a subject pre-pregnancy, during pregnancy and/or during lactation, and preferably during pregnancy and/or during lactation.

13. DGLA or a composition comprising DGLA for use in the manufacture of a composition for use in the prophylaxis of allergic disease in an offspring of a subject wherein said composition is administered to said subject pre-pregnancy and/or during pregnancy and/or during lactation.

14. A kit containing DGLA or a composition comprising DGLA, and (i) and/or (ii):

(i) at least one other omega-6 polyunsaturated fatty acid, preferably selected from the group consisting of LA and GLA or a combination of LA and GLA

(ii) at least one omega-3 polyunsaturated fatty acid preferably wherein the omega-3 polyunsaturated fatty acid is selected from the group consisting of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), or a combination of EPA and DHA,

wherein (i) and/or (ii) are for sequential, separate or simultaneous administration with the DGLA or composition comprising DGLA, and preferably wherein the kit is for use in the prophylaxis of allergic disease in the offspring of a mammalian subject.

FIGURE 1

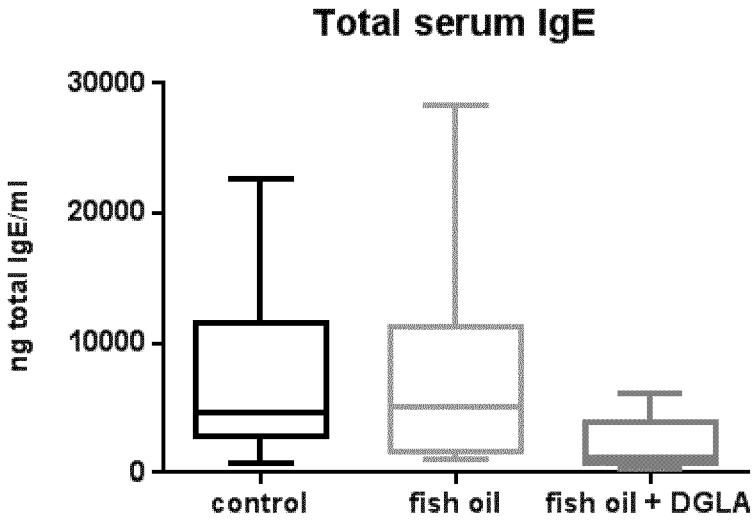

Total serum IgE
ng total IgE/ml
30000
20000
10000
0
control
fish oil
fish oil + DGLA

**FIGURE 2**

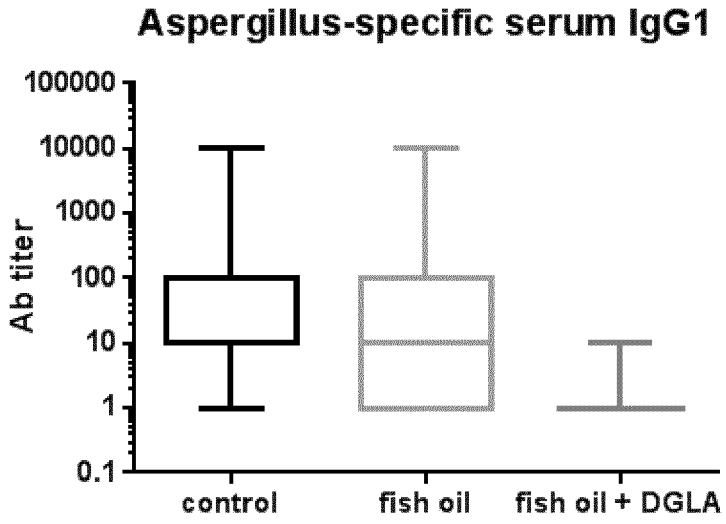

Aspergillus-specific serum IgG1
Ab titer
100000
10000
1000
100
10
1
0.1
control
fish oil
fish oil + DGLA

FIGURE 3

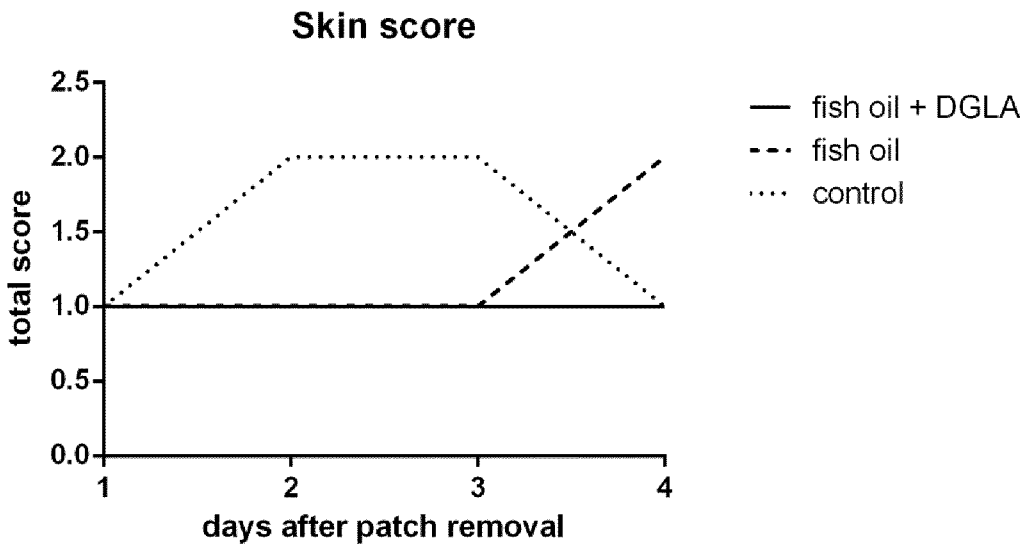

Skin score
total score
2.5
2.0
1.5
1.0
0.5
0.0
1
2
3
4
days after patch removal
fish oil + DGLA
fish oil
control

FIGURE 4

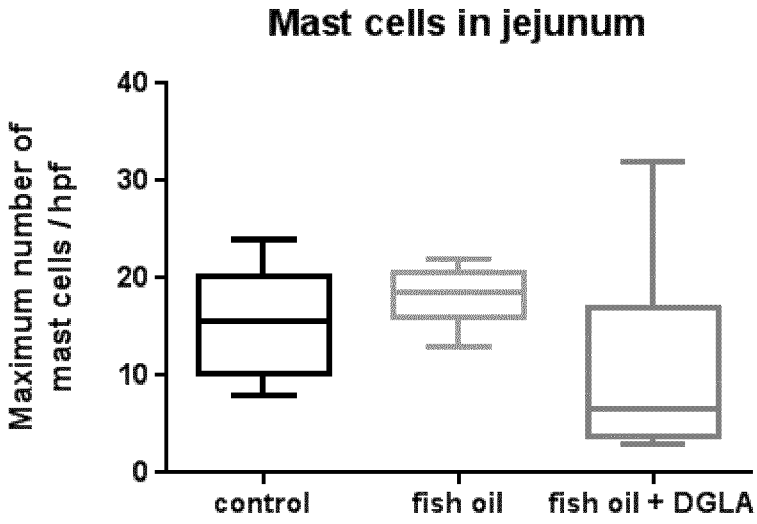
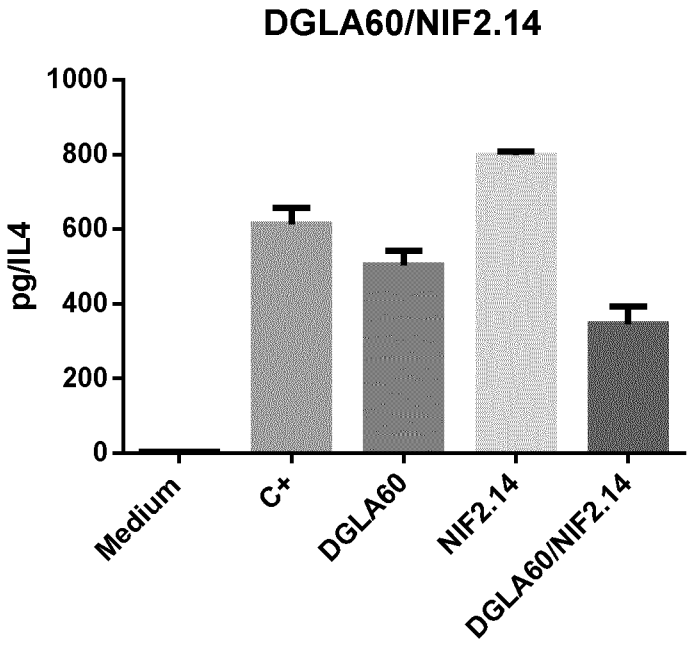

Mast cells in jejunum
Maximum number of mast cells / hpf
40
30
20
10
0
control
fish oil
fish oil + DGLA

FIGURE 5


DGLA60/NIF2.14
pg/IL4
1000
800
600
400
200
0
Medium
C+
DGLA60
NIF2.14
DGLA60/NIF2.14

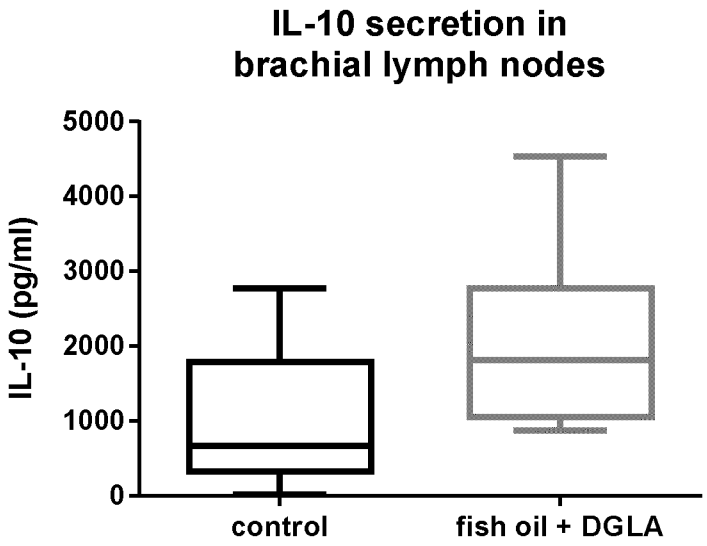

IL-10 secretion in
brachial lymph nodes
IL-10 (pg/ml)
5000
4000
3000
2000
1000
0
control
fish oil + DGLA

Figure 6

# INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/055680

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/202 A61P27/14
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | US 5 591 446 A (MELNIK BODO C [DE] ET AL) 7 January 1997 (1997-01-07) | 1-13 |
| Y | claims 1,4<br>tables 1-4<br>column 4, line 19 - column 5, line 5<br>column 7, lines 52-65 | 14 |
| | ----- | |
| X | US 6 150 411 A (STORDY BARBARA JACQUELINE [GB]) 21 November 2000 (2000-11-21) | 14 |
| Y | column 2, lines 31-57 | 14 |
| | ----- | |

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

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1

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Information on patent family members

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