THE OMEGA 3 FATTY ACIDS AND THE CARDIOVASCULAR SYSTEM:
nutritional benefits and claims

afssa
AGENCE FRANÇAISE DE SECURITE SANITAIRE DES ALIMENTS

FOREWORD

The working group, composed of a balanced number of clinicians and research scientists, conducted a constructive and concordant review of the nutritional benefits of omega-3 fatty acids. However, profound differences of opinion gradually emerged regarding the scope of the functional claims for omega-3 fatty acids.

The qualification criteria for some of the experts, required for the assessment of the claims, meant that some group members were concerned in scientific work financed by industrial companies involved in the development of omega-3 fatty acids. But because of their direct involvement in preparing the reference intakes for the French population (ANC), their participation in the working group was nonetheless deemed desirable in order to achieve consistency between the expert assessment of the ANC and of the claims.

The subtle division which gradually became apparent within the group was mainly centred around two distinct visions of the issues surrounding these claims:

- one group suggested, looking at the subject from the public health standpoint and in line with the ANC, extending functional claims to a maximum number of foods, in order to promote an effective increase in omega-3 fatty acid intake in the widest possible population (the view of the majority of the research scientists),
- the other group, looking at the subject from the standpoint of cardiovascular prevention, proposed accepting functional claims relating to healthy cardiovascular function on a very limited basis (the view of the clinicians).

In view of the small majority for the second option, it would have been possible to put forward the restrictive option with a secondary, minority position presented as well.

However, following further discussion, a consensus position was achieved by identifying an intermediate level of claim reflecting nutritional properties without prejudging the possibility of a cardiovascular benefit. The working group felt that this synthesis was the most relevant in scientific terms, expressing as faithfully as possible the complexity of the interactions between fortification and the vector food.

Because of the importance of the issues and the complexity of the subject, the various options were twice submitted to a second level of collective expert assessment, by the CES "Nutrition Humaine" (Afssa Expert Committee on Human Nutrition). This body also opted for the option containing three levels of claim, rejecting the Manichean view under consideration at one point.

The opinions of external experts were sought, analysed and in some cases included, but this summary report does not necessarily express their overall opinion on the subject.

CONTENTS

LIST OF ACRONYMS AND ABBREVIATIONS

Afssa: *Agence française de sécurité sanitaire des aliments* - French Food Safety Agency
Afssaps: *Agence française de sécurité sanitaire des produits de santé* - French Health Products Safety Agency
ALA: alpha-linolenic acid
ANC: *apports nutritionnels conseillés pour la population française* (reference intakes for the French population)
AOCS: American oil chemist's society
BOCCRF: *Bulletin officiel de la concurrence, de la consommation et de la répression des fraudes* - Official Bulletin of the DG for Competition, Consumer Affairs and Trading Standards
BP: blood pressure
CEDAP: *Commission d'étude des produits destinés à une alimentation particulière* - Interministerial Committee for products intended for particular nutritional uses
CES «Nutrition Humaine»: *Comité d'experts spécialisé «Nutrition Humaine»* (Afssa Expert Committee on Human Nutrition)
CHD: coronary heart disease
CIQUAL: *Centre informatique sur la qualité des aliments (Afssa)* - Informatics Centre for Food Quality
CIV: *Centre d'information des viandes* - Meat Information Centre
CLO: cod liver oil
CNAM: *Conservatoire national des arts et métiers* - National Institute of Technology
CRP: C-reactive protein
CSHPF: *Conseil supérieur d'hygiène publique de France* - French Higher Council for Public Health
DART: study titled **"***Dietary and reinfarction trial"* (Burr et al. 1989)
DGAl: *Direction générale de l'alimentation* - General Directorate for Food
DGCCRF: *Direction générale de la concurrence, de la consommation et de la répression des fraudes* - General Directorate for Fair Trading, Consumer Affairs and Fraud Control
DGS: *Direction générale de la santé* - General Directorate for Health
DHA: docosahexaenoic acid (C 22:6 n-3)
DM: dry matter
DPA: docosapentaenoic acid (C 22:5 n-3)
EPA: eicosapentaenoic acid (C 20:5 n-3)
FDA: Food and Drug Administration
FIDM: fat in dry matter
FO: fish oil

FSA: Food Standards Agency
GC: gas chromatography
GISSI: study by the "Grupo Italiano per la Studio della Sopravvivenza nell'Infarto miocardico" (1999)
GRAS: generally recognised as safe
HDL: high density lipoproteins
HHT: 12-hydroxyheptadecatrienoic acid
IHD: ischaemic heart disease
INCA: *Enquête individuelle et nationale sur les consommations alimentaires* - Individual national dietary survey
INRA: *Institut national de la recherche agronomique* (French National Institute for Agricultural Research)
INSERM: *Institut national de la santé et de la recherche médicale* - French National Institute for Health and Medical Research
ISSFAL: International Society for the Study of Fatty acids and Lipids
ISTNA: *Institut Scientifique et Technique de la Nutrition et de l'Alimentation* - Scientific and Technical Institute for Food and Nutrition
ITERG: *Institut des Corps Gras (Industrial technical centre for the fats and oils industry)*
IUPAC: International Union of Pure and Applied Chemistry
LA: linoleic acid
LC-PUFA: long chain polyunsaturated fatty acid
LDL : Low density lipoproteins
MAFF: UK Ministry of Agriculture, Fisheries and Food
MDA: malondialdehyde
MI: myocardial infarction
MUFA: monounsaturated fatty acid
NIDDM: non-insulin dependent diabetes
NS: not significant
OCA: *Observatoire des consommations alimentaires* (Afssa) - Food Intake Unit
PAF: platelet-activating factor
PNNS: *Programme national nutrition-santé* - National Health and Nutrition Programme
PUFA: polyunsaturated fatty acid
RDA: recommended daily allowances
RDI: recommended daily intakes
SANCO: General Directorate for Health and Consumer Protection (European Commission)
SFA: saturated fatty acid
SU.VI.MAX.: study entitled "Anti-oxidant vitamin and mineral supplements"
TBA: thiobarbituric acid
w**TDE**: total daily energy

TFA: total fatty acids
TG : TAG: triglycerides, triacylglycerols
UENRN: *Unité d'évaluation sur la nutrition et les risques nutritionnels* (Afssa) - Unit for the Assessment of Nutrition and Nutritional Risks
UFCS: *Union féminine civique et sociale* - Women's Civil and Social Union
UHT: ultra-high temperature
USDA: United States Department of Agriculture
« Visa PP »: *« visa Publicité produit»* (product advertising visa)
12-HETE: 12-hydroxyeicosatetraenoic acid

INTRODUCTION

The constant developments in knowledge and the gradual application of the concept of evidence-based medicine are leading, in terms of nutrition, to regular re-examination of the particular benefit of a number of nutrients and the claims which might support their consumption.

The contribution made by the fatty acids in foods to the prevention of or increase in the risk of ischaemic cardiovascular disease is an inexhaustible source of data but also of controversy. A number of experimental and epidemiological studies have suggested that regular consumption of omega-3 fatty acids might be linked to beneficial cardiovascular effects. However, a number of intervention studies, each conducted in particular conditions, have provided somewhat contradictory clarification.

The objective of this working group was not to define a nutrition policy for fatty acids, but, through a review of the mass of current scientific data, to produce an assessment of what was acceptable or unacceptable in terms of the possible claims used to support the promotion of omega-3 fatty acids.

MEMBERSHIP OF THE WORKING GROUP

Members of the CES "Nutrition Humaine":

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REFERRAL 2001-SA-0104

On the basis of a referral dated 27 April 2001 issued by the Direction Générale de la Concurrence, de la Consommation et de la Répression des Fraudes (DGCCRF), the working group was tasked by the CES "Nutrition Humaine" to assess the nutritional benefits and the scientific basis of the claims being formulated in connection with the fortification of foodstuffs with fatty acids of the omega-3 family (ω3 FA), in the form of precursors or derivatives (the working group was set up in July 2001).

More specifically, this project was to supply the information required to formulate a general opinion on the following points:

1) Are the amounts of omega-3 fatty acids found in foodstuffs completely safe for consumers, based on the omega-3 content, the number of product categories concerned, current consumption, the prospects for the future or the modification of the balance between omega-3 and omega-6 fatty acids?

2) What should the omega-3 content of the product be (per 100 g, 100 ml or 100 kcal of product ready for use by the consumer) and what type of omega-3 fatty acid should it contain, to justify:
- the quantitative nutrition claims "source of omega-3", "rich in omega-3"
- the qualitative claims stating the role of omega-3 fatty acids in healthy cardiovascular function?

3) Are claims such as "Omega-3 fatty acids have a beneficial effect on blood fluidity and healthy cardiovascular function" justified? This type of advertising is often accompanied by a picture of a red heart, emphasising the claimed effect;

4) Are claims stating the role played by omega-3 fatty acids in the lowering of cholesterol levels justified?

To avoid spreading its work too widely and given the targeted nature of the referral, the working group focussed exclusively on the adult population, excluding in particular data concerning omega-3 fatty acids and brain development in newborn babies and children. Products such as food supplements or dietary products designed for special medical purposes were also not taken into account. The relationships between omega-3 fatty acids and carcinogenicity were not discussed in detail (elements regarding this point are discussed in an annex) as this is not based on human intervention data which would allow validation of the corresponding concepts.

REFERRAL 2001-SA-0046

The Union Féminine Civique et Sociale (UFCS) consulted Afssa directly on 5 February 2001 regarding the justification for the claim "suitable for cholesterol-lowering diets" and the assessment of the omega-3/omega-6 fatty acid ratio of a margarine. More generally, the UFCS also consulted Afssa on what types of claims would be acceptable for these types of product, on the basis of their omega-3 and omega-6 fatty acid content.

When informed of this referral, the DGCCRF requested a technical dossier from the company concerned in order to enable Afssa to evaluate the points raised by this consumer association (documents sent to Afssa on 8 January 2002).

This request also forms part of referral 2001-SA-0104, (request by the DGCCRF for an assessment of the food safety and nutritional benefits of omega-3 fatty acids found in or added to foodstuffs and the justification of the claims used).

PREAMBLE

The purpose of this preamble is to clarify the discussion and it does not constitute an exhaustive review: the reader can refer to the general articles referred to throughout the text and the chapter in *Apports nutritionnels conseillés pour la population française* (Martin, 2001) specifically devoted to this subject.

I – Omega-3 and omega-6 fatty acids: definition, structure, terminology

Fatty acids are organic molecules comprising one carbon chain ending in a carboxylic group. This carbon chain may have no double bonds and, if so, the fatty acids are described as saturated (SFA). It may also have one or more double bonds, in which case the fatty acids are called monounsaturated fatty acids (MUFA) or polyunsaturated fatty acids (PUFA). While saturated, monounsaturated and some polyunsaturated fatty acids are synthesised in the body, polyunsaturated fatty acids of the omega-6 and omega-3 families, or at least linoleic and alpha-linolenic acids (Figure 1), have to be provided by the diet.

Unsaturated fatty acids can be generally referred to in terms of the first double bond on the terminal methyl group. This means that the fatty acids in the omega-6 (n-6 or ω 6) and omega-3 (n-3 or ω 3) families characteristically have their first double bond located respectively at 6 carbons (n-6) and 3 carbons (n-3) from the terminal methyl group. These two double bonds are impossible to insert in humans and animals. However, humans and animals can add additional double bonds to the two indispensable fatty acids (linoleic acid C18:2 n-6 and alpha-linolenic acid C18:3 n-3) at the terminal carboxyl group and lengthen the chain at this end. The group of derivatives obtained, added to the two precursor indispensable fatty acids, constitute the two families of essential fatty acids, required for the maintenance of a given biochemical, cell or physiological function. There is no metabolic conversion or functional substitution between the two omega-3 and omega-6 families. Finally, as regards the group of polyunsaturated fatty acids, a distinction is made for the sub-group of long-chain fatty acids which must be more than 18 carbon atoms long (LC- PUFA).

Linoleic acid (18:2 n-6): chain of 18 carbons, 2 double bonds, the first bond at 6 carbons from the terminal methyl group

Alpha linolenic acid (18:3 n-3): chain of 18 carbons, 3 double bonds, the first bond 3 carbons from the terminal methyl group

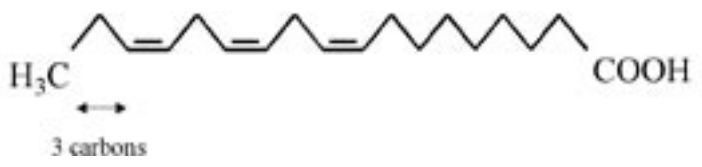


Figure 1: characteristic structure of essential fatty acids

II – Dietary sources of omega-3 and omega-6 fatty acids (Martin, 2001)

Linoleic and alpha-linolenic acid are found in notable quantities in vegetable oils: principally in sunflower seed oil and corn oil in the case of linoleic acid and in rapeseed oil and soya oil in the case of alpha-linolenic acid. In addition, terrestrial animal products provide varying amounts of alpha-linolenic acid.

As regards long chain polyunsaturated fatty acids in our common diet, LC-PUFA of the omega-6 family are provided by the consumption of some terrestrial animal products (meat, eggs) and breast milk. Fish and other marine animal products, breast milk and terrestrial animal products provide varying quantities of LC-PUFA of the omega-3 family.

III - Metabolism of PUFA.

Starting with the initial products, alpha-linolenic acid and linoleic acid, stages of desaturation and elongation succeed each other (Figure 2).

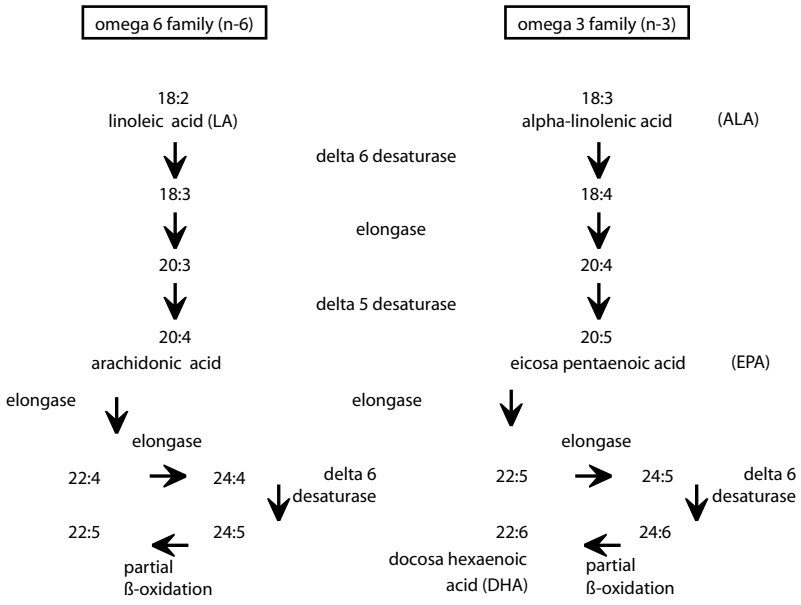


Figure 2: conversion of linoleic and alpha-linolenic acids into long chain PUFA

The metabolism of these two families of fatty acids follows two parallel pathways. Along these metabolic pathways, at least 3 enzymes are involved, delta 6 desaturase, elongase and delta 5 desaturase. During the metabolism of PUFA, compounds such as dihomo-γ-linolenic acid (20:3 n-6), arachidonic acid (20:4 n-6) or in the other family, EPA (20:5 n-3) are synthesised. They will act, respectively, as precursors for the synthesis of prostaglandins series 1, 2 and 3 and participate in the synthesis of thromboxanes and leucotrienes.

Studies *in vitro* and *in vivo* have shown that omega-3 and omega-6 fatty acids compete for the

same enzymes for metabolising PUFA. An excess of the precursor of the omega-6 family is therefore likely to compromise the generation of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from alpha-linolenic acid (ALA).

IV - The ANCs (Reference intakes for the French population)

ANCs for fatty acids have been set for healthy adults based on experimental, epidemiological and clinical data and were updated in 2001.

Table 1: ANCs for fatty acids in adults (Martin, 2001)

In MJ/day (kcal/day)		SFA	MUFA	18:2 n-6	18:3 n-3	LC-PUFA**	Of which DHA*	Total
Adult man	g/day	19.5	49	10	2	0.5	0.12	81
9.2 (2200)	% TDE	8	20	4.0	0.8	0.20	0.05	33
Adult woman	g/day	16	40	8	1.6	0.40	0.10	66.0
7.5 (1800)	% TDE	8	20	4.0	0.8	0.2	0.05	33
Pregnant woman	g/day	18	45.5	10	2.0	1	0.25	76.5
8.6 (2050)	% TDE	8	20	4.4	0.9	0.4	0.1	33.7
Breastfeeding woman	g/day	20	50	11	2.2	1	0.25	84.2
9.4 (2250)	% TDE	8	20	4.4	0.9	0.4	0.1	33.7
Older subjects	g/day	15	38	7.5	1.5	0.40	0.10	62.5
7.1 (1700)	% TDE	8	20	4.4	0.9	0.4	0.1	33.7

*DHA: docosahexaenoic acid (C 22:6 n-3)

** LC-PUFA: long chain fatty acids of the omega-6 and omega-3 family

NB: these values are based on the total daily energy intake (TDE) of the different populations listed in the table, on a total lipid intake of 33% of TDE and a ratio of 18:2 n-6 / 18:3 n-3 equal to 5.

The ANCs were set with a view to optimising intake, based on current scientific knowledge.

In terms of PUFA, in addition to the ANC set for linoleic acid (18:2 n-6) and alpha-linolenic acid (18:3 n-3), it is recommended that the 18:2 n-6 / 18:3 n-3 ratio should tend towards 5, to avoid excessive competition between omega-3 and omega-6 fatty acids[1]. This ratio only applies to the precursors of the fatty acids of the two families and not to the long chain derivatives, due to the uncertainty regarding recommended intake of EPA (20:5 n-3) when ALA intake is optimum. Moreover, an ANC could not be set for every LC-PUFA: the ANC for LC-PUFA concerns all the long chain polyunsaturated fatty acids of the omega-3 and omega-6 families.

Given the close relationship between them, in terms of metabolism and ANC, both the omega-3 and omega-6 families will be covered in the later sections of this document.

[1] *This recommended ratio of 5 applies to adults only.*

1. THE CURRENT SITUATION

1.1. THE CONSUMPTION OF OMEGA-3 AND OMEGA-6 FATTY ACIDS IN FRANCE

In order to come to a conclusion as to the justification for claims leading indirectly to the promotion of the fortification of the diet with omega-3 fatty acids, it was first necessary to improve our knowledge of current levels of consumption of omega-3 fatty acids in the French population and of the balance between omega-6 and omega-3 fatty acids.
It appears that in France, data on consumption levels of omega-3 fatty acids are extremely patchy, mainly due to the intrinsic limitations of food composition tables and the difficulty of assessing dietary lipid intake, especially when this involves small quantities strongly influenced by individual choice and wide inter- and intra-individual variation.

1.1.1. Estimate of linoleic and alpha-linolenic acid intake in the INCA survey and identification of missing data *(Annex 1)*

The Inca survey is an individual dietary survey based on a representative sample (quota method) of French households and carried out during 1998 and 1999. Food consumption was assessed by the 7-day record method (with the use of photographs to identify portion sizes of foods). The study covered 1985 adults (over 15 years old) and 1018 children (over 3 years old) and adolescents and was conducted in phases over 11 months to incorporate seasonal effects.

1.1.1.1 Project objective

As part of the group's work, the Observatoire des Consommations Alimentaires (OCA) (Afssa Food Intake Unit) at Afssa conducted a study to attempt to estimate the distribution of mean intake over one week of alpha-linolenic acid and linoleic acid in a sample of the French population. There is currently no estimate available of this consumption in the French population, as the breakdown of PUFA, when these are quantified, into alpha-linolenic and linoleic acid is incomplete or imprecise, when it exists. Moreover, fats added during the preparation of food, as well as seasoning, are frequently forgotten by the subjects when they record their food consumption.

1.1.1.2 Methodology

The consumption recorded over 7 days in the INCA survey and data supplied by Ciqual (Afssa Informatics Centre for Food Quality), were used by the OCA to attempt the estimation of alpha-linolenic and linoleic acid intakes broken down by age and sex. It should be emphasised that the LC-PUFA content (of fish in particular) is not included in the Ciqual data and was therefore not taken into account in this simulation exercise.

The ALA content of 77 foods and the LA content of 130 foods were provided by Ciqual and used in this study; additional data were obtained for other foods more recently. The OCA composition table comprises 1025 foods, 701 of which include a quantity of PUFA other than zero, with 300 having a PUFA content of more than 1 g/100g. The list of foods for which the content of one of the fatty acids (alpha-linolenic and linoleic) is known, is given in Annex 1 (Table I and Table J with additional data).

Several methodological limitations were observed.
- Incomplete composition data: for example, LA and ALA contents are available for a very few fish, mainly oily fish. Tables G and H show mean consumption of fish and crustacea according to whether their ALA content is known or not.
- Lack of knowledge on the influence of heat and technical processing: the major problem in assessing the PUFA content of fish lies in the availability of data for the raw food but not for the

cooked food. As the impact of storage methods and heat treatment on the PUFA content is not well known (Annex 3), it is very difficult to deduce its content in the cooked food.
- Imperfect designations: this mainly concerns fish and oils. When the type of fish was not listed in the consumption data, the procedure followed used the construction of a "virtual edible fish" based on the consumption levels for different fish. An identical procedure was used for oils. Then the dietary intake of PUFA was estimated from the total quantity of food consumed and taking account of each meal time.

Intakes of alpha-linolenic acid and linoleic acid and the LA/ALA ratio were broken down by sex and age group for the 3003 participants in the INCA survey. Following exclusion of under-reporters, these analyses were carried out on 2492 subjects.

1.1.1.3 Results

- Very low mean intakes of **alpha-linolenic acid** (Tables A and B, Annex 1) were observed, between 0.10 and 0.20 g/day, regardless of age and sex. These mean intakes are slightly higher in men and tend to increase with age in both sexes, going, in the male population, from 0.10 g/d in the youngest children to 0.20 g/d in 45-64 year olds. There is high inter-individual variability, with minimum values which can be 0 g/d and maximum values which can reach 1.5 g/d. However, values for the 95th percentile only rarely exceed 0.4 g/d in women and remain below 0.5 g/d in men.

- Mean intakes of **linoleic acid** (Tables C and D, Annex 1), for which the content is known in a greater number of foods, are markedly higher, generally comprised between 1 and 2 g/d. As with alpha-linolenic acid, mean intakes are slightly higher in men and tend to increase with age in both sexes, going from 1.04 g/d in the youngest boys to 2.18 g/d in men aged 45 to 64. Variability of these intakes between individuals is very high, with maximum values as much as 25 g/d. Values for the 95th percentile are generally comprised between 3 and 7 g/d.

- These mean intakes result in too high an **LA/ALA ratio**, on average greater than 10 and which can be even higher (Tables E and F Annex 1).

Intakes of linoleic and alpha-linolenic acid, expressed for men and women as a percentage of total energy intake and the 18:2 n-6 / 18:3 n-3 ratio are given in Table 2.

Table 2: intakes of linoleic and alpha-linolenic acids in France (data from INCA)

		Min	P5	Mean	P95	Max	ANC (2001)
18:2 n-6 (% of TDE)	M	0.02	0.11	**0.70**	1.93	8.80	4.0
	W	0.01	0.11	**0.75**	2.24	18.65	4.0
18:3 n-3 (% of TDE)	M	0.002	0.013	**0.068**	0.16	0.48	0.8
	W	0.001	0.014	**0.072**	0.17	0.47	0.8
Ratio 18:2 n-6 / 18:3 n-3	M	1.7	4.0	**11.5**	27.5	64.9	5
	W	1.0	3.9	**12.7**	31.6	65.0	5

- These observations have to be weighted by the low number of foods for which the alpha-linolenic acid content is known.
- The quality of the estimates supplied during this study requires improvement, notably through improved knowledge of content levels in the main vector foods for alpha-linolenic acid. Nonetheless, analysis of the CIQUAL tables shows that with the exception of some oils (rapeseed and corn), very of the few foods regularly consumed in the diet provide significant quantities of alpha-linolenic acid in nutritional terms which logically results in a low intake of this fatty acid in the general population.

Because of the under-estimation bias in the omega-3 intake and the high degree of extrapolation required to compensate for it, the INCA survey cannot provide a reliable basis to conclude on the benefits of a possible fortification of the diet with alpha-linolenic acid. The same applies to the assessment of intakes of 3 LC-PUFA.

1.1.2. Estimate of linoleic and alpha-linolenic acid intakes in the SU.VI.MAX study

(*Annex 2*)

1.1.2.1. Methodology

Among the 12,735 participants in the SU.VI.MAX study, 5008 volunteers (2119 men aged between 45 and 60 and 2889 women aged between 35 and 60) completed 10 one-day records over a period of 2.5 years between the end of 1994 and the end of 1998, which formed the basis of this project. The amounts of food consumed were estimated based on notebooks containing photographs of food portions. The content of the foods, in terms of the two fatty acids under consideration, was determined based on tables produced by CIQUAL, MAFF, the USDA, the CIV (for meat) and ITERG (for fats and oils) and completed from original works. The foods were selected from a pre-prepared list, source of one of the study's limitations: the type of vegetable oil used is generally not specified. Another limitation is that the types of margarine and therefore their composition, are also generally unknown.

1.1.2.2. Linoleic and alpha-linolenic acid intakes

The data collected concern the intakes of linoleic (18:2 n-6) and alpha-linolenic (18:3 n-3) acids and are shown in table 3. The distribution graphs for the intakes of these fatty acids are shown in Annex 2 (Figures I and II).

Table 3: intakes of linoleic and alpha-linolenic acid in France (provisional data from SU.VI.MAX)

		Min	P5	Mean	P95	Max	ANC (2001)
18:2 n-6 (% of TDE)	M	1.53	2.81	**4,26**	6.21	10.54	4.0
	W	1.62	2.91	**4.38**	6.31	11.63	4.0
18:3 n-3 (% of TDE)	M	0.21	0.30	**0.39**	0.52	1.52	0.8
	W	0.19	0.32	**0.41**	0.55	1.11	0.8
Ratio 18:2 n-6 / 18:3 n-3	M	5.5	7.5	**11.1**	16.1	33.8	5
	W	4.5	7.3	**10.8**	5,7	34.6	5

The mean 18:2 n-6 / 18:3 n-3 ratio is 11 for both sexes and greater than 5 for over 95% of the sample studied.

1.1.2.3. Dietary sources of linoleic and alpha-linolenic acids

Intakes of these two fatty acids are closely correlated to the total fat intake in the diet.
Unlike linoleic acid, the contribution made by vegetable fats (oils and margarines) to the intake of alpha-linolenic acid is slight: 6.7 % on average, which is less than fruit and vegetables (about 10%). However, the contribution of vegetable fats to alpha-linolenic acid intake increases when this intake rises.
The main foods contributing to alpha-linolenic acid intake (men and women combined) are animal products (dairy products, meat, poultry, meat products) which account for some 40% of intake.

The contribution of foods to linoleic and alpha-linolenic acid intake and the differences in contribution between the 1st and 5th quintiles of ALA intake are shown in Annex 2 (Figures III, IV and V).

1.1.2.4. Intake of long chain omega-3 PUFA

The SU.VI.MAX study has not provided information on EPA, DPA (docosapentaenoic acid) or DHA intakes at the current stage of data analysis.

The following points arise from the SU.VI.MAX. study:

- The linoleic acid needs of the population studied are largely covered. In contrast, with the methodological limitations mentioned above, almost none of the individuals in the population studied reached the ANC for alpha-linolenic acid and the ratio of the intakes of precursors is always greater than 5. In particular, 38% of individuals in the sample have an alpha-linolenic intake below 0.4% of TDE, with a precursor ratio greater than 10.

- Distribution of alpha-linolenic acid intake is less scattered than that of linoleic acid. However, given the limitations of the study, it is probable that the lowest intakes of this fatty acid are even lower.

- In the population studied, vegetable fats do not seem to be, in statistical terms, major contributors to alpha-linolenic acid intake, a large proportion of which comes from animal products.

- **Finally, as with the INCA study, the intake of long chain omega-3 fatty acids in the population of the SU.VI.MAX study is unknown.**

1.1.3. Estimate of alpha-linolenic acid intake in France based on other studies

According to the **Transfair study** (Hulshof et al., 1999), on the intake of *trans* fatty acids in Europe, mean alpha-linolenic acid intake was 0.6 ± 0.3 g/d and 0.5 ± 0.2 g/d in the 300 French men and 463 French women included in the study and the LA/ALA acid ratio was approximately 13.8 in both sexes. Mean intake of alpha-linolenic acid in France is relatively low compared with other European countries as in men, for example, it is 0.8 g/d in Italy while it reaches 1.4 ± 0.7 g/d in Sweden and 1.8 ± 0.9 g/d in Finland.

The **"Aquitaine study"** (Combe and Boué, 2001) provides additional information.
The population studied was 140 women living in Aquitaine (61 parturient women and 79 non-parturient women), aged from 18 to 50 and who were undergoing surgery (caesarean section or gynaecological surgery). One month before surgery the subjects completed a questionnaire (7-day record) and during the surgery, samples of abdominal sub-cutaneous adipose tissue and blood were taken.
This study confirmed that intake of alpha-linolenic acid (18:3 n-3) was insufficient in the population studied (on average 0.34 +/- 0.1% of TDE or 0.7 +/- 0.2 g/d and 42% of ANC). This study is therefore highly informative, as, on this point, observations are scarce or rely on very indirect protocols ("economic" studies of total consumption).
This work also consistently showed that alpha-linolenic acid intake is mainly of animal origin (75%) as the contribution made by vegetable oils is low (9%). In fact, consumption of rapeseed oil (rich in alpha-linolenic acid) is very low in France, particularly in the population studied.

1.1.4. Conclusions

Overall, it appears from these studies that:

- estimation of daily intakes of omega-3 fatty acids (alpha-linolenic acid and long chain omega-3 fatty acids) in the French population is imprecise, due to the limitations of the consumption studies caused by the lack of information in the tables and the difficulty of accurately recording nutrients consumed in small quantities and which vary greatly from one day to another.

- in spite of the imprecision and the methodological limitations of the different studies, the data converge and result in the view that alpha-linolenic acid intake is too low in France and is far from covering ANC, in particular as regards the linoleic acid / alpha-linolenic acid balance (Martin 2001).

- in the current situation terrestrial animal products certainly constitute a not inconsiderable source of alpha-linolenic acid for the French population and might constitute potential vector foods for fortification with this fatty acid.

- intakes of ω3 LC-PUFA are undoubtedly too low as mean consumption of marine products in the population is close to 35 g/day/person with a large confidence interval. (Tables G and H, Annex 1 and Volatier, 2000).

This insufficient intake of ω3 PUFA (alpha-linolenic acid and ω3 LC-PUFA) leads to support for an increase in intake through the promotion of foods which contain them naturally and possibly through fortification.

It would be essential for industrial companies fortifying their foods with omega-3 fatty acids to inform Ciqual of the exact composition of their product (based on effective analysis and not extrapolations based on calculations).

1.2. OMEGA-3 FATTY ACIDS AND THE CARDIOVASCULAR SYSTEM

A literature review, carried out when Apports nutritionnels conseillés pour la population française (Martin, 2001) was updated, provided an inventory of the scientific data on the biological and physiological effects of omega-3 fatty acids to which the reader can refer.
The working group decided to present a summary of the literature, covering only clinical studies **conducted in adults from 1990 to 2002.**

1.2.1. Compilation of observational and intervention studies

A summary table of the studies carried out in humans (meta-analyses, intervention or observation studies), published in journals listed in the PubMed database and concerning the evaluation of the impact of omega-3 PUFA on cardiovascular disease (mortality, relapse, risk markers) is shown in Annex 4.

1.2.1.1. Observational studies

Annex 4 lists 22 observational studies. Most of these studies were conducted using questionnaires on the dietary habits (mainly fish consumption) of the population samples studied. The others are based on the measurement of levels of omega-3 fatty acids in the plasma or tissue. Cardiovascular risk was estimated by the mortality or the incidence of ischaemic coronary complications. The observation studies were conducted on populations of up to 80,000 people and for maximum periods of 30 years.

Two-thirds of the studies based on eating habits show an inverse relationship between seafood

consumption and cardiovascular risk. This relationship becomes stronger with the length of the observation period. Links with reduced rates of cardiovascular mortality of up to 50% were observed (Angerer and Von Schaky, 2000; Leaf and Weber, 1988).

However, the methodology of this type of study does not permit either a causal relationship to be established or an assessment of the extent of the role of omega-3 fatty acids and other constituents of the foods, or indeed of the role of health and dietary habits associated with the consumption of these products.

1.2.1.2. Intervention studies

These studies provide decisive data in terms of causal relationships. Annex 4 lists 45 intervention studies. The majority relate to intermediate criteria (LDL cholesterol, blood pressure, bleeding time, etc.) and small sample sizes. The effects are of moderate intensity, sometimes erratic and often studied in populations suffering from cardiovascular or metabolic disorders.
In subjects given omega-3 PUFA supplements, some studies have shown:

- a reduction in blood pressure from 3 to 6 mm Hg in hypertensive subjects,
- a reduction in triglyceridaemia with large doses, most often in subjects who were themselves hypertriglyceridaemic,
- LDL-cholesterol levels, considered as a critical coronary risk factor, were not altered by these intakes.

However, four major controlled single blind studies which each included at least 300 people with a surveillance period of more than 1 year, showed a clear and significant reduction in cardiovascular mortality in situations of secondary prevention. Cardiovascular morbidity and mortality were reduced with the administration of products fortified with alpha-linolenic acid in the Lyon study (Lyon Diet Heart Study, a controlled intervention study comprising, among others, a fortified margarine administered double blind) and in the Indo-Mediterranean study (Singh et al., 2002). A reduction in the lethality of infarctions (DART study [Diet and Reinfarction Trial] and GISSI study[2], 1999) was observed, with no reduction in the incidence of non-fatal infarctions during these latter two studies which used fish or long chain omega-3 fatty acids.
In addition, two meta-analyses confirmed the reduction in cardiovascular mortality. The first, carried out using the Cochrane criteria (eleven intervention studies involving long chain omega-3 PUFA, Annex 5) showed, in secondary prevention only, a reduction in cardiovascular risk following supplementation with omega-3 PUFA: the effect on the cardiovascular mortality criterion is significant due to a reduction in the risk of sudden death, but no significant reduction was observed in the incidence of infarctions (Yzebe, 2000). The recent meta-analysis by Bucher (2002), which takes into account both the trials conducted with alpha-linolenic acid and those conducted with omega-3 LC-PUFA, found a 30% reduction in sudden deaths and a 20% reduction in total mortality.

The mechanism(s) of the cardiovascular benefit observed during the Lyon Diet Heart Study, attributed at least in part to fortification of the diet with alpha-linolenic acid, is/are controversial.
This study has been partially reinforced by the Singh et al intervention study which found, with similar intakes of alpha-linolenic acid, a 67% reduction in the risk of sudden death and a 53% reduction in non-fatal infarctions. However, interpretation of this study is complicated by the fact that the dietary measures led to severe modifications in the intake of a number of nutrients, as is shown by the reduction in the daily intake of cholesterol from 210 to 125 mg/d.
A workshop organised by the UK Food Standards Agency (FSA) in March 2002 brought together several experts to review current research into whether alpha-linolenic acid presented an equivalent benefit, in terms of cardiovascular risk, to EPA and DHA. It appears from the studies presented at that workshop that any benefit which could be attributed to alpha-linolenic acid would be limited, even in terms of secondary prevention. According to Sanderson's report, prepared before the Singh study was published, the results obtained with fish oil supplementation were not repeated when fortification with alpha-linolenic acid was employed (Sanderson et al., 2002).

[2] *This study demonstrated a 15 % reduction in mortality in patients who had already suffered an infarction and had consumed approximately 800 mg EPA and DHA for 3 to 5 years.*

Surprisingly, it appears that the GISSI study, which included the largest number of subjects (11,324 individuals), presented a methodology which was not entirely rigorous, because it was not conducted in double blind, although the administration of omega-3 supplement in capsule form did permit it (Yzebe, 2000).

Very few studies have been conducted in primary prevention situations to establish the benefits for the general population of the fortification of food with omega-3 fatty acids. A recent Dutch study (Bemelmans et al., 2002) compared, over two years, the effects of the administration of margarines rich in alpha-linolenic acid or rich in linoleic acid in terms of the estimated IHD risk (administration of these margarines being accompanied by nutritional education in some but not all cases). After only two years of follow-up, the number of cardiovascular events was found to be lower (2 *vs.* 9) in the case of the margarine rich in alpha-linolenic acid in comparison with the margarine rich in linoleic acid. This is only a trend, in view of the limited statistical power of the study, but it is consistent with the results of the *Lyon Diet Heart Study*. The Singh study, which comprised a large proportion of subjects with coronary heart disease who had not yet presented an infarction, did not include a sub-group analysis enabling a reduction in risk to be shown. In view of its limited power, this analysis is not likely to be very informative in statistical terms.

1.2.2. Effects observed with low doses of omega-3 fatty acids

Most of the intervention studies were conducted with high doses of long chain omega-3 fatty acids, reaching from 2 to 10 times the ANC in the case of DHA. The supplementary doses of alpha-linolenic acid employed in the *Lyon Diet Heart Study* and the Singh study were lower, of the order of 1.8 g/d and corresponding to dietary intakes (ANC 2 g/d for alpha-linolenic acid for adult men). It appears that doses nearer the recommended intakes could have biological effects measured *ex-vivo* in controlled studies in humans.

In subjects over 68 years old, compared with young subjects (21-43 years), platelet hyperactivity was observed (Véricel et al., 1992), associated with an increase in levels of certain oxygenated metabolites derived from arachidonic acid (HHT and 12-HETE), an increase in levels of malondialdehyde (MDA), a marker for lipid peroxidation, and a reduction in levels of vitamin E and in glutathion peroxidase activity

Administration of 150 mg DHA and 30 mg EPA (in triglyceride form) in elderly subjects for 42 days produced a reduction in platelet peroxidation (Véricel et al., 1999)[3].

Conversely, these fatty acids provided in large quantities (of the order of several grams) expose subjects to lipid peroxidation phenomena, especially when the subjects have reduced antioxidant capacities (elderly persons, for example) (Brown, 1990).

Therefore, according to these data, omega-3 fatty acids, in particular EPA and DHA, may be the object of lipid peroxidation phenomena and interact with the cascade of reactions which result in the production of oxygenated derivatives of arachidonic acid. In high concentrations, EPA and DHA are in fact pro-oxidant in the platelets, while they are antioxidant in low concentrations (Véricel, 1999). This antioxidant activity is associated with a reduction in platelet aggregability, at least in elderly people. In low concentrations, DHA is preferentially esterified in the plasmalogen molecules which have antioxidant properties.
These data suggest that pharmacological intakes are not necessary and that moderate doses as observed in the common diet might have beneficial effects, as illustrated by the favourable results of the DART study and in the two intervention studies which employed doses close to the ANC for alpha-linolenic acid (De Lorgeril, 1994; Singh, 2002). These studies also suggest that,even limited, intakes from additional dietary sources might have an impact.

[3] *The effects of an intake of 100 mg EPA in triglyceride form on platelet hyperactivity in elderly individuals has also been studied (Croset et al., 1990).*

1.3. REGULATIONS APPLICABLE TO CLAIMS

It must be remembered that product composition must conform to current legislation and that any claim relating to omega-3 fatty acids which appears on the label or the advertising for a foodstuff must be scientifically substantiated. No claim may ever state properties of prevention, treatment or cure for human diseases, or mention such properties.

1.3.1. Conformity of product composition: regulations applicable to methods of fortifying foods with omega-3 fatty acids

There are different methods recorded for the fortification of foodstuffs with omega-3 fatty acids. These fortification strategies are subject to different regulations. Whatever the circumstances, the manufacturer is under an obligation to ensure that the product placed on the market complies with current regulations (Article L 212.1 of the Consumer Code). The DGCCRF is responsible for verifying compliance with these provisions.

- the fortification may be direct:
  - through the use, in the formulation of the foods, of ingredients intrinsically rich in omega-3 fatty acids and permitted in food for human consumption (for example, tuna oil);
  - *via* fatty acids permitted in food for human consumption based on the amended Decree of 15 April 1912[4];
  - *via* fatty acids which may fall within the scope of EC Regulation 258/97 concerning novel foods and novel food ingredients insofar as these substances do not have a previous history of consumption in human food within the European Union.

- the fortification may be indirect via animal feed: for example, feeding linseed which is naturally rich in alpha-linolenic acid, resulting in an increase in the 3 PUFA content of the animal products. The ingredients added to the animal feed must be permitted for use in such feed.

It should be noted that the fortification with omega-3 fatty acids of foods that are also subject to specific regulations, may have consequences as regards the legal name of the product. For example, when milk is fortified directly with an ingredient rich in omega-3 fatty acids, the legal name is "milk drink" and not "milk" (B.I.D. N° 6, 2000). Conversely, if the fortification takes place at an earlier point (genetic modification of the animal, cows fed a diet fortified with specific nutrients) the legal name does not change.

1.3.2. Justification of claims regarding the presence of omega-3 fatty acids

1.3.2.1. Fair and non-misleading information to the consumer

Any claim on the label or advertising of a foodstuff mentioning the presence of or the role of these fatty acids in normal functions of the body or their health benefits must comply with the provisions on non-misleading advertising (Article L 121.1) and the provisions relating to deception (Article L 213.1 and 213.2) laid down in the Consumer Code. In other words, all claims must be scientifically substantiated and must not be liable to mislead the consumer
The manufacturer must therefore be in a position to scientifically substantiate the claims related to its

[4] *Decree of 15 April 1912 laying down government regulations implementing the Law of 1 August 1905 on the prevention of fraud in the sale of merchandise and the adulteration of foodstuffs.*

products. At the present time there are no specific regulations on claims. However, a draft regulation on nutrition, functional and health claims is in preparation at Community level (Working document SANCO /1832/2002). pWhile this regulation is pending, statutory definitions of functional claims and health claims remain unavailable.

• Quantitative nutrition claims

These claims are defined by Decree No. 93-1130[5] of 27 September 1993 concerning nutritional qualities of foodstuffs. A nutrition claim is defined as any description or advertisement which states, suggests or implies that a foodstuff has particular properties due to the energy it provides or the nutrients it contains or does not contain or which it provides in reduced or increased amounts: for examples "source of", "rich in" "free of", "reduced", etc.

When quantitative nutrition claims are used, the manufacturer is under an obligation to display a nutritional labelling, as provided for by the above-mentioned Decree No. 93-1130 concerning nutritional qualities of foodstuffs, and its implementing order of 3 December 1993[6], and the content of omega-3 fatty acids.

In addition, in anticipation of the publication of the EC regulation on claims which will set conditions of use, notably for these claims, the Opinion of 8 July 1998 (BOCCRF of 31 August 1999) from the Commission d'études des denrées destinées à une alimentation particulière (CEDAP), has set the threshold levels for establishing quantitative nutrition claims (such as "source", "rich", "free of", "reduced", etc.) for certain nutrients and the calorific value. However, neither CEDAP's recommendations nor the draft proposal on claims include omega-3 fatty acids.

As regards the claims "natural source of" or "naturally rich in", the DGCCRF has agreed that these claims may be used when the raw material contains, without any technical modification, the claimed content of omega-3 fatty acids. In terms of foods of animal origin, it is accepted that these claims may be used when the source of omega-3 fatty acids incorporated in the animal feed is acknowledged as traditionally used in feed for the animal species concerned.

• Functional claims

The draft EC regulation on nutrition, functional and health claims, uses the following definition for these claims in its initial version (Working Document SANCO/1832/2002): "Functional claim" means any claim that states, suggests or implies the role of a food category, a food or one of its constituents in growth, development or normal physiological functions of the body."
The CEDAP opinion of 18 December 1996 (BOCCRF of 7 October 1997) lays down criteria for using functional claims solely concerning certain vitamins and minerals. In this opinion CEDAP estimated that several verbs such as "participates", "contributes", "involved in" "permits", "plays a role in", etc. were equivalent and acceptable in the wording of a functional claim. This opinion does not set out word for word claims but scientific concepts acknowledged as not misleading.

• Health claims

The definition proposed by the above-mentioned draft EC regulation on claims, in its current version, is as follows:

- **"Health claim** means any claim that states, suggests or implies that a relationship exists between a food category, a food or one of its constituents and health.
- **Enhanced function claim** means any health claim that states, suggests or implies that the consumption of a food category, a food or one of its constituents has a specific beneficial effect, beyond that normally obtained from the diet, on physiological functions of the body.

[5] *Decree No. 93-1130 of 27 September 1993 concerning labelling relating to the nutritional qualities of foodstuffs.*
[6] *Order of 3 December 1993 implementing Decree No. 93-1130 of 27 September 1993 concerning labelling relating to the nutritional qualities of foodstuffs.*

- **Reduction of disease risk factor claim** means any health claim that states, suggests or implies that the consumption of a food category, a food or one of its constituents significantly reduces a major risk factor in the development of a human disease."

The concept of "suggesting" properties beneficial to health is subject to interpretation: for example, what does the use of a red heart on the labels of products fortified with omega-3 fatty acids suggest to consumers?

• Therapeutic claims

Therapeutic claims mean any claim on the label or in the advertising of a foodstuff which states preventive, treatment or curative properties for disease or which mentions such properties. These claims are prohibited (Article R 112.7 of the Consumer Code).

Therefore, the questions posed by the DGCCRF in its referral to Afssa relate to the regulations mentioned above, with opinions required on the following points:

- **What contents of omega-3 fatty acids justify quantitative, functional and health claims?**
- **Does modification of the balance between omega-3 fatty acids and the other polyunsaturated fatty acids have any consequences for health? At what content do these fatty acids constitute a danger to health (safety limit)?**
- **Is there a validated scientific argument enabling demonstration of claims like those stating a role for omega-3 fatty acids in "healthy cardiovascular function" or "reducing cholesterol levels", or claiming their "beneficial effect on blood fluidity"?**
- **Is the selection of the foodstuff carrying the claim always appropriate?**
- **Secondarily, does the depiction of a red heart on the label of fortified products or in their advertising constitute a source of confusion for the consumer?**

1.3.2.2. The "visa PP"

Article L. 5122-14 of the Public Health Code imposes advertising controls (an a priori check on advertising aimed at the general public before an advertising certificate known as the "visa PP" is issued and an a posteriori check when the advertising is aimed at health professionals, which must be submitted for approval) on:
- claims presenting a product as promoting the diagnosis, prevention or treatment of diseases;
- claims presenting a product as promoting changes in the physical or physiological condition;
- claims presenting a product as promoting the modification, correction or restoration of a function of the body.

Under the terms of Article L. 5122-14 of the Public Health Code, the product is presented as having a participatory role in the process ("promotes", "helps to", contributes to"), which distinguishes the claim in Article L. 5122-14 from the therapeutic claim in Article L. 5111-1 (disease prevention or treatment).

A "visa PP" is granted for a determined advertising medium (packaging, TV film, press advertisement, etc.) for a period of three years for packaging deemed to constitute advertising (primary, secondary packaging, instructions for use) and for two years for all other media.

This procedure is the responsibility of the Agence française de sécurité sanitaire des produits de santé (Afssaps).

1.3.3. Positions of other national and international bodies concerning the claims relating to omega-3 fatty acids

1.3.3.1. Positions of other countries

The following information has been gathered.

As regards the European countries, **Norway** has no specific regulations concerning nutrition, functional or health claims for omega-3 fatty acids. Declaration on the labelling of the content of omega-3 fatty acids is possible. The quantity and type of fatty acid may be declared if the total fat content, the saturated, monounsaturated and polyunsaturated fat contents and the cholesterol content are also stated. The National Council on Nutrition and Physical Activity in Norway has also made recommendations on nutrient intakes for the general population: in particular, omega-6 and omega-3 PUFA should form at least 3% of total energy intake and 0.5% of the energy should come from omega-3 fatty acids. When omega-3 fatty acids are consumed in the form of tablets, capsules or through the fortification of a food providing an intake of more than 3g, the product is deemed to be a medicine.

Similarly, in the **United Kingdom,** there are no recommendations on nutrition, functional or health claims for omega-3 fatty acids. However, the Committee on Medical Aspects of Food Policy recommends the weekly consumption of 1.5g of omega-3 fatty acids from oily fish.

In 1993 in the **United States,** the Food and Drug Administration (FDA) had not authorised the use of the claim alleging an effect from omega-3 fatty acids on a reduction of the risk of coronary heart disease, either for "conventional" foods or for dietary supplements. However, the same agency approved the following claim in 2000, for dietary supplements only: "*the scientific evidence about whether omega-3 fatty acids may reduce the risk of coronary heart disease (CHD) is suggestive, but not conclusive. Studies in the general population have looked at diets containing fish and it is not known whether diets or omega-3 fatty acids in fish may have a possible effect on a reduced risk of CHD. It is not known what effect omega-3 fatty acids may or may not have on risk of CHD in the general population*". The claim was defined as follows: "*consumption of omega-3 fatty acids may reduce the risk of coronary heart disease. FDA evaluated the data and determined that, although there is scientific evidence supporting the claim, the evidence is not conclusive*". This claim may be used for dietary supplements containing EPA and DHA, provided that these supplements do not recommend on their labels and do not provide, under normal consumption conditions, daily intakes greater than 2g EPA and DHA per day. In fact, the FDA even recommends that manufacturers limit the recommended quantities to provide 1 gram or less of EPA and DHA per day, to ensure an additional safety margin and due to the possible benefits of intakes below 1 g/d.

1.3.3.2. Work of the Codex Alimentarius Commission[7]

A draft directive on the use of health and nutrition claims is in preparation. However, this draft does not specifically deal with the conditions for using nutrition, functional or health claims concerning omega-3 fatty acids.

As a general rule, neither the countries consulted by the working group nor the Codex Alimentarius have in place scientific recommendations or technical regulations concerning the conditions for using nutrition, functional or health claims for foods fortified with 3 PUFA for the general population. However, the United States has approved a claim relating to the role of omega-3 fatty acids in cardiovascular health, for dietary supplements only.

[7] *Combined committee of the United Nations Food and Agriculture Organisation (FAO) and the World Health Organisation (WHO)*

1.4. OPINIONS ISSUED BY THE ASSESSMENT BODIES

1.4.1. Review of the "visas PP" granted by Afssaps

To date, only one product (a margarine) claiming the benefits of omega-3 fatty acids for the cardiovascular system has obtained a "visa PP". This was in July 2000, for a booklet aimed at consumers. The margarine contains omega-3 fatty acids of both plant and marine origin.
The following claims for the effects of omega-3 fatty acids were made for this product:

- protection for the heart, arteries and cardiovascular system: "to protect your heart and arteries", "omega-3: a plus for the heart", "omega-3: a potential protective role for the cardiovascular system";
- contribution to reducing cardiovascular risk and a favourable effect on clotting: "a number of studies have shown that a sufficiently high intake of either of these components [EPA or alpha-linolenic acid] contributes to a reduction in the risk of cardiovascular disease and has a favourable effect on clotting".

The applicant stated that the claims were made in the context of diets suggested in case of excess cholesterol ("included in recommendations for cholesterol-lowering diets").

Previously, in 1991, a "visa PP" was granted for a TV advertisement for this margarine fortified with omega-3 fatty acids by the Ministry of Health, which was responsible for awarding the "visa PP" at the time[8]. In this case, the claim "included in recommendations for cholesterol-lowering diets" was approved.

These two certificates have now expired, as they were granted for a period of two years.

1.4.2. Review of opinions issued by Afssa

(Annex 6)

No dossier relating to claims on the subject "omega-3 fatty acids and health" has been examined by the CSHPF or CEDAP.
To date, opinions concerning commonly-eaten foods[9] have been issued by Afssa in response to two referrals.

- **opinion dated 11 July 2000, 17 January 2001 and 15 May 2002, concerning assessment of the nutritional role of a regular milk fortified with omega-3 fatty acids from fish oil**

The product was fortified with fish oil containing long chain omega-3 PUFAs (18% of the fatty acids added in were in the form of DHA and 12% in the form of EPA; 60 mg omega-3 fatty acids in 100 ml[10]) and claimed that "omega-3 contribute to healthy cardiovascular function". This claim was deemed acceptable in scientific terms (Opinion of 17 January 2001, Annex 6) on the basis of the documented effects of omega-3 fatty acids.

[8] *Afssaps has in fact only been responsible for granting PP certificates since 1999 (Law of 1 July 1998 and implementing decrees of March 1999).*
[9] *The CES "Nutrition Humaine" refers to commonly-eaten foods as all food that can be made available to the consumer, in all markets and for all members of the population regardless of:*
- age (including children of 2 years and above, adults and older people);
- physiological state (including, for example, pregnant women or highly physically active people);
- nutritional typology, including regional.
[10] *One 300 ml portion therefore provided 180 mg omega-3 fatty acids, which is a value close to the RNI fixed for DHA.*

- **opinion dated 28 May 2001 concerning the assessment of the claims for a special seasoning oil with a guaranteed content of vitamin E and rich in omega-3 fatty acids**

The product was a mixture of several food oils (rapeseed, walnut, grapeseed, olive, wheatgerm and fish). This product contained 7.5g omega-3 fatty acids in 100g oil[11]: 7.3g alpha-linolenic acid, 0.07g EPA, 0.13g DHA. The LA/ALA ratio in the product was 4.4.
Two claims were made:

- "omega-3 fatty acids contribute to healthy cardiovascular function"
- "omega-3 fatty acids are included in recommendations for cholesterol-lowering diets"

Only the first claim was deemed acceptable in scientific terms, the second claim was considered misleading as it implies that omega-3 fatty acids are cholesterol-lowering agents, an effect with no scientific basis.

Several claims have therefore been approved through the granting of "visa PP", but these have now expired.
At the present time, only the claim concerning the effect of omega-3 fatty acids on healthy cardiovascular function has been considered by Afssa as scientifically substantiated, in the case of a regular milk fortified with fish oil and a mixture of oils.

2. CONCERNING FORTIFICATION

2.1. METHODS OF FORTIFYING FOODS WITH OMEGA-3 POLYUNSATURATED FATTY ACIDS

Increasing the consumption of omega-3 PUFA can be envisaged using several different methods (see 1.3.1.).

2.1.1. Increasing intake of alpha-linolenic acid

Increasing dietary intake of alpha-linolenic acid can be achieved through increased consumption of oils naturally rich in these fatty acids, such as rapeseed oil and soy oil or specially manufactured oils (blend of oils) and even fortified margarine. Fortified oils and margarines also provide significant amounts of vitamin E (from 20 to 70 mg per 100g oil or margarine).
At the present time, terrestrial animal products constitute a proportionately large source of alpha-linolenic acid. Increasing or stabilising levels of this fatty acid in animal tissue can be obtained by changing the diet fed on farms (linseed in the animal feed) to obtain commonly-eaten foods fortified with omega-3 polyunsaturated fatty acids.

2.1.2. Increasing intake of long chain omega-3 PUFA (principally EPA and DHA)

2.1.2.1. Increasing the consumption of products naturally rich in long chain omega-3 PUFA

This involves promoting increased consumption of fish and derived products compared with average current consumption, in so far as resources of these products permit and with the hope that their contaminant levels do not negate the expected benefit[12].

[11] *Consumption of a portion of 20 g/d of oil provided 1.6 g omega-3 fatty acids, a value close to the RNI fixed for alpha-linolenic acid.*

[12] *Afssa Opinion dated 21 October 2002 concerning the assessment of the health risks from mercury exposure for pregnant and lactating women and young children;*
and Guallar 2002.

A list of vector foods for fats of animal or mixed origin has been prepared from the INCA survey (contributions from the different foods based on contents and consumption levels) (OCA technical note, 2000). While the PNNS recommends twice weekly consumption of fish, fried fish cakes appears in 45th position and contribute 0.4 g/day of fat, steamed salmon is at 76th position and contributes an average of 0.23 g/day of fat and other fish (not specified) appear in 90th position with 0.17 g/day of fat. These current intakes are therefore insufficient in the general population to meet the requirements for omega-3 LC-PUFA.

2.1.2.2. Using farm animals to fortify commonly-eaten foods

Eggs are one example as the fatty acid content of phospholipids is greatly influenced by the diet of the laying hen. Eggs fortified with omega-3 polyunsaturated fatty acids have been produced. In the INCA survey and in adults, eggs are in 12th, 24th and 60th position (plain omelette, fried eggs, hard-boiled eggs). They contribute 2.2 g/d to average fat intake. Phospholipids from eggs fortified with long chain omega-3 PUFA can be included in manufactured biscuits and pastries. They can also be added to dairy desserts. Their benefits have, however, to be viewed in the light of the high cholesterol content of egg yolk.

The second example is that of cow's milk or meat from animals (pigs, sheep, cattle) whose diet has been enriched with linseed. This process enriches the intramuscular fats of these animals not only with alpha-linolenic acid (2.1.1) but also with long chain polyunsaturated fatty acids from the endogenous metabolism of the alpha-linolenic acid. However, the potential benefits from fortification can be counteracted by the occurrence of peroxidation of the long chain polyunsaturated fatty acids which reduce the storage options for the meat and may possibly alter their organoleptic properties.

2.1.2.3. Fortifying manufactured products with long chain polyunsaturated fatty acids

This involves adding omega-3 PUFA of marine origin during manufacture. The addition is either in the form of a liquid (oil) or in the form of micro-capsules. Powdered milk, salad oils and fruit and vegetable juice have been fortified in this way with long chain omega-3 PUFA (Kolanowski et al., 1999). However, the addition of these products of marine origin in excessive concentrations has unpleasant effects on the flavour and odour of the fortified products. Moreover, enrichment of aqueous products with an acid pH promotes oxidation of the fatty acids which raises the issue of the sensitivity to peroxidation of omega-3 LC-PUFA.

2.2. ASSESSING THE PEROXIDATION RISK IN OMEGA-3 FATTY ACIDS

This aspect is of primordial importance in the development of fortified products, as these must have a stable composition and their consumption must be safe and potentially beneficial. All products enriched with polyunsaturated fatty acids and containing water and/or peroxidant agents are liable to be peroxidised through the action of a catalyser or when energy is applied (heating for example). They require consideration of the potential hazards to the consumer from the presence of peroxidation products in the foods.

2.2.1. The oxidation process in fatty acids and the principal decompositionproducts

Omega-3 fatty acids are theoretically more sensitive to oxidation than omega-6 fatty acids, as they have one or two additional double bonds in their molecule. For this reason, they are more subject to auto-oxidation and photo-oxidation which result, through some complex dynamic processes, in a whole series of intermediate and final products.

Table 4: major compounds originating from the oxidation of linoleic and linolenic acids (Aruoma et al., 1997)

	Auto-oxidation	Photo-oxidation
Linoleate	Hexanal; pentane; 2,4-decadienal	2-Heptenal; hexanal
Linolenate	2,4-Heptadienal; ethane	Propanal; 2-butenal

In the initial phases of the oxidation process, peroxides develop, conjugated dienes form and oxygen absorption is observed. Following accumulation of these initial products, their decomposition is observed, with, among other results, the appearance of carbonyl groups and lipid peroxidation products, which may be volatile (e.g. aldehydes, ketones, alcohols) or reactive (e.g. epoxide derivatives). These phenomena are influenced by the metal content (Fe, Cu), the fatty acid concentration and the type of matrix, which can influence the accessibility of the fatty acid molecules to the pro- and antioxidant agents.

Due to this dynamic aspect and the multiplicity of intermediate and final products (more than one hundred can be enumerated in a profoundly oxidised fish oil), only a very rough assessment can easily be achieved of the state of oxidation of an oil containing polyunsaturated fatty acids, in particular omega-3 fatty acids.

2.2.2. Methods of assessing oxidation levels

Generally, methods for assessing oxidation are based on the measurement of either the primary or the secondary oxidation products. The primary oxidation products are the precursors of the secondary products, some of which are the source of characteristic odours, particularly fish odours. It is therefore the peroxidation products and not the polyunsaturated fatty acids themselves which produce unpleasant odours in foods.

Table 5: principal types of primary and secondary oxidation products and applicable methods of analysis (Aruoma et al. 1997)[13]

	Products/process	Analyses
Primary oxidation	Hydroperoxides Oxygen absorption Double bond migration	Peroxide index Conjugated dienes
Secondary oxidation	Carbonyl group compounds Hydrocarbons, aldehydes, ketones	TBA index GC of volatiles Sensory tests

[13] *TBA index (or TBARS): this index is defined as the increase in absorbance measured at 530 nm following the reaction of an equivalent of 1 mg of sample in 1 ml volume with 2-thiobarbituric acid (AOCS official method Cd 19-90, Reapproved 1997). The value of the TBA index is a measure of the secondary oxidation products of oils and fats.*
Peroxide index: number of micromoles of active oxygen contained in a gram of fat likely to oxidise potassium iodide with release of iodine. This criterion enables the evaluation of the first stages in oxidative deterioration (in Karleskind, 1992).

The initial oxidation products such as hydroperoxides are considered precursors for odour molecules. They can be estimated by measurement of the peroxide index or the level of conjugated dienes.
The secondary oxidation products can be measured by analysis of the carbonyl group compounds or the volatile products using gas chromatography. Their effect can also be assessed using sensory tests carried out by groups of experts. Appropriate analytical methods can be used to identify what type of molecules have been detected by these groups of experts.
It has been observed that there is no close correlation between fish odours and the traditional parameters for measuring oxidation, namely measurement of the peroxide index and the anisidine index[14]. There is also no correlation with the values given by electronic noses.
Gas chromatography techniques coupled with mass spectrometry can enable the detection of three molecules which are present in very low concentrations (of the order of one part per billion), markers for the state of peroxidation of long chain omega-3 fatty acids. These are 4-heptanal, 2,6 nonadienal and 3,6 nonadienal (Macfarlane and Muggli, ISSFAL, 2000).

2.2.3. The other peroxidation products of omega-3 fatty acids

As a general rule, study of the peroxidation products of oils containing omega-3 fatty acids focuses almost exclusively on the degradation products of these fatty acids. Very little attention has been paid to analysis of the glycerides containing residues of oxidised fatty acids. It might be assumed that oil purification techniques enable these products to be eliminated. The fact remains that no information is available concerning these molecules which may also appear in products to which oils rich in omega-3 fatty acids have been added.

The situation is completely different as regards glycerophospholipids containing omega-3 fatty acids. Following an oxidant action, glycerophosphatidylcholines can generate molecules related to PAF-acether (platelet-activating factor), a mediator of platelet aggregation and certain inflammatory reactions (Tokumura et al. 2000).
These oxidised glycerophospholipids could be an active principle at the origin of the pro-atherogenic power of oxidised LDL. However, their exact bioavailability is hard to evaluate. The phospholipases may be most active on the oxidised glycerophospholipids which would then be converted into lyso-glycerophospholipids (McLean et al., 1993). This type of preferential action by the digestive phospholipases could protect the body against a PAF-acether effect of foods containing glycerophospholipids rich in omega-3 fatty acids.

2.2.4. Unknown elements in the risk assessment of the peroxidation products of omega-3 fatty acids

It must be emphasised that, as part of the substantiation for fortification, the applicant is already required to provide test results on the level of the nutrient of interest in the food and on its variability during recommended storage times and conditions of use.

- Assessment of the toxicological risk from the peroxidation products of polyunsaturated fatty acids in humans is problematic, due to the lack of data on the following points:the conditions for producing peroxidation products in the food: cooking, storage methods (cf. Annex 3 on studies specifically devoted to fish), etc.;
- the stability of the oxidation products in the food (influence of storage, etc.);
- the possible arrival of these products intact in the intestine following ingestion;
- the influence of other foods ingested at the same time and/or other molecules of the food matrix such as antioxidants, enzymes, etc.;
- the possible absorption of the peroxidation products through the intestinal barrier;
- the question of whether these products reach certain zones or organs where they might induce harmful effects;
- and consequently, the attribution of a real toxic effect in humans to the various peroxidation products identified at the concentrations expected in the foods.

[14] *Anisidine index: the anisidine index is defined as being 100 times the absorbency measured at 350 nm under a thickness of 1 cm of a solution containing 1g triglycerides of omega-3 fatty acids in 100 ml of a mixture of solvents and reagents (using a specific method) (in* Pharmeuropa *vol 12., n°3, July 2000). It enables evaluation of the state of rancidity of the fat or oil.*

2.2.4.1. In qualitative terms

Not all the molecules which might be produced by oxidation of omega-3 fatty acids have been identified. Their potential toxicity is therefore unknown and a matter of debate.

2.2.4.2. In quantitative terms

Major peroxidation products of omega-3 fatty acids have been identified (Table 4), but precise data on their toxicity thresholds are not available.

However, while omega-3 fatty acids contain up to 50% more double bonds than omega-6 fatty acids, in a food in which the omega-6/omega-3 ratio is 5, the very high proportion of omega-6 fatty acids statistically provides three times more double bonds and therefore potential oxidation sites than the omega-3 fatty acids. The assessment of the risk fromconsumption of oxidised omega-3 fatty acids is therefore at present more qualitative than quantitative and so falls within the general framework of polyunsaturated fatty acids protection against peroxidation. In particular, EPA and DHA peroxidation products (nonatrienal and decatrienal) are characteristic of the odours of oxidised fish oil.
Although the standard analyses of lipid oxidation levels sometimes provide results which are not really representative of the product quality, they constitute one element of quantitative assessment which must nevertheless not be overlooked.

2.2.5. Recommendations on the measurement of peroxidation levels

The aim was to identify, if possible, which analytical methods should be required and which threshold levels should be set to limit the health risks from ingestion of oxidised omega-3 PUFA.

In the first instance, the same control methods should be required which are traditionally used to evaluate oxidation levels of fats in the fats and oils industry.
As indicated in table 5, these methods should use the measurement of the primary and secondary oxidation products: measurement of the peroxide index for example (AOCS official method Cd 8-53, re-approved 1997 and Cd 86-90, re-approved 1997, revised 2000; IUPAC 2.501) and the anisidine index (AOCS official method Cd 18-90, re-approved 1993; IUPAC 2.504, 7th edition).
The development of more sensitive and more specific methods of analysis such as those quantifying 4-heptanal, 4,6 nonadienal and 3,6 nonadienal, should provide more reliable elements for evaluating the oxidation level of omega-3 fatty acids in products containing them.

The addition of omega-3 fatty acids to a preparation containing other fatty acids is problematic: as the indices of oxidation are related to total fat content, the addition of a small quantity of omega-3 fatty acids, possibly in a highly oxidised form, will not greatly modify the value of the indices, even though the potential benefit may be non-existent.
Therefore the tests carried out on the finished product must be related to the quantity of omega-3 fatty acids added, so as to evaluate the possible pro-oxidant effect arising specifically from the addition.

Omega-3 fatty acids are particularly susceptible to peroxidation (numerous unsaturations) and there are no nutritional benefits (even potential harmfulness) from their intake in a peroxidised form. Therefore, the working group also considered that evidence must be provided that almost all the initial and added omega-3 fatty acids (so at least 90%) are found in the finished ready-to-eat product and at the end of its shelf life.

In addition, the working group emphasised the need to:

- **conduct studies to develop new sensitive and specific peroxidation markers for quantification of the peroxidation level of omega-3 fatty acids, in order to set threshold values for product approval;**
- **determine whether the peroxidative risk with similar contents of omega-3 fatty acids in the finished products depends on the food fortification method (direct or indirect), in order to improve assessment of the matrix effect.**

2.3. UPPER INTAKE LIMIT FOR OMEGA-3 FATTY ACID INTAKE

There are a few data available on the chronic ingestion of massive amounts of essential fatty acids. Eskimo populations such as the Inuit eat a large amount of marine animals (marine mammals, fish). The Inuit's traditional diet therefore provides large quantities of omega-3 fatty acids, in particular EPA and DHA, which reduce platelet aggregation. This population, in spite of a high fat intake, has a very low incidence of IHD.
A greater bleeding time linked to high consumption of omega-3 fatty acids has been reported, with a potential risk of haemorrhagic complications. Clinical studies (Annex 4) have shown that even if bleeding time is increased, this increase does not seem substantially to influence haemorrhagic risk in the general population. The effect shown on platelet aggregation is much less marked than that observed in individuals treated with aspirin.
It must also be emphasised that in intervention studies, doses of the order of 1g/d of EPA-DHA were administered for prolonged periods without any undesirable effects being reported. However, the longest studies were limited to a few years.

No safety limit can be determined with certainty on the basis of the data available

However, it seems unreasonable not to set any maximum limit for the intake of omega-3 fatty acids and to rely on self-regulation based on technological constraints (alteration of the organoleptic qualities with high contents of omega-3 fatty acids). In effect, the lack of a threshold value could result in the massive and generalised fortification of foods with these fatty acids, which is not desirable. Moreover, food manufacturing techniques are becoming more and more sophisticated: fish oils are more refined and the technique of micro-encapsulation, used in fortification, enables organoleptic obstacles to be overcome (taste and smell).

In the absence of a clearly definable safety limit, one option is to determine a maximum reasonable intake based on nutritional benefit. In this situation, a distinction should be made between alpha-linolenic acid and the LC-PUFA.
In view of its metabolism and energy use and limited capacities for elongation, there is no need to recommend a restriction on intake of alpha-linolenic acid under normal conditions of consumption. However, there remains the issue of where to set the LC-PUFA fortification limit beyond which there would be no nutritional benefits.
With this in mind, one could consider the maximum values for intakes of omega-3 fatty acids found in epidemiological studies (upper quintile or decile of consumption and intake levels used in intervention studies without any notable side effects being reported during prolonged administration), namely approximately 2 to 4 g/d for omega-3 LC-PUFA (cf. Annex 4). In particular, in the GISSI study, doses of omega 3 LC-PUFA were used over a period of 3.5 years (randomised study) with no notable side effects being signalled.
Moreover, daily consumption of 100g fish provides, depending on the species, up to 2 or 3 g/d of LC-PUFA.
In the study on the Inuit of Nunavik in Quebec, mean consumption of EPA and DHA exceeded 2g/d (24hr dietary recall, n=426) and exceeded 3g/d in the highest quintile (Dewailly, 2001). Similar rates of chronic intake have been observed in Japanese fishing villages where, moreover, the incidence of IHD is low (EPA+DHA: 2.9 g/d) (Yamada, 2000).
Finally, the FDA has granted GRAS status to menhaden oil (Brevoortia) on condition that daily intake of EPA and DHA from this oil is less than 3g/d (ISSFAL statement on omega 3 polyunsaturated fatty acids and heart disease, Oct. 2000).

The working group considers that:

- **the maximum permitted intake for LC-PUFA should be 2.0g/d, a level close to the mean values observed in populations which consume large amounts of marine products on a daily basis: when a product is fortified with long chain omega-3 fatty acids, a consumption simulation study of heavy consumers (90th or 95th percentile) should be used to verify that daily intake of long chain omega-3 fatty acids will remain below the limit of 2g/d (including both intake from the fortified food and from other sources);**
- **there is no justification for setting a maximum intake for alpha-linolenic acid.**

Rather than see this value of 2g/d as a safety limit for omega-3 LC-PUFA beyond which there would be a risk, it should be emphasised that there is no proven nutritional benefit in recommending a daily intake near or above it. For this reason, the accumulation of several daily consumption units of fortified foods, each at a level corresponding to 100% of the ANC for adult men (set for DHA at 120 mg/d) appears to be safe even if other LC-PUFA such as EPA are consumed at the same time. The risk of reaching the maximum permitted intake of 2.0/g/d is weak in this case. However, levels of fortification greater than 100% of the ANC per unit of daily consumption (ANC for DHA for adult men) are considered hazardous due to accumulation of doses in particular when there is simultaneous ingestion of other omega-3 LC-PUFA.

2.4. TYPES OF OMEGA-3 FATTY ACIDS TO BE USED FOR FORTIFICATION

Does fortification have the same nutritional benefit and does a claim have the same validity whatever the form of intake (fish oil or triacylglycerols containing omega-3 fatty acids) and regardless of the method of fortification (direct or indirect) with omega-3 fatty acids at an identical level? Should the preferred recommendation be the addition of the precursor or derivatives or both these forms of omega-3 fatty acids? Should these molecules preferably be added in the form of triacylglycerols or phospholipids?

The theory:

Although the different omega-3 fatty acids (in particular alpha-linolenic acid, EPA, DPA and DHA) result from one another, in humans, from biosynthetic and catabolic pathways, their biological effects can be different. Moreover, their biological "weight" is very different given the low yields from the endogenous biosynthetic route. For these reasons, it might seem appropriate to treat these omega-3 fatty acids differently, but this would result in enormous complexity in technical terms. From a pragmatic standpoint, one could consider that intake of ALA (an essential fatty acid precursor of the omega-3 series), requires particular attention. Moreover, intake of DHA(end product of the biosynthesis of omega-3 fatty acids from alpha-linolenic acid) could enable compensation for a deficit in endogenous biosynthesis of DHA in a fraction of the population or make up for a limited ALA intake. EPA and DPA intakes could be viewed as arising from the same process as DHA intake, with certain disadvantages attributable to interference between the metabolisms of EPA and arachidonic acid.
The working group, based on the patchy data in the literature, decided on a biological equivalency factor of 10 for EPA and DHA to the precursor (alpha-linolenic acid) (Pawlosky et al., 2001, Brenna 2002). This value is the result of an approximation, in the knowledge that bio-equivalence can be variable depending on physiological condition (especially age, etc.).

As regards the benefits of fortification with the precursor and/or long chain derivatives, comparison of the results of the *Lyon Diet Heart Study* and the GISSI study suggests that an intake of alpha-linolenic acid results in a reduction in the risk of cardiovascular morbidity and mortality greater than can be observed with long chain omega-3 fatty acids (Annex 4). However, the studies are not directly transposable and comparable and this hypothesis requires validation by a specific study, especially since this point is controversial (Sanderson, 2002; Renaud and Lanzmann-Petithory, 2002).

As a general rule, with the omega-3 fatty acids, the further one goes up the food chain, there is biological selection in favour of DHA and the chemical forms with which it is associated gradually change from the triacylglycerol form to that of glycerophospholipid, which can differ in terms of bioavailability and biological effects. Omega-3 fatty acids added to chicken feed induce the presence of omega-3 in the form of phospholipids in the birds' flesh. Conversely, in the case of direct fortification, omega-3 fatty acids are provided in the form of triglycerides, even ethyl esters. This means that depending on the route chosen for fortification (direct or indirect fortification) the forms of omega-3 fatty acids provided via the diet will be different and may have a different bioavailability. In particular, as regards the effect of purified omega-3 fatty acids, the GSSI study (1999) reported a 15% reduction in mortality in patients who had already suffered an infarction and who consumed approximately 800 mg of EPA and DHA for 3.5 years (Annex 4). In that study, the EPA and DHA were provided in the form of ethyl esters which have a lower bioavailability (of the order of 50%) than triacylglycerols and phospholipids.

In practice:

Not enough evidence is available to enable the recommendation of one particular omega-3 fatty acid for fortification, as intervention studies have been conducted either with the precursor (*Lyon Diet Heart Study* and Indo-Mediterranean study) or with long chain products (meta-analysis, Annex 5), or with fish which naturally contains a high level of long chain omega-3 fatty acids (as in the DART study). The studies which used alpha-linolenic acid showed the most marked reductions in relative risk, but they used a smaller population, their period of observation was shorter and although the intervention concerned alpha-linolenic acid, it did not do so exclusively.
Moreover, no factorial study has compared the chronic effects of the administration of alpha-linolenic acid with those of long chain omega-3 fatty acids.

Therefore, in the current state of knowledge, the working group is recommending approval of the use for fortification of either the precursor (alpha-linolenic acid) or the long chain derivatives (such as DHA or EPA) or both. The restriction of certain claims based on the specific functional properties of the different omega-3 PUFA will be discussed on a case by case basis in the corresponding paragraph (see Part 3).

3. CONCERNING CLAIMS

Optimisation of intake, with no harmful effects from fortification in terms of consumer safety and without misleading consumer information constitute general elements to be taken into account in the discussion of the validity of quantitative, functional and health claims for omega-3 fatty acids.

To evaluate the beneficial effect of fortification on the cardiovascular system, two types of criteria have been defined:

- intermediate criteria, such as triglyceridaemia, blood pressure and haemostasis;
- terminal ("hard") criteria, such as cardiovascular complications and cardiovascular and/or total mortality.

Based on current scientific data, there is no justification for attributing the protective effect on the cardiovascular system exclusively to alpha-linolenic acid or to the EPA/DHA pair specifically, as each of these three molecules can have beneficial effects.

There are not enough current intervention studies concerning the beneficial effects of omega-3 PUFA on the development of pathologies such as psoriasis, asthma, glomerulopathies or cancer to substantiate claims relating to functional properties or a risk reduction effect concerning these pathologies (all claims relating to the prevention, treatment or cure of a disease are prohibited, see 1.3.2.1).

3.1. POSSIBLE VECTOR FOODS FOR FORTIFICATION

In France, the vector foods fortified with omega-3 currently on the market are notably: dairy products, including milk, butter, crème fraîche, margarine, eggs, oils, meat and meat products and bread.

More generally, the working group considered the benefits of the vector foods which could be used to enrich the diet in omega-3 fatty acids.

The following points have to be considered when identifying the best vector foods for fortification:

a) What is the target population?
b) Are there markers of consumption available for the nutrient and what is their level in the target population?
c) What are the threshold values to be achieved but not exceeded to ensure optimum consumption in terms of optimisation of the risk/benefit ratio for health?
d) What amounts should be consumed and how often to achieve (and maintain) the result?
e) Is there any possibility that nutritional prevention messages could become confused when the composition of the vector food is not consistent with the optimum recommendations in terms of cardiovascular prevention or, more generally, with nutrition policy recommendations[15]?

3.1.1. Identifying a target population

The objective of compensating for a dietary deficit in omega-3 fatty acids would have a far greater chance of achievement if the foods selected for fortification were those most commonly consumed

15 *One of the priority objectives of the PNNS is to reduce total fat intake to less than 35% of total daily energy input, with a reduction by a quarter of mean consumption of saturated fatty acids in the population (less than 35% of total fat intake).*

by the target population with this deficit, with levels of fortification based on the quantities and rates of consumption observed in this population. This would mean that low consumers of fish, in particular, would be likely to get the most benefit from the fortification of foods with omega-3 PUFA. Because of the limitations of the OCA study (see 1.1.1.) it is difficult to define the target populations on the basis of nutritional criteria. Another approach would consist in taking all adults with a medium to high cardiovascular risk as the target population.

3.1.2. Nutritional criteria and interpretation of claims

One of the points to be resolved is whether all foods could be enriched with omega-3 PUFA, in particular foods with a fat content mainly consisting of saturated fatty acids. Consideration might also be given to whether it is better to consume a food with a moderate content of omega-3 fatty acids on a regular basis rather than eat a food highly fortified with these fatty acids once a week, or even once a month.

To attempt to answer these questions, two vector foods were discussed in detail by the working group: milk and butter.
Some products which contain notable proportions of saturated fatty acids, such as dairy products fortified with omega-3 fatty acids, claim or might claim that *"omega-3 fatty acids contribute to* or *support healthy cardiovascular function"*. Insofar as the claims give the products a positive image, an increase in consumption levels of the fortified food is possible. The example of whole milk products is a particularly sensitive one as they combine nutritional advantages with an unfavourable saturated fatty acid content in terms of cardiovascular prevention. Whole milk is not therefore an optimum vector food for fortification with omega-3 PUFA. The example of semi-skimmed milk is intermediate (this is a useful vector food due to its high consumption levels in the general population, namely 60%; its saturated fatty acid content is lower and the amounts provided in absolute terms are low). The opinion of the working group favours skimmed milk. The other argument against milk as a vector food concerns its conditions of use (mainly heating), which do not always guarantee the nutritional qualities of the omega-3 PUFA if the quantity of antioxidants added is insufficient. However, if its stability is proven, there could be an inconsistency between the authorised vitamin D fortification of whole milk and the refusal of the fortification of whole milk with omega-3 fatty acids (Order of 11 October 2001[16], following the Afssa Opinion of 1 June 2001[17]).
As regards butter, fortification with omega-3 fatty acids might seem reasonable for a regular butter consumer. However, like milk, the fortification of butter with omega-3 fatty acids (and the use of a claim regarding these fatty acids) might lead to an increase in the consumption of this product, prompted by the claim, when it already represents a major source of saturated fats in the French diet. Moreover, butter , due to the saturation of its fatty acids, is often taken to a high temperature during cooking and the stability of the omega-3 PUFA might then be place in doubt. Would this strategy make a genuine contribution to public health when alternative vector foods are available?

The issue of the selection of suitable vector foods raises the question of the impact of the claims on the consumer. A claim is likely to guide the consumer in his dietary choices. However, it is difficult to know whether it leads the consumer to increase consumption of the fortified food or simply to modify his choices between similar products, with no effect on the total consumed.
It is therefore necessary:

- to establish with the minimum degree of uncertainty whether, in practice, the nutrition or functional claims (and even the health claims), stated on foodstuffs encourage the consumer towards substitution rather than an increase in consumption,
- and to highlight the determining factors in this behaviour.

16 *Order of 11 October 2001 concerning the use of vitamin D in commonly eaten milk and fresh milk products (yoghurts and fermented milks, soft cheeses).*
17 *Afssa Opinion of 1 June 2001 concerning the evaluation of the draft Order on the use of vitamin D in commonly eaten milk and fresh milk products (yoghurts and fermented milks, soft cheeses).*

In view of the risk of a counter-productive effect (saturated fatty acids / polyunsaturated fatty acids ratio) and of confusion regarding medical advice on cardiovascular prevention, it might be preferable to restrict functional claims for omega-3 fatty acids to products which naturally contain low levels of saturated fat and cholesterol. However, the logical vector food for a family of fatty acids is precisely a frequently consumed fat. So the other option would be not to lay down strict requirements on the composition of the vector foods before fortification, in order to raise the probability of increasing average intake of omega-3 fatty acids in the population as a whole.

The approach proposed by the working group is to approve the quantitative nutrition claims "source" or "rich" based on criteria relating solely to the content of omega-3 fatty acids and to restrict functional and health claims to foods whose composition is currently considered consistent with cardiovascular prevention measures, the nutritional recommendations for the French population (ANCs) and the Programme national nutrition-santé [PNNS].

3.2. QUANTITATIVE NUTRITION CLAIMS

(Annex 7)

At the present time, the CEDAP Opinion of 8 July 1998 concerning quantitative claims for the vitamin and mineral content of foods is used by industry, by default, to support fortification with omega-3 PUFA, using the ANC in the absence of a defined RDA for these fatty acids.
This opinion states that:

- a food is a "source of vitamin X" if it contains more than 15% of the RDA of vitamin X per 100 g, or 7.5% of the RDA / 100 ml or 5% of the RDA / 100 kcal;
- a food is "rich in vitamin X" if it contains more than twice the threshold value set for "source", namely 30% of the RDA of vitamin X per 100 g, or 15% of the RDA / 100 ml or 10% of the RDA / 100 kcal;

The approach followed by CEDAP to set these levels was based firstly on the assumption of an average diet providing 2000 kcal daily and secondly on an analysis of the foods highest in vitamins and minerals and their level of consumption.
However, in terms of regulations, the Order of 3 December 1993[18] states, in Article 2, that nutritional labelling and nutrition claims, as defined in the Decree of 27 September 1993 on nutrition labelling, can apply to the vitamins and mineral salts listed in the Annex to the Order if they cover at least 15% of recommended daily allowance per 100g or 100 ml of the foodstuff in question or per pack, if it contains only one serving.
At the present time, not all the products on the French market claiming their "richness" in omega-3 fatty acids are fortified at the same level. The question is, therefore, to determine at what level a food can be considered a "source of omega-3 fatty acids" or "rich in omega-3 fatty acids".
Minimum levels of fortification are needed to claim a content which is considered significant in terms of cardiovascular physiology. For omega-3 fatty acids, these levels are established by consensus and are not based on convincing scientific data. They do not take account of differences in bioavailability, which varies according to the molecular structures containing these fatty acids (phospholipid *vs.* triacylglycerol, Sn1 or Sn2 position). The fortification of food with omega-3 fatty acids differs from fortification with vitamins and minerals, as these fatty acids are metabolised substrates and undergo beta-oxidation, unlike vitamins and minerals.
These threshold levels are nonetheless essential to prevent confusion and clarify matters for consumers.

By analogy with the 1998 CEDAP opinion, which applies to vitamins and minerals, and with the Order of 3 December 1993, but taking into account the specific features of the composition of foods also containing significant amounts of fat, the following statements are proposed:

- **a food is a "source of omega-3 fatty acids" if it contains more than 15% of the ANC for adult men of the omega-3 fatty acids concerned in 100 g, 100 ml or 100 kcal;**
- **a food is "rich in omega-3 fatty acids" if it contains more than twice the threshold value set for "source", namely 30% of the ANC for adult men of the omega-3 fatty acids concerned in 100 g, 100 ml or 100 kcal;**

[18] *Order implementing Decree No. 93-1130 (see 1.3.2.1.)*

3.3 - FUNCTIONAL CLAIMS AND HEALTH CLAIMS

The consequences of the application of these threshold levels have been evaluated in a CIQUAL simulation (Annex 7).

These levels apply to both DHA andalpha-linolenic acid.
If the option of combined fortification (alpha-linolenic acid and long chain omega-3 fatty acids) is selected, the condition concerning the percentage of ANC must be verified for alpha-linolenic acid or for DHA. However, combined fortification is not required anyway: a product of plant origin is not required to provide long chain omega-3 fatty acids if it contains sufficient alpha-linolenic acid and inversely an animal product is not required have an alpha-linolenic acid content higher than the threshold level if it satisfies the requirement for DHA.

No fortified product should by itself provide doses which, when one daily portion is ingested, exceed the ANC of DHA for adult men, in order to avoid an accumulation of excessive daily doses (see 2.3.).

Within the context of a nutrition policy intended to promote an increase in intake of omega-3 fatty acids, the working group considers that it would be illogical to ban all functional claims, especially as there are intervention studies available which cover both intermediate and hard criteria (morbidity and mortality) suggesting a possible cardiovascular benefit to the consumer.
However, the attribution of a generic claim stating a beneficial cardiovascular effect must be consistent with the effect sought and some vector foods are not suitable when their composition does not comply with standard dietary recommendations on cardiovascular prevention.

The working group carried out an evaluation of the various functional claims used to date by industry and considered the issues surrounding the subject.

The definition of nutritional criteria enabling the approval of functional claims for omega-3 fatty acids is not a simple matter. There are two frames of reference available:

- **one concerns populations at high cardiovascular risk, aimed at the primary and secondary prevention of cardiovascular disease.**
  This is based on the national, European and international consensus on nutrition recommendations which include overall, in terms of dietary lipids: a reduction in cholesterol intake to less than 300 mg/d, a reduction in the proportion of saturated fats and re-balancing of the omega-6 / omega-3 ratio *(recommendations of the Second Joint Task Force of European and other Societies on coronary prevention, 1998; Expert panel on detection evaluation and treatment of high blood cholesterol in adults, 2001)*;

- **the other is aimed at the general population in good health**
  This is based on the ANC (Martin, 2001) which recommend reference values, in particular for total fatty acids, saturated fatty acids, linoleic and linolenic acids. However, the ANC do not recommend a value for dietary cholesterol, for three reasons: the low impact of dietary cholesterol on cholesterolaemia in a healthy population, the lack of a proven benefit from this reduction in a population with no risk factor and the unrealistic prospects of such a recommendation being followed by an unaffected population.

3.3.1. Substantiation of claims concerning the normal functions of the body

3.3.1.1. The claim "Omega-3 fatty acids contribute to or support healthy cardiovascular function"

Afssa has already deemed this claim to have a scientific basis in the responses to two referrals (Annex 6 and Section 1.4.2).
Omega-3 fatty acids and not the product itself are presented as playing a role ("contribute to"), which agrees with, firstly, the clinical and experimental data on intermediate criteria and secondly,

clinical trials on the action of omega-3 PUFA in terms of cardiovascular mortality (GISSI study and *Lyon Diet Heart Study* in particular).
In addition, this claim relates to general function and not to a particular physiological parameter ("cardiovascular function"): it expresses the pleiotropic effects of omega-3 fatty acids. Finally, this claim makes no direct reference to any preventive effect.

In addition to the quantitative nutrition claims "source" and "rich" ("Level 1" claim), two other claim levels were put forward by the working group. They are distinguished by the number of criteria required.

> *Level 3 (the highest): requires the strictest criteria (including cholesterol) with an implicit prevention strategy directed at subjects at cardiovascular risk.*

This approach is based:

- on the fact that cardiovascular disease is the main cause of death in France and that therefore by definition there is a high number of individuals at cardiovascular risk within an apparently healthy population;
- on the transposition to a particular product of recommendations aiming at an overall diet. Because a balanced diet is composed of foods which are in themselves unbalanced, in order to obtain a health benefit it is logical that products which benefit from the "privilege of a claim" should be factors in the re-balancing of the overall diet as regards fat intake. This means that the criteria for a given product must be at least as strict as those applied to the overall diet.

It is recommended that products are promoted with the claim "Omega-3 fatty acids contribute to / support healthy cardiovascular function" should fulfil the following conditions:

- sufficient content of omega-3 fatty acids: ≥ 15% of the ANC for adult men (the ANC fixed for alpha-linolenic acid at 2g/d or for DHA at 120 mg/d) per 100 g or 100 ml or 100 kcal;

- **if the product is fortified with the precursor (alpha-linolenic acid) the linoleic acid / alpha-linolenic acid ratio must be less than or equal to 5,**
- **if the fortified food contains linoleic acid and is not fortified with alpha-linolenic acid, it must be sufficiently rich in or fortified with EPA and/ or DHA so that the weighted ratio including omega-3 LC-PUFA is also lower than or equal to 5 (the alpha-linolenic acid biological equivalency factor for omega-3 LC-PUFA is set at 10, see 2.4.)**

and

- total fat composition in accordance with current recommendations on cardiovascular prevention:

If the food is high in fat[19] (fat calories ≥ 33% of the calorie content of the food):

- **ratio of (saturated fatty acids) / (total fatty acids) less than 30%,**
- **and cholesterol content ≤ 150 mg/100 g or 100 ml.**

If the food provides little energy of fat origin (fat energy ≤ 33% of the food's energy content):

- **cholesterol content ≤ 150 mg/100 g or 100 ml**

advantages

- the elements substantiating the claim of the role played by omega-3 fatty acids on healthy cardiovascular function take account of composition criteria other than the content of omega-3 fatty acids alone, so that the product meets nutritional recommendations for cardiovascular prevention,
- foods are promoted which contribute to cardiovascular prevention measures,
- a restricted number of products is concerned, clarifying the consumer's perception.

[19] *All fatty acids (excluding cholesterol).*

disadvantages

- there is a risk of an increase in the fortification of vector foods which have a very low fat content, but which have the sole positive point of complying with the criteria.
- the probability that consumption of the very few products meeting all the above criteria might significantly modify the intake of omega-3 fatty acids in the general population and have a significant health impact is also low.

Level 2 (intermediate): no application of the composition criterion concerning the cholesterol content of the vector food but a requirement for minimum criteria set in terms of fat composition.

The fortification of basic products (eggs for example) could enable a useful increase in intake in the general population, on condition that there is substitution of non-fortified products with fortified products.
However, if these fortified products claim the role played by omega-3 fatty acids on healthy cardiovascular function and this functional claim leads to an increase in the consumption of these products, it is debatable whether the result would be beneficial in cardiovascular terms. Furthermore, cardiovascular prevention messages would then be blurred, as the consumer would associate products possibly high in saturated fatty acids and cholesterol with a possible cardiovascular benefit implicitly suggested by the wording of the claim. In addition, given the range of products which would benefit from the claim, its promotional effects would be reduced.
However, the French recommendations for the general population in good health do not include a restriction on the intake of dietary cholesterol.

It is proposed that products which comply with the following conditions may claim a re-balancing effect for the diet in terms of omega-3 fatty acids, with a claim such as "helps re-balance the intake of omega-3 fatty acids"[20]:

- sufficient content of omega-3 fatty acids: ≥ 15% of the ANC for adult men (the ANC fixed for alpha-linolenic acid at 2 g/d or for DHA at 120 mg/d) per 100 g or 100 ml or 100 kcal;

- **if the product is fortified with the precursor (alpha-linolenic acid) the linoleic acid / alpha-linolenic acid ratio must be less than or equal to 5,**
- **if the fortified food contains linoleic acid and is not fortified with alpha-linolenic acid, it must be sufficiently rich in or fortified with EPA and/or DHA so that the weighted ratio including omega-3 LC-PUFA is also lower than or equal to 5 (the alpha-linolenic acid biological equivalence factor for omega-3 LC-PUFA is set at 10)**

- and, if the food provides a large amount of energy from fat (fat calories ≥ 33% of the food calorie content): (saturated fatty acids) / (total fatty acids) ratio less than 30%.

The consequences of the application of the threshold levels mentioned above concerning the omega-6 and omega-3 fatty acid ratio, saturated fatty acids and, in the case of the claim relating to the role played by omega-3 fatty acids in healthy cardiovascular function, cholesterol, were evaluated in the CIQUAL simulation presented in Annex 7.

Application of the criteria listed above produces three groups of foods based on the three levels of claim envisaged:

- **level 1**, products which are sources of or rich in omega-3 fatty acids,
- **level 2**, products which are sources of or rich in omega-3 fatty acids, and which contribute to re-balancing the intake of omega-3 fatty acids and omega-6 fatty acids, and:
  - are low in fat

  or
  - are high in fat but provide relatively little saturated fatty acid

[20] This claim complies with the model established by CEDAP in its opinion of 18 December 1996 (opinion on recommendations concerning the non-misleading nature of claims), meaning that it includes:
- a verb, which may be "contributes", "supports", "plays a role in", etc.
- a scientific concept normaly re-balancing the intake of Omega-3 fatty acids.

- **level 3**, products which are sources of or rich in omega-3 fatty acids, and which contribute to re-balancing the intake of omega-3 fatty acids and omega-6 fatty acids, and:
    - are low in fat and cholesterol

    or
    - are high in fat but provide relatively little saturated fatty acid and cholesterol

The working group considers that it is inconceivable that products would be able to make a functional claim concerning the role played by omega-3 fatty acids on healthy cardiovascular function which directly or indirectly implied a benefit for cardiovascular health from the consumption of the fortified products, whilst at the same time their composition (at least the fat composition) caused them to be classed as products incompatible with the French nutritional recommendations on ANC, recommendations on cardiovascular prevention and the nutrition objectives set by the PNNS.
The fat composition criteria established by the working group constitute a set of necessary reference points within the context of considerations on the clarification of claims concerning omega-3 fatty acids.
However it is also desirable that the vector foods selected for fortification should provide a general benefit in terms of nutrition, other than their content of omega-3 fatty acids. This falls outside the context of the considerations concerning omega-3 fatty acids, rather it is part of more general considerations on the selection of vector foods, whatever the nutrient of interest. This general review is currently underway at Afssa and a specification to define a general framework characterising the vector foods and their criteria of choice should be available shortly. For foods meeting the criteria permitting level 2 or 3 claims, but having a debatable nutritional benefit, the CES "Nutrition Humaine" will have to be consulted on a case by case basis.

It also makes sense to provide evidence, when making Level 3 claims, that an increase in the consumption of the vector food which results in substantial coverage of the ANC for alpha-linolenic acid or DHA (50% of the ANC), if confirmed, is *not associated with a detrimental change in the main markers for cardiovascular risk*: LDL cholesterol, HDL cholesterol, blood pressure, glycaemia, CRP, homocysteinaemia, fibrinogen. This study should cover the target population involved.

3.3.1.2. The claim "Omega-3 fatty acids play a structural role in the cell membranes, omega-3 fatty acids play a role in platelet function and controlling lipaemia"

This claim refers to proven biochemical data. It is however not relevant and seems out of place. Not every contribution to an elementary function implies a health benefit: for example, cholesterol also plays a structural role in the membrane, however an excess intake has clearly been associated with an increase in cardiovascular complications in epidemiological observation studies and the selective lowering of cholesterol levels through treatment provides proven benefit to the cardiovascular system.
As regards the reference to lipaemia and platelet function, these points are discussed below (review of the claims concerning cholesterol, triglycerides and blood fluidity).

3.3.2. Substantiation of claims concerning improvement of a function

The other claims concerning health-related physiological properties can only be approved on a case by case basis, in the light of appropriate evidence.

This evidence must include several elements:

(a) proof of the existence of a modification in the targeted function (platelet aggregation, for example), when the fortified product is consumed in reasonable amounts, meaning simply as a substitute for the non-fortified food (these amounts can be estimated based on the 50th percentile of the consumption distribution of the similar non-fortified product); the rest of the diet should consist of the subjects' normal diet (change in a single factor due to a controlled food).

(b) proof that at these intakes, the other validated markers for cardiovascular risk are unchanged (cf. 3.3.1.1.).

- *"Omega-3 fatty acids enable improved cardiovascular function" / "omega-3 to maintain a healthy heart and arteries"*

The term "improved function" is certainly envisageable in reference to a dietary imbalance (excess of omega-6 fatty acids, insufficient intake of omega-3 fatty acids). However, the reference to "improved function" or the maintenance of a satisfactory condition ("maintain… healthy") could be considered a therapeutic or prevention claim, which the regulations do not permit for a food product and is not supported by the data from clinical trials including foods fortified with omega-3.

- *"Consumed regularly, omega-3 fatty acids make the blood more fluid"*

This claim refers to a biological effect on platelet aggregation, badly expressed in the terms selected.
The data relating to a possible effect of omega-3 fatty acids on platelet aggregation or bleeding time at nutritional doses are not sufficient to substantiate such a claim. The affirmation that these fatty acids have a modest anti-aggregant effect on the platelets, and only at high doses, should be balanced against the powerful anti-aggregant effect of aspirin. The claim is even less acceptable in scientific terms, because when combined with the claim concerning healthy cardiovascular function (cf. 3.3.1.1.), it could lead to the belief that improved cardiovascular function relies on the anti-aggregant effect, which has not been established.

However, the working group considers that this claim could be substantiated, but only if the manufacturer wishing to make it proves this effect through a series of platelet aggregation tests carried out following administration of the product promoted by this claim, in a randomised clinical trial at the recommended doses and under the conditions of use suggested by the applicant.
If the study provides conclusive evidence of this effect, the same options regarding substantiation for the generic functional claim concerning the role of omega-3 fatty acids on healthy cardiovascular function (3.3.1.1.), with the exception of the threshold value for content of omega-3 fatty acids, would also apply.

- *"Omega-3 fatty acids are included in recommendations for cholesterol-lowering diets"*

This claim refers to dietary recommendations issued for cases of excess cholesterol (in particular, increasing the consumption of fish containing omega-3 fatty acids) and has some logical basis. However, this claim has already been ruled unacceptable by Afssa, as it implies that omega-3 fatty acids are cholesterol-lowering, which is incorrect. In fact, these fatty acids do not lower cholesterol, but do lower triglyceride levels (although this effect is not generally the most often observed when supplementation is by means other than capsules of long chain omega-3 fatty acids, see the discussion below on triglyceridaemia).

In conclusion, the working group considers that no claim alleging a cholesterol-lowering effect of omega-3 fatty acids is justified. The claim "included in recommendations on cholesterol-lowering diets" is unacceptable, unless it is also explicitly stated that omega-3 fatty acids do not reduce blood cholesterol levels and unless the type of diet suggested is indicated.

The most logical claim, stating that omega-3 fatty acids are "suitable for diets intended to reduce the risk of ischaemic heart disease" is problematic. It is in conformity with the spirit of the nutrition recommendations but it implicitly includes a reference to a cardiovascular prevention effect (risk reduction). As the preventive effect has been achieved in studies which do not necessarily correspond to those used in the food industry and with different vectors, this claim can only be accepted on a case by case basis:

- **in scientific terms, validation would be required by means of a clinical cardiovascular prevention trial using the product at the recommended doses and in the conditions of use suggested by the applicant,**

- **in regulatory terms this category of claim is not acceptable at the present time under current legislation.**

If the effect is demonstrated and the regulations change, the same options regarding substantiation of the generic functional claim concerning the role of omega-3 fatty acids on healthy cardiovascular function (3.3.1.1.), with the exception of the threshold value for content of omega-3 fatty acids, would also apply.

- *"Omega-3 fatty acids contribute to lowering triglyceride levels"*

The effect of omega-3 fatty acids on the regulation of triglyceride levels is well documented. This effect is dose dependent: at an intake below 1 g omega-3 fatty acids per day, the effect is not shown; however, above that threshold (and especially at an intake of 4 g/d), this effect has been demonstrated.
However, the effect is less well-established in the general population than in the hypertriglyceridaemic population.

The effect is achieved with doses close to or higher than the acceptable upper intake limit (cf. Part 2.3.). Nevertheless, the fixing of a possible threshold for the use of this claim (consumption of the fortified product resulting in an intake of 1 g/d of omega-3) is not justified, insofar as the term "contributes" means that the product alone does not itself provide the dose required to observe that effect.
However, the effect was not observed when the supplementation was by means other than capsules of long chain omega-3 fatty acids. Moreover, it is not certain that the effect would be observable if the vector food simultaneously provided large quantities of fructose.
This claim can be substantiated only if it is supported by a controlled clinical trial with the finished product at the recommended doses and in the conditions of use suggested by the applicant.
If the effect is properly demonstrated, the same options regarding substantiation for the generic functional claim concerning the role of omega-3 fatty acids on healthy cardiovascular function (3.3.1.1.), with the exception of the threshold value for content of omega-3 fatty acids, would also apply.

3.3.3. Substantiation of other claims suggesting a health benefit

- *"Helps you feel better day-to-day"*

This claim is too vague and has no identified scientific basis.

- *"Asset for a healthy heart"*

The working group as a whole agreed that this claim was too vague and liable to interpretation. It suggests an improvement in function and there is no proof that omega-3 fatty acids improve cardiac function in a clinically significant fashion even if one echographic parameter was able to be improved independently during a controlled clinical study.

Images such as a red heart depicted on the packaging or in the advertising for a foodstuff did not raise any particular observations on the part of the working group as they concern a subjective assessment dependent on individual perception. Evaluation of this type of presentation is the responsibility of the supervisory authorities and not a matter of scientific expertise. Nevertheless, the working group felt that in the mind of certain consumers, there is probably little or no difference between the terms cardiovascular or heart, as used in the text of the claims, and the image of the heart. Precise studies are required on the impact of this type of message on consumers.

3.4. RESEARCH RECOMMENDATIONS

There remains a need for more scientific data on the situation of the French population and the protective effects of omega-3 fatty acids for cardiovascular health. The working group emphasised the benefits of:

- conducting studies:
    - designed to provide a better estimate of the intake of omega-3 fatty acids in the French population (data on food composition, particularly fish, and consumption data),
    - on the development of new markers permitting improved checks on low levels of omega-3 PUFA peroxidation in finished products,
- putting in place an intervention trial on cardiovascular prevention involving alpha-linolenic acid to supplement the results of the *Lyon Diet Heart Study* which covered a limited population and had a relatively short follow-up period and because the Singh study concerned a special population in which the majority is vegetarian and very few are undergoing treatment with hypolipidaemic drugs;
- supplementing this trial with a study comparing the effects of the administration of alpha-linolenic acid or long chain omega-3 fatty acids by stratifying the inclusions based on the presence of a statin;
- carrying out comparative studies of the different forms of intake of omega-3 fatty acids (triglycerides, phospholipids, diglycerides, etc.);
- putting in place additional cardiovascular prevention studies, required to provide better evidence that nutritional doses of omega-3 fatty acids can have a clinical effect on primary cardiovascular disease prevention.

As regards the claims concerning omega-3 fatty acids, the working group emphasised the value of conducting studies designed to provide improved information on the impact on French consumption of the introduction of a claim. In this context, it would be useful for applicants to carry out a consumption study (after the placing on the market of their products fortified with omega-3 fatty acids), to verify that the fortified products benefiting from claims are being consumed:
- as a substitute for and not in addition to similar non-fortified products (for example: as the PNNS recommends restricting consumption of added fats, steps must be taken to prevent the use of the claim resulting in the consumption of butter fortified with omega-3 fatty acids being in addition to the "habitual" consumption of non-fortified butter, possibly leading the consumer to increase intake excessively);
- as an addition to and not a substitute for other products of nutritional benefit (for example: steps should be taken to prevent a reduction in the consumption of fish when it is replaced by consumption of butter fortified with omega-3).

CONCLUSIONS

The working group, in view of the variable levels of proof on which it has based its proposals, decided to give each of its recommendations a rating (A maximum to C minimum)[21]. This approach illustrates the fact that the recommendations formulated reflect a consensus opinion within the group based on a synthesis of the data available in 2002. It is therefore liable to be adapted later on as more information becomes available, requiring regular updating of this review and the proposals resulting from it. The basis of the approach to claims concerning omega-3 fatty acids remains the ANC updated in 2000.

NUTRITIONAL BENEFITS

- The fortification of commonly eaten foods with omega-3 fatty acids, in the form of the precursor or long chain derivatives, is recommended as long as:
  - it may enable improved coverage of requirements, currently insufficient in the French population **(B)**.
  - it may also have a beneficial effect on cardiovascular disease prevention in subjects at cardiovascular risk whose requirements are covered but who might benefit from an increased intake of omega-3 fatty acids **(B)**.

  The working group considers that the benefit of omega-3 fatty acid supplementation, although mainly established in secondary prevention, might well be transposed to primary prevention and to a fortification strategy **(C)**.

CLAIMS

Points 2,3 and 4 of the referral

In view of the deficit situation in alpha-linolenic acid and omega-3 LC-PUFA intake and the potentially beneficial effects of food fortification, the use of claims to promote the consumption of naturally rich or fortified foods is acceptable and logical.

- Within the context of a nutrition policy seeking to promote an increase in omega-3 fatty acid intake, the working group takes the view that it would be illogical to prohibit all **functional claims**, especially since there are intervention studies available based on intermediate and hard criteria (morbidity and mortality) which suggest a possible cardiovascular benefit for the consumer.

  However, the attribution of a generic claim stating a beneficial cardiovascular effect must be consistent with the effect sought and some vector foods are inappropriate when their composition does not comply with the standard dietary recommendations for cardiovascular prevention.

  Two frames of reference are available for the definition of nutrition criteria permitting the approval of functional claims for omega-3 fatty acids:
  - **one concerns populations at high cardiovascular risk, aimed at the primary and secondary prevention of cardiovascular disease.**

[21] Points based on literature are rated from I to IV.
I: meta-analysis of randomised controlled studies, or at least one powerful randomised controlled study
II: non-randomised controlled studies
III: descriptive correlation studies
IV: expert committee report, expert opinions, clinical experience of recognised authorities
Recommendations are rated A to C.
A: recommendation based on concordant Level I studies, indicating established scientific proof
B : recommendations based on Level II and III studies indicating the existence of scientific arguments
C : recommendations based on weak scientific arguments at Level IV drawn from expert agreement or professional consensus

This is based on the national, European and international consensus on nutrition recommendations which include overall, in terms of dietary lipids: a reduction in cholesterol intake to less than 300 mg/d, a reduction in the proportion of saturated fats and re-balancing of the omega-6 / omega-3 ratio *(recommendations of the Second Joint Task Force of European and other Societies on coronary prevention, 1998; Expert panel on detection evaluation and treatment of high blood cholesterol in adults, 2001)*;

- **the other is aimed at the general population in good health**
  This is based on the ANC (Martin, 2001) which recommend reference values, in particular for total fatty acids, saturated fatty acids, linoleic and linolenic acids. However, the ANC do not recommend a value for dietary cholesterol, for three reasons: the low impact of dietary cholesterol on cholesterolaemia in a healthy population, the lack of a proven benefit from this reduction in a population with no risk factor and the unrealistic prospects of such a recommendation being followed by an unaffected population.

Functional claims concerning the role of omega-3 fatty acids in healthy cardiovascular function are justified as they are based on a number of experimental facts supported by a series of intervention studies.
The claim "omega-3 fatty acids contribute to / support healthy cardiovascular function" is justified both for the precursor (alpha-linolenic acid) and for the omega-3 LC-PUFA, given the available epidemiological data and clinical trials **(A).** It must be emphasised that it is important that the wording of the claim clearly indicates that the role is attributable to the omega-3 fatty acids and not to the fortified product, to avoid misleading the consumer.

- **Several levels of claim** were set out by the group, differentiated by the number of criteria required.

> *Level 3 (the highest): requires the strictest criteria (including cholesterol) with an implicit strategy of prevention directed at subjects at cardiovascular risk.*

This approach is based:
- on the fact that cardiovascular disease is the main cause of death in France and that therefore by definition there is a high number of individuals at cardiovascular risk within an apparently healthy population;
- on the transposition to a particular product of recommendations aiming at an overall diet. Because a balanced diet is composed of foods which are in themselves unbalanced, in order to obtain a health benefit it is logical that products which benefit from the "privilege of a claim" should be factors in the re-balancing of the overall diet as regards fat intake. This means that the criteria for a given product must be at least as strict as those applied to the overall diet.

It is recommended that products are promoted by the functional claim "Omega-3 fatty acids contribute to / support healthy cardiovascular function" should fulfil the following conditions (C):

- sufficient content of omega-3 fatty acids: ≥ 15% of the ANC for adult men (the ANC fixed for alpha-linolenic acid at 2 g/d or for DHA at 120 mg/d) per 100 g or 100 ml or 100 kcal;
  - *if the product is fortified with the precursor* (alpha-linolenic acid) the linoleic acid / alpha-linolenic acid ratio must be less than or equal to 5,
  - *if the fortified food contains linoleic acid and is not fortified with alpha-linolenic acid*, it must be sufficiently rich in or fortified with EPA and/or DHA so that the weighted ratio including omega-3 LC-PUFA is also lower than or equal to 5 (the alpha-linolenic acid biological equivalency factor for omega-3 LC-PUFA is set at 10, see 2.4.)

and

- total fat composition in accordance with current recommendations on cardiovascular prevention:

If the food is high in fat[22] (fat calories ≥ 33% of the calorie content of the food):

- ratio of (saturated fatty acids) / (total fatty acids) less than 30%,
- and cholesterol content ≤ 150 mg/100 g or 100 ml.

If the food provides little energy of fat origin (fat energy ≤ 33% of the food's energy content):

- cholesterol content ≤ 150 mg/100 g or 100 ml

It also makes sense to provide evidence, when making Level 3 claims, that an increase in the consumption of the vector food which results in substantial coverage of the ANC for alpha-linolenic acid or DHA (50% of the ANC) is not associated with a detrimental change in the main markers for cardiovascular risk: LDL cholesterol, HDL cholesterol, blood pressure, glycaemia, CRP, homocysteinaemia, fibrinogen.

Level 2 (intermediate): no application of the composition criterion concerning the cholesterol content of the vector food but a requirement for minimum criteria set in terms of fat composition.

The fortification of basic products could enable a useful increase in intake in the general population, on condition that there is substitution of non-fortified products with fortified products.
Nevertheless, if these fortified products claim the role played by omega-3 fatty acids on healthy cardiovascular function and this functional claim leads to an increase in the consumption of these products, it is debatable whether the result would be beneficial in cardiovascular terms. Furthermore, cardiovascular prevention messages would then be blurred, as the consumer would associate products possibly high in saturated fatty acids and cholesterol with a possible cardiovascular benefit implicitly suggested by the wording of the claim. In addition, given the range of products which would benefit from the claim, its promotional effects would be reduced.
However, the French recommendations for the general population in good health do not include a restriction on the intake of dietary cholesterol.

It is proposed, therefore, that products which comply with the following conditions may claim a re-balancing effect for the diet in terms of omega-3 fatty acids:

- sufficient content of omega-3 fatty acids: ≥ 15% of the ANC for adult men (the ANC fixed for alpha-linolenic acid at 2 g/d or for DHA at 120 mg/d) per 100 g or 100 ml or 100 kcal;

- *if the product is fortified with the precursor* (alpha-linolenic acid) the linoleic acid / alpha-linolenic acid ratio must be less than or equal to 5,
- *if the fortified food contains linoleic acid and is not fortified with alpha-linolenic acid*, it must be sufficiently rich in or fortified with EPA and/or DHA so that the weighted ratio including omega-3 LC-PUFA is also lower than or equal to 5 (the alpha-linolenic acid biological equivalence factor for omega-3 LC-PUFA is set at 10)

- and, if the food provides a large amount of energy from fat (fat calories ≥ 33% of the food calorie content): (saturated fatty acids) / (total fatty acids) ratio less than 30%.

The fat composition criteria established by the working group constitute a set of necessary reference points within the context of considerations on the clarification of claims concerning omega-3 fatty acids.
However it is also desirable that the vector foods selected for fortification should provide a general benefit in terms of nutrition, other than their content of omega-3 fatty acids. This falls outside the context of the considerations concerning omega-3 fatty acids, rather it is part of more general considerations on the selection of vector foods, whatever the nutrient of interest. This general review is currently underway at Afssa and a specification to define a general framework characterising the

[22] *All fatty acids (excluding cholesterol).*

vector foods and the criteria of choice should be available shortly. For foods meeting the criteria permitting level 2 or 3 claims, whilst having a debatable nutritional benefit, the CES "Nutrition Humaine" would have to be consulted on a case by case basis.

Level 1 (the lowest): sets minimum criteria for the content of omega-3 fatty acids.

Quantitative nutrition claims concerning the presence of omega-3 fatty acids are useful, whether they concern a product naturally containing these fatty acids or a product fortified with these substances **(B)**.

The claim **"source of omega-3 fatty acids"** is substantiated whenever the product contains 15% of the ANC for adult men (ANC set for alpha-linolenic acid at 2 g/d or for DHA at 120 mg/d) in 100 g or 100 ml or 100 kcal. **(C)**

The claim **"rich in omega-3 fatty acids"** is substantiated whenever the product contains 30% of the ANC for adult men defined for alpha-linolenic acid or DHA in 100 g or 100 ml or 100 kcal. **(C)**

- The **other claims concerning health-related physiological properties** can only be approved on a case by case basis in the light of the appropriate evidence.

  This evidence must comprise several elements:

  (a) proof of the existence of a modification in the targeted function (platelet aggregation, for example), when the fortified product is consumed in reasonable amounts, meaning simply as a substitute for the non-fortified food (these amounts can be estimated based on the 50th percentile of the consumption distribution of the similar non-fortified product); the rest of the diet should consist of the subjects' normal diet (change in a single factor due to a controlled food).

  (b) proof that at these doses, the other validated markers for cardiovascular risk are unchanged (cf. 3.3.1.1.).

  In scientific terms, claims concerning an effect on blood fluidity cannot be generically accepted as the term is too general and too vague and it constitutes an interpretation of the effects of omega-3 fatty acids on some haemostatic processes **(C)**.
  Claims such as "Consumed regularly, omega-3 fatty acids make the blood more fluid" can only be approved if the applicant demonstrates this effect through a series of platelet aggregation tests following administration of the product, in a randomised clinical trial at the recommended doses and under the conditions of use suggested by the applicant. If the study provides conclusive evidence of this effect, the same options regarding substantiation for the generic functional claim concerning the role of omega-3 fatty acids on healthy cardiovascular function ("Level 3") shall apply, with the exception of the threshold value for the content of omega-3 fatty acids.

  Claims concerning the cholesterol-lowering properties of omega-3 fatty acids are unjustified on the basis of the data available from controlled trials **(A)**. The claim "Omega-3 fatty acids are included in recommendations for cholesterol-lowering diets" is unacceptable, unless it is also explicitly stated that omega-3 fatty acids do not reduce blood cholesterol levels and unless the type of diet suggested is indicated, since this claim causes confusion in the consumer, as it implies cholesterol-lowering properties which do not exist.

  Claims concerning a triglyceride-lowering effect are in fact essentially valid in hypertriglyceridaemic subjects, at doses higher than the ANC and solely with omega-3 LC-PUFA **(B)**. In view of the very variable effects depending on the type of vector food, they should be systematically supported by a controlled clinical study carried out in humans using the finished product under regular consumption conditions and comply with the options regarding substantiation stated above, with the exception of the threshold value for the content of omega-3 fatty acids.

Claims concerning a reduction in the risk of the occurrence of cardiovascular disease could only be envisaged with reference to the fortification of a specific vector food which has been the subject of a probing intervention study with objective criteria concerning cardiovascular morbidity and mortality. These claims are not authorised by the current regulations.

Images such as a red heart depicted on the packaging or in the advertising for a foodstuff did not raise any particular observations on the part of the working group as they concern a subjective assessment dependent on individual perception.

METHODS OF FORTIFICATION
Point 1 of the referral

- Indirect fortification (*via* animal feed) is likely to provide the same benefits as direct fortification (use of ingredients rich in omega-3 fatty acids) and possibly better bioavailability **(B)**. The fortification method (direct or indirect) must be specified to the consumer. Claims are acceptable for both types of fortification.

- Fortification can be with either the precursor or omega-3 LC-PUFA or a combination of alpha-linolenic acid and omega-3 LC-PUFA, in order to combine the potential advantages of the different fortification strategies, in the knowledge that it is not possible to define the pre-eminence of one strategy based on absolute criteria, but that alpha-linolenic acid is more resistant to cooking and peroxidation **(C)**.
  As a general rule, it would be essential for manufacturers fortifying foods with omega-3 fatty acids to inform CIQUAL of the exact composition of their product (based on effective analyses and not calculation-based extrapolations), to enable better evaluation of the intake of omega-3 fatty acids in the French population.

VERIFYING SAFETY OF USE
Point 1 of the referral

- As regards peroxidation products:
  - Given the particular peroxidative susceptibility of omega-3 fatty acids (a high number of unsaturations) and the absence of any nutritional benefit from an intake of omega-3 fatty acids in peroxidated form, the product must satisfy the standard oxidation tests **(C)**.
  - The tests carried out on the finished product must be reported in terms of the added omega-3 fatty acids, so as to evaluate the possible pro-oxidant effect of the addition whilst guaranteeing the good quality of the supplement used.
  - In addition, given this susceptibility to peroxidation, proof must be provided that almost all the omega-3 fatty acids (initial and added), so at least 90%, are found in the finished ready-to-eat product and at the end of its shelf life **(C)**.

- In the absence of a clearly definable safety limit, it seems reasonable to restrict levels of fortification in such a way that total daily intake remains below 2 g/d for omega-3 LC-PUFA **(C)**. This threshold level of 2 g/d of omega-3 LC-PUFA is not a safety limit beyond which a clearly established risk appears, rather a value beyond which a dietary intake of these fatty acids would be of no nutritional benefit and beyond which there are no reference points concerning safety. No fortified product should provide by itself doses resulting in the ANC for DHA being exceeded when a single portion is consumed **(C).**
  When the product is fortified with long chain omega-3 fatty acids, it is therefore recommended that a consumption simulation study on heavy consumers (90th or 95th percentile) is used to verify that the daily intake of long chain omega-3 fatty acids would remain below the limit of 2 g/d (including both intake from the fortified food and from other sources).

  As regards alpha-linolenic acid, in view of its metabolism with energy use and the limited capacities for elongation, no threshold value has been defined corresponding to an acceptable upper intake limit **(C)**.

MORE GENERAL CARDIOVASCULAR PREVENTION MEASURES

- The working group would like to point out that dietary and health measures for preventing cardiovascular disease are unlikely to be based on one nutrient or family of nutrients, however attractive this may seem, but on a group of positive measures. Although the increase in omega-3 fatty acids could be part of a cardiovascular prevention process, it cannot be the only element in that process **(B)**. It must be emphasised that, in all trials which have shown a cardiovascular benefit from an increase in intake of omega-3 fatty acids, the subjects included were taking a number of additional nutritional measures designed to prevent cardiovascular disease.

The working group would like to emphasise that the consumption of fish provides an excellent source of omega-3 LC-PUFA, with no prior fortification, the benefits of which, in terms of cardiovascular prevention have been supported by a controlled clinical study **(A)**. Eating fish at least twice a week is recommended. The working group would also like to draw attention to the fact that an intake of precursor represented by oils rich in alpha-linolenic acid (rapeseed oil, for example) also constitutes an effective means of balancing intake of omega-3 fatty acids **(A).**

SYNTHESIS

Omega-3 fatty acids and the cardiovascular system: nutritional benefits and claims

Summary of the working group report, written and revised by
Esther Kalonji, Céline Dumas and Jean-Louis Berta

The new developments in knowledge and the gradual application of the concept of evidence-based medicine are leading, in terms of nutrition, to regular re-examination of the particular benefit of a number of nutrients and the claims which might support their consumption. This process is made all the more essential by the fact that the "health" selling point is now one of the main differentiation criteria for food products sold to consumers.

Therefore, in view of the **upsurge on the market of the number of foods fortified with omega-3 fatty acids claiming beneficial effects for the consumer, in particular for the cardiovascular system,** and in the absence of specific statutory regulations providing a framework for these practices, Afssa (French Food safety Agency) was asked to evaluate the relevance of these substances in nutritional terms.

Exclusively based on the adult population, this review was not aimed at defining a nutrition policy for essential fatty acids. The main objective was to assess what is acceptable or unacceptable in terms of the claims used to promote products providing omega-3 fatty acids as far as the cardiovascular system is concerned.
More precisely, this project was to supply the information required to formulate a general opinion on the following points:

- do products fortified with omega-3 fatty acids offer any nutritional benefits for the consumer? does their consumption have an impact on dietary balance, in view of current consumption and the prospects for development?
- are the omega-3 fatty acid contents in these foods, and the sum of them, based on the number of product categories concerned, likely to pose any risk to consumer health?
- what criteria can be used to substantiate the quantitative nutrition claims "source of omega-3 fatty acids" and "rich in omega-3 fatty acids" and the functional and health claims referring to healthy cardiovascular function, blood fluidity and lowering of cholesterol levels, as these claims are frequently found on products fortified with omega-3 fatty acids?

Brief overview of the essential polyunsaturated fatty acids *(Figure 1)*

Two families of polyunsaturated fatty acids are described as essential: the omega-6 family (linoleic acid (LA) and arachidonic acid) and **the omega-3 family** (alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)). Linoleic and alpha-linolenic acids are also indispensable, as the human body cannot synthesise them.
No metabolic conversion or functional substitution is possible between these two families. However, linoleic and alpha-linolenic acids, precursors for these two families, compete for the enzymes responsible for metabolising polyunsaturated fatty acids (production of derivatives, the long chain polyunsaturated fatty acids: LC-PUFA, notably arachidonic acid, EPA and DHA). This means that an excessive intake of linoleic acid can compromise the production of EPA and DHA from alpha-linolenic acid. It is therefore recommended that, as part of the overall diet, intake of linoleic acid should be equivalent to approximately 5 times the intake of alpha-linolenic acid (Martin et al., Nutritional Recommendations for the French Population, 2001).
The ANC (reference intake for the French population) for alpha-linolenic acid is set at 2 g/day for adult men and 1.6 g/d for adult women and intake of DHA is 0.12 g/day for adult men and 0.10 g/day for adult women.
The polyunsaturated fatty acids of the omega-3 family are mainly found in rapeseed and soya oil (linolenic acid), marine animal products and human breast milk (EPA, DHA). Terrestrial animal products, in view of their levels of consumption, can constitute not inconsiderable sources of omega-3 fatty acids. The polyunsaturated fatty acids of the omega-6 family are provided by sunflower seed and corn oils (linoleic acid), terrestrial animal products and human breast milk (arachidonic acid).

Omega-3 polyunsaturated fatty acids and the cardiovascular system: the scientific viewpoint

In the light of clinical surveys, it seems that fortification with omega-3 fatty acids leads to a number of beneficial effects on the cardiovascular system. Two types of criterion are generally used to assess them:

- intermediate criteria, such as triglyceridaemia, blood pressure and haemostasis;
- terminal or "hard" criteria, such as cardiovascular complications and cardiovascular and/or total mortality.

Most intervention studies concern the intermediate criteria and are based on limited populations. The effects reported are of moderate intensity and often inconsistent. These studies show that fortification with omega-3 fatty acids can lead to a reduction in blood pressure in hypertensive subjects and to a reduction in triglyceridaemia in hypertriglyceridaemic subjects with no modification of plasma levels of LDL-cholesterol, a parameter considered as a critical coronary risk factor.
As regards the hard criteria, the studies listed showed that nutritional intervention comprising the consumption of products rich in alpha-linolenic acid enabled cardiovascular morbidity and mortality to be significantly reduced in subjects previously presenting with cardiovascular or metabolic disorders (subjects who had already suffered an infarction, for example). A reduction in infarction lethality (reduction in the risk of sudden death) with no reduction in the incidence of non-fatal infarctions has also been demonstrated following the consumption of fish or of long chain omega-3 fatty acids (fish oil).
It is important to note that these results are confirmed by two recent meta-analyses.
This work as a whole suggests that **supplementation with omega-3 fatty acids can have a beneficial effect on cardiovascular health in secondary prevention.**
In contrast, the benefit derived from the consumption of omega-3 fatty acids in terms of reduction of cardiovascular risk is plausible in the general healthy population (primary prevention) but cannot currently be considered to have been established, due to a lack of bibliographical data.

Provided in large quantities, omega-3 fatty acids, in particular DHA and EPA, can undergo peroxidation phenomena, particularly in subjects with reduced antioxidant capacities. For this reason, the beneficial effect of omega-3 fatty acids at nutritional doses has also been researched: it appears that **pharmacological intakes are not strictly necessary, as intakes close to those recommended for the daily diet can also be sufficient.**

Fortification of foods with omega- 3 fatty acids

Justification for the French population

The estimation of consumption levels of omega-3 fatty acids in the French population is an essential element in determining whether or not there are valid grounds for fortifying our food with these nutrients.
Two principal studies, the INCA survey[1] (survey based on a representative sample of the French population, data on subjects over 15 years old) and the SU.VI.MAX[2] study (study based on volunteer adults aged from 35 to 60), were used as the basis for an estimate of omega-3 fatty acid intake in the adult French population. Estimation of omega-3 fatty acid intake suffered from a certain number of methodological limitations: the difficulty of evaluating fat intake due to small quantities subject to wide intra and inter-individual variability; imprecise information in the food composition tables (incomplete composition data; lack of knowledge of the effects of technological and heat treatments, imperfect designations for the foods).
However, it appears from these studies that mean intake of alpha-linolenic acid is very low (about 0.1% of total daily energy in the INCA survey and 0.4% in the SU.VI.MAX study) and that the intakes for almost all the individuals did not cover the ANC (0.8% of total daily energy). It seems that this intake is essentially provided by animal products, as rapeseed oil is consumed relatively little in France. Mean intakes of linoleic acid were more than 10 times higher than mean intakes of alpha-linolenic acid, showing an imbalance in the intake of these two families of fatty acids.

[1] INCA: individual national dietary survey
[2] SU.VI.MAX: study entitled «Anti-oxidant vitamin and mineral supplements»

This insufficient intake of alpha-linolenic acid has been confirmed by other studies, notably the Transfair study (multicentre European study) and the Aquitaine study (women aged from 18 to 50).
In terms of long chain omega-3 fatty acids (EPA and DHA), food composition data is much too patchy to allow a reliable estimate of their intake.
It therefore appears that **a policy designed to increase intake of omega-3 fatty acids in the French population would be beneficial.**

Methods of fortification and types of omega-3 fatty acids to be used

Increasing intake of omega-3 fatty acids can be envisaged using different methods:

- either by promoting the consumption of naturally rich foods
    - raising alpha-linolenic intake through increased consumption of rapeseed or soya oil or specially manufactured oils (blended oils)
    - raising consumption of long chain omega-3 polyunsaturated fatty acids (EPA and DHA) by increasing the consumption of fish
- or by fortifying certain foods
    - indirect fortification through the use of linseed in animal feed
    - direct fortification through the use of ingredients or extracts intrinsically rich in omega-3 fatty acids (fish oil)

These fortification strategies are subject to specific regulations and manufacturers must ensure that the product placed on the market complies with them. The fortification of foods with omega-3 fatty acids can affect the product's legal name: for example, the legal name for a milk fortified directly with an ingredient rich in omega-3 fatty acids becomes "milk drink" whereas indirect fortification does not affect the legal name.

Even if it is conceivable, theoretically, that omega-3 fatty acids (ALA, EPA, DHA) can present different biological effects although they result from one another in humans, the level of proof which might have led to a recommendation on a particular omega-3 fatty acid for fortification, precursor or derivative, is low (there are no factorial studies available which have compared the chronic effects of the administration of alpha-linolenic acid with those for EPA and DHA). Moreover, the form of intake (triacylglycerols, phospholipids, ethyl esters) and the fortification method selected are likely to have an impact on the bioavailability of the omega-3 fatty acids.
The working group is therefore recommending the acceptance, for fortification with omega-3 fatty acids, of the use of either the precursor or the long chain derivatives, or both. However, because of the low efficiency of the endogenous biosynthesis route, **a mean biological equivalency factor of 10 was adopted for the conversion of EPA and DHA into alpha-linolenic acid.**

Peroxidation risk

All products rich in polyunsaturated fatty acids and which contain water and/or peroxidant agents (iron, copper) have some susceptibility to peroxidation.
However, in view of the multiplicity of oxidation products of fatty acids (more than one hundred in a highly oxidised fish oil) and uncertainties surrounding assessment of the associated toxicological risk, a foodstuff's state of peroxidation is very difficult to assess.
For this reason, the working group considers that the lack of nutritional benefit and the potential harmfulness of fatty acids in peroxidised form necessitate, unless the applicant is required to produce a detailed qualitative and quantitative evaluation of the peroxidation products of the omega-3 fatty acids: 1) implementation of conventional methods for assessing the oxidation levels of fats (measurement of the primary and secondary oxidation products) which constitutes a not inconsiderable element for evaluating the peroxidation levels of fortified products; 2) that the analyses must be performed on the finished product and that any pro-oxidant effect must be related to the quantity of omega-3 fatty acids added and not the total quantity of fatty acids in the product; 3) that almost all, 90% as a minimum, of the omega-3 fatty acids in the final, ready-to-use product must be stable throughout its shelf-life until final consumption.
The putting in place of an effective system to protect omega-3 fatty acids from peroxidation is therefore strongly recommended, especially for DHA and EPA, whose specific oxidation products are the source of the characteristic odours of oxidised fish oil.

Upper intake limit

The scarcity of available data on the effects of the chronic ingestion of massive quantities of omega-3 fatty acids has prevented a safety limit for these nutrients from being determined with any certainty. In the studies listed, a greater bleeding time (intermediate criterion) was observed in situations of high intakes of omega-3 fatty acids (up to 9g EPA and DHA per day), without, however, any substantial influence on haemorrhagic risk being demonstrated for the general population.
However, in the interests of taking maximum precautions and in order not to encourage massive and generalised fortification of foods with omega-3 fatty acids, **the working group opted for the establishment of an upper intake limit. This limit should be considered as a daily intake level above which the nutritional benefits of omega-3 fatty acids are no longer proven.** This is not a safety limit, meaning a limit beyond which there is a health risk. Due to the limited elongation capacities of alpha-linolenic acid (low efficiency of conversion into EPA and DHA), **a restriction on alpha-linolenic acid intake cannot be recommended under normal consumption conditions. As regards LC-PUFA (EPA and DHA), a maximum limit has therefore been established at approximately 2 g/day**. This value is close to the mean intakes used in epidemiological studies, for prolonged administration and with no significant side effects having been signalled (a value close to those observed in populations with high consumption levels of marine products). It must be noted that in the USA (Food and Drug Administration), GRAS (Generally recognised as safe) status has been granted to menhaden oil for which the daily intakes of EPA and DHA are estimated at less than 3 g/day. Furthermore, **the working group is recommending that LC-PUFA content per daily portion of the fortified food should be less than 100% of the ANC for adult men, as higher levels are considered hazardous. The applicant's supporting dossier of evidence must therefore include simulation data concerning compliance with the upper intake limit.**

Substantiation of claims

Informing the consumer of the beneficial role played by a nutrient in physiological functions or even in general health is an inevitable corollary to the promotion of the nutrient on product labels.

Regulations applicable to the use of claims

There are currently no specific regulations applicable to claims concerning omega-3 fatty acids. Consequently, until the introduction of a regulation on nutrition, functional and health claims, now at the draft stage at Community level, any communication on foodstuffs (labelling or advertising stating the presence of nutrients or their role in normal functions of the body or regarding their favourable impact on health) is subject to the provisions of the Consumer Code (requirement on non-misleading advertising and provisions regarding deception). So manufacturers must be able to scientifically substantiate the claims promoting the products they place on the market, claims which must be such that they do not mislead the consumer.

At present, three main categories of claim are accepted:

- **quantitative nutrition claims**, concerning the energy or nutrient content (micro- and macro-) of foodstuffs; these are subject to the provisions of Decree No. 93-1130 of 27 September 1993;
- **functional claims**, concerning the roles played by a foodstuff or one of its constituents in the growth, development or normal functions of the body; the opinion of the Commission d'étude des denrées alimentaires destinées à une alimentation particulière (CEDAP) [Interministerial Committee for Products intended for particular nutritional uses] of 18 December 1996 lays down the criteria governing their use as regards vitamins and minerals;
- **health claims**, concerning the relationship between a foodstuff, or one of its constituents, and health; at the present time, these are assessed on a case by case basis by Afssa.

When an advertising campaign is planned to promote a product bearing a claim relating to health, an additional control is provided by the Public Health Code: the product then requires a "visa Publicité Produit" (product advertising certificate), a procedure carried out by the Agence française de sécurité sanitaire des produits de santé (Afssaps) [French Health Products Safety Agency].

Finally, all claims concerning the prevention, treatment or cure of a disease are prohibited.

Potential vector foods

A number of different vector foods are used by the food industry for fortification with omega-3 fatty acids: milk and dairy products, eggs, butter, margarine, crème fraîche, meat, bread, meat products, etc.
However, the working group decided to **undertake a general review of the suitability of the potentially useable vector foods.**

Identification of the best vectors for fortification requires a certain number of points to be examined.
Identification of a target population is one way of measuring the effectiveness of a programme to compensate for an insufficient intake of omega-3 fatty acids. This population is likely to benefit more from the fortification if the vectors used are selected from the foods it consumes most frequently. Because of **the limitations on the estimate of omega-3 fatty acid intake in the French population, it is difficult to define target populations on the basis of nutritional criteria. The alternative consists of taking adults with a proven cardiovascular risk as the target population.**
The choice of suitable fortification vectors also raises the question of the impact of the claims insofar as they give products a positive image, likely to result in increased consumption levels for the fortified food. Milk (notably whole milk) and butter vectors illustrate this problem well, as an increase in their consumption levels would lead to an increased intake of saturated fatty acids, with potentially unfavourable consequences in cardiovascular terms. **It is therefore of primordial importance that the overall composition of the fortified food is taken into account.**
Another argument to be considered in the selection of the vector food concerns storage methods and the conditions of use of the fortified food, which are likely to alter the **bioavailability** of the omega-3 fatty acids.

The approach proposed by the working group is:

- to approve the quantitative nutrition claims "source" or "rich" based on criteria relating solely to the content of omega-3 fatty acids;
- to restrict functional and health claims to foods whose composition is currently considered consistent with cardiovascular prevention measures, the nutritional recommendations for the French population (ANCs) and the Programme national nutrition-santé (PNNS) [National health and nutrition programme].

Quantitative nutrition claims *(Figure 2)*

At the present time, the products on the French market promoted with quantitative nutrition claims for omega-3 fatty acids do not contain the same contents of this nutrient. The issue was therefore to determine the content required for a food to be considered as «a source of omega-3 fatty acids» and «rich in omega-3 fatty acids», with this content being significant in terms of cardiovascular physiology.
In the working group's view, **these claims are the ones which require the lowest level of requirements in terms of scientific proof. It was agreed that these would be referred to as Level 1 claims.**

Constrained by the lack of definitive scientific data (bioavailability of the different forms of fatty acid, phospholipid *vs* triacylglycerol, position of the fatty acid; specificity of beta-oxidation) and the need to establish this type of marker (to avoid causing confusion for consumers), the working group, on the basis of a consensus, has set the content required for quantitative nutrition claims by analogy with the CEDAP opinion of 8 July 1998 on vitamin and mineral content.

Therefore, the following justifications are proposed:

- **a food is a "source of omega-3 fatty acids" when it contains 15% of the ANC for alpha-linolenic acid (2 g/day) or DHA (0.12 g/day) for adult men per 100 g, or 100 ml or 100 kcal;**
- **a food is "rich in omega-3 fatty acids" when it contains more than twice the threshold value set for the "source" claim, namely 30% of the ANC for alpha-linolenic acid or DHA for adult men per 100 g, or 100 ml or 100 kcal.**

It is logical that the use of this type of claim should make compulsory the nutrition labelling of the nutritional qualities of foodstuffs (Decree No. 93-1130 of 27 September 1993).

The consequences of the application of the threshold levels envisaged for these Level 1 claims were evaluated as part of a study by the Centre informatique sur la qualité des aliments [Informatics Centre for Food Quality] (Ciqual/Afssa). This was based on available composition data for about 50 food products, some of which were fortified with omega-3 fatty acids and some not, belonging to a variety of food families: oils, eggs, margarine, bread, meat, milk, fish, etc. The aim of this project was to position the different food families in terms of the thresholds envisaged to:

- verify whether the products usually accepted as providing notable quantities of omega-3 fatty acids were properly characterised as "source of" or "rich in" these fatty acids (verification of the consistency of the threshold levels envisaged with the consumer's dietary perception);
- verify the realistic and practical nature of the threshold levels envisaged (evaluation of the quantity of alpha-linolenic acid or DHA to be added to achieve the thresholds set for the claims "source of" or "rich in" omega-3 fatty acids; selection of foods already fortified with omega-3 fatty acids through the application of the envisaged thresholds).

Qualitative functional claims and "health" claims *(Figure 2)*

The working group did not confine itself solely to a general review of the justification of qualitative claims but also evaluated the claims currently used by producers.

General approach

Two frames of reference were proposed to define the nutrition criteria for evaluating qualitative claims referring to omega-3 fatty acids:

- **One is aimed at the general population in good health; it is based on the ANCs defined for total fatty acids, saturated fatty acids and linoleic and alpha-linolenic acids and on rebalancing the omega-6/omega-3 ratio;**
- **The other concerns populations at high cardiovascular risk and is designed to put in place a primary and secondary prevention approach to cardiovascular disease; it is based on the national and international consensus on nutritional recommendations and, as regards dietary fats, a reduction in cholesterol intake (less than 300 mg/day), a reduction in saturated fat intake and a rebalancing of the omega-6/ omega-3 ratio.**

Therefore in addition to the omega-3 fatty acid content of the products, and based on these two frames of reference, **the nutrition criteria for fat composition used in the substantiation of qualitative claims are:**

- **the linoleic acid/omega-3 fatty acids ratio**, in which the omega-3 fatty acids include alpha-linolenic acid, DHA and EPA; the biological equivalency factor set at 10 enabling conversion of DHA and EPA contents into alpha-linolenic acid; this ratio should be called in more general terms the linoleic acid/alpha-linolenic acid equivalent ratio (LA/ALA equivalent ratio)
- **energy content of fat origin**
- **the proportion of fat intake in the form of saturated fatty acids** (saturated fatty acids/total fatty acids ratio)
- **cholesterol content**

Two levels of claim, based on an accumulation of required criteria, were envisaged:

- **Level 2 claim**
  - **the food product is a "source of omega-3 fatty acids" or is "rich in omega-3 fatty acids"**
  - **the LA/equivalent ALA ratio is less than or equal to 5**

This criterion, stricter than the one proposed as part of the ANC, is based on the requirement that products benefiting from a positive image as a result of a claim should play a part in re-balancing the total dietary intake of fats.

  - **the product provides fats in reasonable quantities (content <33 % of the food energy content) or the product is rich in fat (content ≥33 %) but provides reasonable quantities of saturated fatty acids (content <30 %)**

When all these conditions are met, the claim "the product contributes to/supports rebalancing of the omega-3 fatty acid intake" can be made.

- **Level 3 claim**
  - **The food product is a "source of omega-3 fatty acids" or is "rich in omega-3 fatty acids"**
  - **the product contributes to/supports rebalancing of the omega-3 fatty acid intake**
  - **the product contains a maximum of 150 mg cholesterol per 100 g or 100 ml**

When all these conditions are met, the claim "omega-3 fatty acids contribute to/support healthy cardiovascular function" can be made.

This claim has been assessed by Afssa as being scientifically substantiated on two occasions. It has been demonstrated that omega-3 fatty acids play a role in healthy cardiovascular function, with a beneficial effect on intermediate criteria and on cardiovascular mortality. Moreover, this claim does not concern a particular physiological parameter and does not refer to any preventive effect whatsoever.

It appears therefore that, for a food fortified with omega-3 fatty acids, fulfilment of the criteria enabling the use of level 2 and 3 claims is closely linked to the demonstration of an undeniable nutritional benefit (inclusion of the overall composition of the vector food). **The working group is therefore recommending that a specific evaluation be required when the nutritional benefit is debatable.** Such an evaluation could refer to the specification for the choice of a nutrient/vector food pair (document in the process of being finalised at Afssa).

The consequences of the application of the criteria for these different levels of claim were also verified by Ciqual in order to ensure that foods likely to carry Level 3 claims are fully consistent with recommendations on cardiovascular prevention and clinical practice.

Substantiation of other claims

The working group considers, in the current state of knowledge, that certain claims used by manufacturers are not fully substantiated, in particular:

- **"Omega-3 fatty acids enable improved cardiovascular function", "omega-3 fatty acids to maintain a healthy heart and arteries"**

These claims are considered therapeutic claims or claims stating preventive properties, which are not permitted by the regulations or supported by scientific data.

- **"Consumed regularly, fatty acids make the blood more fluid"**

Data on the effect of omega-3 fatty acids at nutritional doses on platelet aggregation or bleeding time are insufficient to substantiate such a claim.

- **"Omega-3 fatty acids are included in recommendations for cholesterol-lowering diets"**

Even though this claim refers to dietary recommendations made for situations of excess cholesterol, it is unacceptable as it implies that omega-3 fatty acids have cholesterol-lowering properties which is incorrect: solely a hypotriglyceridaemic effect in hypertriglyceridaemic subjects, and not in the general population, has been attributed to them. However, it can be accepted with two conditions: 1) an explicit indication regarding the absence of a cholesterol-lowering effect from omega-3 fatty acids and 2) a precise indication on the type of diet being suggested.

- **"asset for a healthy heart"**

This claim is not justified as there is no evidence that omega-3 fatty acids cause an overall improvement in cardiac function in a clinically significant manner.

The working group would like to emphasise that the claims evaluated as part of this review do not in any sense constitute an exhaustive list of those considered, to date, as partially or wholly justified in scientific terms. For this reason, the specific evaluation of partially demonstrated claims and emergent claims is in no sense being called into question.

CONCLUSIONS

The working group would like to point out that the recommendations made in this report are based on variable levels of scientific proof and to reaffirm that the recommendations formulated are based on a consensus. Regular updating of this review and the proposals arising from it is therefore inevitable.
In view of the context, formulation of these recommendations was essential to limit the confusion surrounding the development of claims and to ensure the consumer is not misled.

While the working group accepts the principle of the fortification of foods with omega-3 fatty acids and the use of quantitative and qualitative claims arising from it, it would like to point out that dietary and health measures for preventing cardiovascular disease are unlikely to be based on one nutrient or family of nutrients, however attractive this may seem, but on a group of positive measures. As part of an overall nutrition policy, the consumption of fish with low mercury content, at least twice a week and the consumption of rapeseed oil, constitute effective means of rebalancing intakes of omega-3 fatty acids.

FIGURE 1
METABOLISM OF OMEGA-6 and OMEGA-3 POLYUNSATURED FATTY ACIDS

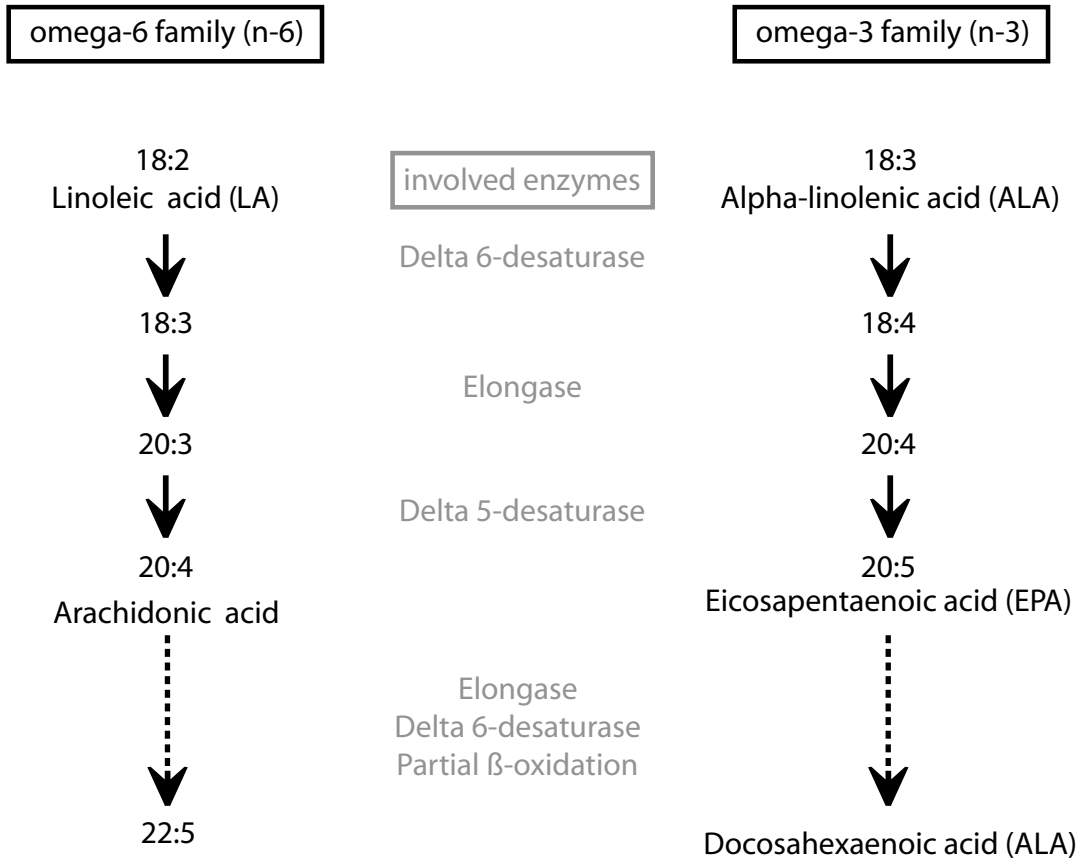
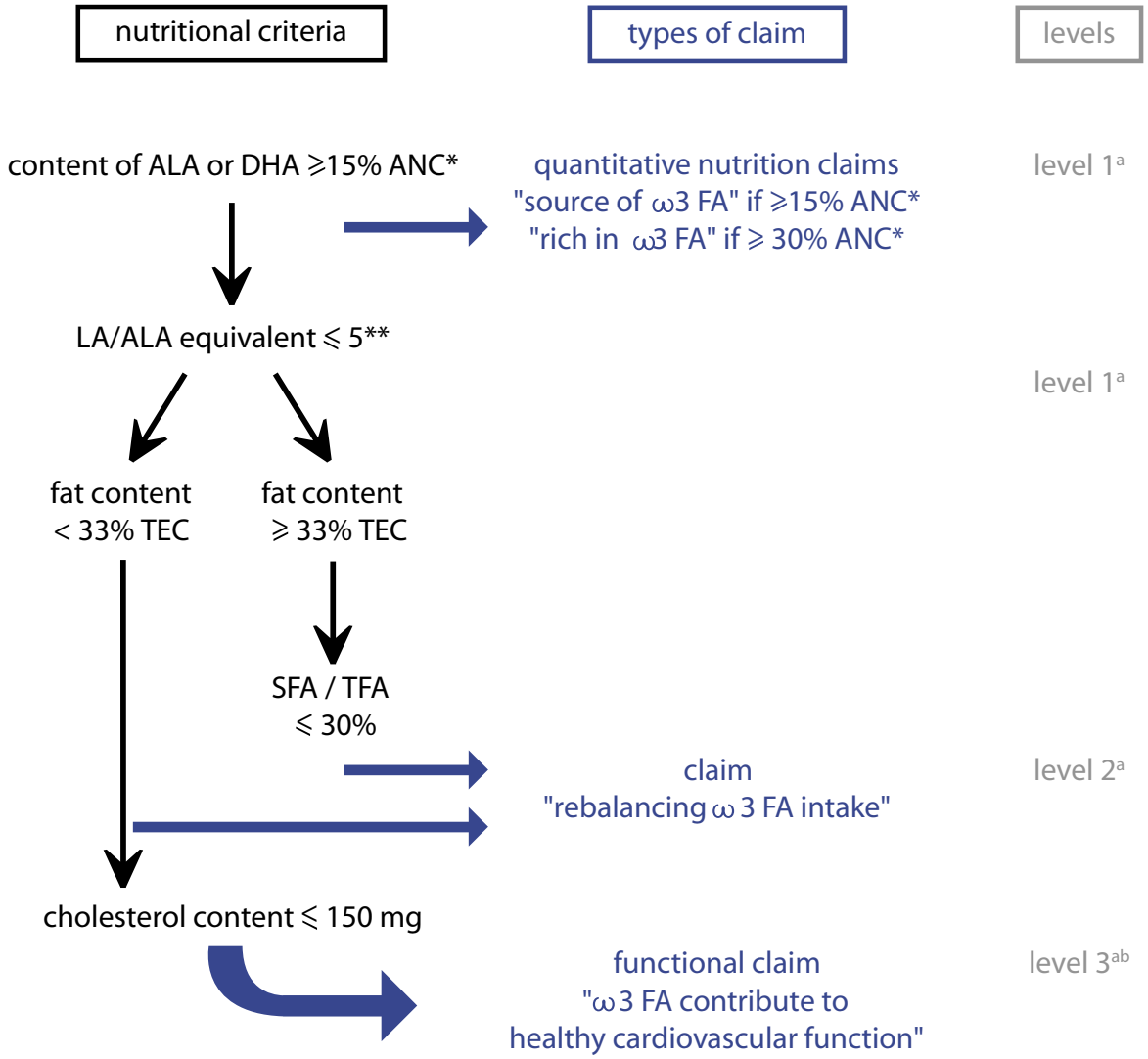


FIGURE 2
OMEGA-3 FATTY ACIDS: TYPES OF CLAIM POSSIBLE BASED ON THE NUTRITIONAL QUALITY OF FOOD



ω 3 FA: omega-3 fatty acids
LA: linoleic acid; ALA: alpha-linolenic acid; DHA: docosahexaenoic acid
SFA: satured fatty acids; TFA: total fatty acids; TEC: total energy content
*: ANC for adult men set at 2 g/day for ALA and 120 mg/day for DHA
**: the bio-equivalency factor for DHA and EPA to alpha-linolenic acid is set at 10
a: the general population in good health is used as a reference
b: populations at high cardiovascular risk (primary and secondary) are used as a reference

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REFERENCES

Scientific books and articles

Angerer P. and Von Schaky C. (2000). *N-3 polyunsaturated fatty acids and the cardiovascular system*. Curr. Opin. Lipidol. 11: 57-63.

Aruoma O.I. and Cuppet S.L. Editeurs (1997). *Antioxidant methodology. In vivo and in vitro concepts*. AOCS Press, Champaign, Illinois, 228-229.

Bemelmans W., Broer J., Feskens E., Smit A., Muskiet F., Lefrandt J., Bom V., May J. and Meyboom-de Jong B. (2002). *Effect of an increased intake of alpha-linolenic acid and group nutritional education on cardiovascular risk factors: the Mediterrean Alpha-linolenic Fortified Groningen Dietary intervention (MARGARIN) study*. Am. J. Clin. Nutr. 75: 221-227.

Brenna T.J. *Efficiency of conversion of alpha-linolenic acid to long chain n-3 fatty acids in man*. (2002). Curr. Opin. Clin. Nutr. Metab. Care 5:127-132.

Brown J.E. and Wahle K.W. (1990). *Effect of fish-oil and vitamin E supplementation on lipid peroxidation and whole-blood aggregation in man*. Clin. Chim. Acta. Dec.14; 193(3): 147-156.

Bucher H.C., Hengstler P., Schindler C. and Meier G. (2002). *N-3 polyunsaturated fatty acids in coronary heart disease: a meta-analysis of randomised controlled trials*. Am. J. Med.; 112(4): 298-304.

Burr M.L., Fehily A.M., Gilbert J.F., Rogers S., Holliday R.M., Sweetnam P. M., Elwood P.C. and Deadman N.M. (1989). *Effects of changes in fat, fish and fiber intakes on death on myocardial reinfarction: diet and reinfarction trial (DART)*. Lancet. 2(8666):757-761.

Combe N. and Boué C. (2001). *Apports alimentaires en acides linoléique et alpha-linolénique d'une population d'Aquitaine.* OCL 8 (2): 118-121.

Croset M., Vericel E., Rigaud M., Hanss M., Courpron P., Dechavanne M. and Lagarde M. (1990). *Functions and tocopherol content of blood platelets from elderly people after low intake of purified eicosepentaenoic acid*. Thromb. Res. 57 (1): 1-42.

De Lorgeril M., Renaud S., Mamelle N., Salen P., Martin J.L., Monjaud I., Guidollet J., Touboul P. and Delaye J. (1994). *Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease*. Lancet 343(8911): 1454-1459.

Dewailly E., Blanchet C., Lemieux S., Sauve L., Gingras S., Ayotte P. and Holub B.J. (2001). *N-3 fatty acids and cardiovascular disease risk factors among the Inuit of Nunavik*. Am. J. Clin. Nutr. 74: 4 464-473.

Expert panel on detection, evaluation and treatment of high blood cholesterol in adults. (2001). *Executive summary of the third report of the National cholesterol education program (NCEP) expert panel on detection evaluation and treatment of high blood cholesterol in adults (adult treatment panel III)*. J.A.M.A., May 16 ; 285(19): 2486-2497.

Fernandez-Reiriz M.J., Pastoriza L., Sampedro G. and Herrera J.J. (2000). *Changes in lipids of whole and minced rayfish (Raja clavata) muscle during frozen storage*. Z. Lebensm. Unters. Forsch. 200: 420-424.

Garcia-Arias M.T., Sanchez-Muniz F., Castrillon A. and Pilar Navarro M. (1994). *White tuna canning, total fat and fatty acid changes during processing and storage*. Journal of Food Composition and Analysis. 7: 119-130.

Guallar E., Sanz-Gallardo M.I., Veer P. v.'t, Bode P., Aro A., Gomez-Aracena J., Kark J.D., Riemersma R.A., Martin-Moreno J.M. and Kok F.J., for the Heavy Metals and Myocardial Infarction Study Group (2002). *Mercury, fish oils and the risk of myocardial infarction.* N. Eng. J. Med. Nov 28 ; 347: 1747-1754.

Grupo Italiano per la Studio della Sopravvivenza nell'Infarto miocardico (GISSI) (1999). *Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction : results of the GISSI-Prevenzione trial*. Lancet 354 (9177): 447-455.

Gunstone F., Harwood J. and Padley F. (1994). *The Lipid Handbook*. Chapman and Hall (Londres) 2e édition.

Hulshof K., van Erp-Baart M.A., Anttolainen M., Becker W., Church S.M., Couet C., Hermann-Kunz E., Kesteloot H., Leth T., Martins I., Moreiras O., Moschandreas J., Pizzoferrato L., Rimestad A.H., Thorgeirsdottir H., van Amelsvoort J.M.M., Aro A., Kafatos A.G., Lanzmann-Petithory D. and van Poppel G. (1999). *Intake of fatty acids in Western Europe with emphasis on trans fatty acids: the TRANSFAIR study*. Eur. J. Clin. Nutr., 53: 143-157.

Karleskind A. coordonnateur (1992). *Manuel des corps gras*. Ed. Tec&Doc, Paris.

Kolanowski W., Swiderski F. and Berger S. (1999*). Possibilities of fish oil application for food products fortification with omega-3 PUFA*. Int. J. Food Sci. Nutr., 50(1): 39-49.

Koletzko B., Thiel I. and Abiodun P.O. (1992). *The fatty acid composition of human milk in Europe and Africa*. J. Pediatr 120: S62-70.

Leaf A. and Weber P. (1988). *Cardiovascular effects of n-3 fatty acids*. N. Eng. J. Med. 318:549-557.

Macfarlane N. and Muggli R. (2000). *Fast Index : a sensory index of marine oil quality.* Proceedings of the 4 th congress of ISSFAL, Tsukuba, Japan.

Martin A. coordonnateur (2001). *Apports nutritionnels conseillés pour la population française.* Tec & Doc, 3e édition, Paris.

McLean L.R., Hagaman K.A and Davidson W.S. (1993). *Role of lipid structure in the activation of phospholipase A2 by peroxidized phospholipids.* Lipids 20: 505-509.

Medina I., Sacchi R. and Aubourg S. (2000). *Application of 13C NMR to the selection of the thermal processing conditions of canned fatty fish*. Eur. Food Res. Technol. 210: 176-178.

Pawlosky R.J., Hibbeln J., Novotny J.A. and Salem N. Jr. (2001). *Physiological compartmental analysis of -linolenic acid metabolism in adult humans*. J. Lipid Res. 42: 1257-1265.

Renaud S.C. and Lanzmann-Petithory D. (2002). *The beneficial effect of alpha-linolenic acid in coronary artery disease is not questionable.* Am. J. Clin. Nutr. 76 (4): 903-904 (letter to the editor).

Rueda F.M., Hernandez M.D., Egea M.A., Aguado F., Garcia B. and Martinez F.J. (2001). *Differences in tissue fatty acid composition between reared and wild sharpsnout sea bream, Diplodus puntazzo (Cetti, 1777)*. Br. J. Nutr. 86: 617-622.

Ruiz-Roso B., Cuesta I., Perez M., Borrego E., Perez-Olleros L. and Varela G. (1998). *Lipid composition and palatability of canned sardines. Influence of the canning process and storage in olive oil for five years*. J. Sci. Food Agric. 77: 244-250.

Sanderson P., Finnegan Y.E., Williams C.M., Calder P.C., Burdge G.C., Wootton S.A., Griffin B.A., Milward D.J., Pegge N.C. and Bemelmans W.J.E. (2002). *UK Food Standards Agency -linolenic acid workshop report*. Br. J. Nutr., 88: 573-579.

Second Joint Task Force of European and other Societies on coronary prevention (1998). *Prevention of coronary heart disease in clinical practice*. Eur. Heat J.; 19 (10): 1434-1503.

Singh R.B., Dubnov G., Niaz M.A., Ghosh S., Singh R., Rastogi S.S., Manor O., Pella D., and Berry E.M.L. (2002). *Effect of an Indo-Mediterranean diet on progression of coronary artery disease in high risk patients (Indo-Mediterranean Diet Heart Study): a randomised single-blind trial.* Lancet; 360 (9344): 1455-1461.

Souci S.W., Fachmann W. and Kraut H. (2000). *La composition des aliments. Tableaux des valeurs nutritionnelles*. 6e édition revue et complétée. Medpharm Scientific Publishers, CRC Press, Boca Raton.

Tokumura A., Sumida T., Toujima M., Kogure K. and Fukuzawa K (2000). *Platelet-activating factor (PAF)-like oxidized phospholipids : relevance to atherosclerosis*. Biofactors 13: 29-33.

Varela G., Pérez M. and Ruiz-Roso B. (1990). *Changes in the quantitative and qualitative composition of fat from fish, due to seasonality and industrial and culinary processing*. Bibl Nutr. Dieta. Basel, Karger, 46 : 104-109.

Vericel E., Rey C., Calzada C., Haond P., Chapuy P.H. and Lagarde M. (1992). *Age-related changes in arachidonic acid peroxidation and glutathione-peroxidase activity in human platelets.* Prostaglandins 43 (1): 75-85.

Vericel E., Calzada C., Chapuy P. and Lagarde M. (1999). *The influence of low intake of n-3 fatty acids on platelets in elderly people*. Atherosclerosis 147(1): 187-192.

Volatier J.-L. coordonnateur (2000). *Enquête INCA individuelle et nationale sur les consommations alimentaires*, Tec & Doc, Paris.

Voldrich M., Dobias J., Kalac P. Jr. and Curda D. (1991). *Changes of fatty acid composition during the processing of fish*. Die Narhung, 35: 663-664.

Yamada T., Strong J.P., Ishii T., Ueno T., Koyama M., Wagayama H., Shimizu A., Sakai T., Malcom GT. and Guzman M.A. (2000). *Atherosclerosis and omega-3 fatty acids in the populations of a fishing village and a farming village in Japan.* Atherosclerosis, 153(2): 469-481.

Yzebe D. (2000). *Efficacité des oméga-3 dans la prévention secondaire du risque cardio-vasculaire : méta-analyse d'essais thérapeutiques randomisés*. PhD Thesis at the University of Lyon 1.

Legislation

EC Regulation No. 258/97 of 27 January 1997 concerning novel foods and novel food ingredients (OJEC of 14 February 1997).

Council Directive 90/496/EEC of 24 September 1990 on nutrition labelling for foodstuffs (OJEC of 6 October 1990).

Decree of 15 April 1912 laying down government regulations implementing the Law of 1 August 1905 on the prevention of unfair commercial practices and the adulteration of foodstuffs.

Decree No. 93-1130 of 27 September 1993 concerning the labelling of nutritional qualities of foodstuffs (French OJ of 29 September 1993).

Order of 3 December 1993 implementing Decree No. 93-1130 of 27 September 1993 concerning the nutritional labelling of foodstuffs (French OJ of 26 December 1993).

Order of 11 October 2001 concerning the use of vitamin D in concerning the use of vitamin D in commonly eaten milk and fresh milk products (yoghurts and fermented milks, soft cheeses). (French OJ of 19 October 2001).

Public Health Code, Articles L. 5111-1 et 5122-14.

Consumer Code, Articles R 112.7, L 121.1, L 213.1 et L 213.2.

Information and documentation bulletin published by the Direction Générale de la Concurrence, de la Consommation et de la Répression des Fraudes No. 6. Official opinion 2000-147, *Lait de consommation enrichi en huile de poisson*, p 6. [Regular milk fortified with fish oil]

Draft regulation

Working document SANCO/1832/2002. Draft Proposal for Regulation of the European Parliament and of the Council on nutrition, functional and health claims made on foods.

Opinions issued by CEDAP

CEDAP opinion of 8 July 1998 concerning the non-misleading nature of threshold levels for nutrition claims. BOCCRF 31/08/99.

CEDAP opinion of 18 December 1996 on the recommendations regarding the non-misleading nature of functional nutrition claims. BOCCRF 7/10/97.

Afssa opinions and documentation

Afssa opinion dated 11 July 2000, 17 January 2001 and 15 May 2002, concerning the nutritional role of a regular milk fortified with omega-3 fatty acids from fish oil.

Afssa opinion dated 28 May 2001 concerning the evaluation of claims for a special salad oil with a guaranteed content of vitamin E and rich in omega-3 fatty acids.

Afssa opinion dated 1 June 2001 concerning the evaluation of a draft order on the use of vitamin D in commonly eaten milk and fresh milk products (yoghurts and fermented milks, soft cheeses).

Afssa opinion dated 21 October 2002 concerning the assessment of the health risks from mercury exposure to pregnant and lactating women and young children.

OCA technical note (2000). *Les aliments vecteurs de lipides d'origine animale ou mixte [Vector foods for fats of animal or mixed origin]*

Websites

ISSFAL statement on 3 Polyunsaturated Fatty Acids and Heart Disease, Oct. 2000, http://www.issfal.org.uk/heart_statement.htm

FDA website: http://www.cfsan.fda.gov

ANNEXES

ANNEXES

Annex 1: estimate of the alpha-linolenic and linoleic acid intakes in the population of the INCA survey and identification of missing data

Table A: values of the distribution parameters of the intake (g/d) of α-linolenic acid in the female population of the INCA study, by age group. (P: percentile)

Age group	Number	Mean ± standard deviation	[min-max]	median	5th P	10th P	90th P	95th P
3-5 yrs	111	0.09 ± 0.07	[0.00-0.34]	0.08	0.008	0.022	0.200	0.220
6-8 yrs	129	0.12 ± 0.08	[0.01-0.45]	0.12	0.026	0.034	0.226	0.279
9-11 yrs	113	0.13 ± 0.09	[0.01-0.52]	0.11	0.026	0.034	0.218	0.312
12-14 yrs	135	0.14 ± 0.09	[0.00-0.47]	0.11	0.029	0.041	0.280	0.319
15-24 yrs	140	0.16 ± 0.10	[0-0.46]	0.14	0.021	0.047	0.281	0.372
25-44 yrs	323	0.18 ± 0.16	[0-1.50]	0.14	0.026	0.049	0.310	0.389
45-64 yrs	206	0.17 ± 0.13	[0-0.81]	0.14	0.031	0.043	0.313	0.415
≥ 65 yrs	133	0.14 ± 0.11	[0.006-0.55]	0.11	0.023	0.045	0.287	0.404

Table B: values of the distribution parameters of the intake (g/d) of α-linolenic acid in the male population of the INCA study, by age group. (P : percentile)

Age group	Number	Mean ± standard deviation	[min-max]	median	5th P	10th P	90th P	95th P
3-5 yrs	132	0.10 ± 0.07	[0.01-0.49]	0.08	0.021	0.025	0.184	0.213
6-8 yrs	140	0.13 ± 0.12	[0.00-0.83]	0.11	0.018	0.030	0.224	0.352
9-11 yrs	125	0.16 ± 0.10	[0-0.52]	0.13	0.021	0.042	0.291	0.335
12-14 yrs	133	0.17 ± 0.12	[0-0.66]	0.14	0.026	0.062	0.295	0.419
15-24 yrs	114	0.20 ± 0.18	[0.00-1.59]	0.17	0.021	0.036	0.360	0.403
25-44 yrs	263	0.21 ± 0.16	[0-1.15]	0.17	0.046	0.058	0.401	0.487
45-64 yrs	183	0.20 ± 0.15	[0.00-1.31]	0.17	0.046	0.057	0.378	0.463
≥ 65 yrs	112	0.17 ± 0.15	[0-1.11]	0.14	0.007	0.040	0.319	0.439

Table C: values of the distribution parameters of the intake (g/d) of linoleic acid in the female population of the INCA study, by age group. (P: percentile)

Age group	Number	Mean ± standard deviation	[min-max]	median	5th P	10th P	90th P	95th P
3-5 yrs	111	0.93 ± 0.90	[0.05-4.23]	0.58	0.17	0.22	2.09	3.08
6-8 yrs	129	1.05 ± 1.04	[0.11-9.95]	0.79	0.24	0.28	1.87	2.33
9-11 yrs	113	1.22 ± 1.32	[0.08-8.56]	0.82	0.24	0.28	2.56	3.70
12-14 yrs	135	1.19 ± 1.24	[0.10-10.91]	0.85	0.16	0.28	2.32	3.57
15-24 yrs	140	1.52 ± 1.55	[0.07-8.99]	1.03	0.17	0.27	3.25	5.18
25-44 yrs	323	1.79 ± 2.53	[0.03-24.97]	1.20	0.23	0.38	3.50	5.64
45-64 yrs	206	1.94 ± 2.48	[0.02-19.30]	1.08	0.25	0.31	4.70	6.77
≥ 65 yrs	133	1.97 ± 3.83	[0.09-36.65]	0.83	0.23	0.33	4.26	9.08

Table D: values of the distribution parameters of the intake (g/d) of linoleic acid in the male population of the INCA study, by age group. (P : percentile)

Age group	Number	Mean ± standard deviation	[min-max]	median	5th P	10th P	90th P	95th P
3-5 yrs	132	1.04 ± 1.12	[0.06-8.44]	0.68	0.20	0.23	2.27	3.03
6-8 yrs	140	1.04 ± 0.83	[0.10-4.46]	0.80	0.19	0.25	2.14	3.00
9-11 yrs	125	1.59 ± 1.82	[0.09-10.97]	1.05	0.24	0.34	3.13	4.35
12-14 yrs	133	1.62 ± 2.19	[0-13.69]	1.00	0.27	0.38	2.41	5.59
15-24 yrs	114	1.72 ± 1.61	[0.10-9.43]	1.29	0.23	0.32	3.85	4.99
25-44 yrs	263	2.00 ± 2.26	[0-16.87]	1.28	0.34	0.45	4.02	5.66
45-64 yrs	183	2.18 ± 2.69	[0.06-24.80]	1.29	0.28	0.45	4.50	6.33
≥ 65 yrs	112	1.81 ± 3.28	[0-24.95]	0.91	0.20	0.30	3.01	5.97

Table E: values of the distribution parameters of the linoleic acid / α-linolenic acid ratio in the female population of the INCA study, by age group. (P: percentile)

Age group	Number	Mean ± standard deviation	[min-max]	median	5th P	10th P	90th P	95th P
3-5 yrs	111	13.6 ± 17.6	[2.5-134.6]	8.2	4.3	5.0	22.9	52.3
6-8 yrs	129	9.1 ± 6.9	[3.1-69.6]	7.3	4.3	5.0	14.9	16.9
9-11 yrs	113	10.4 ± 14.6	[3.3-155.5]	8.1	4.6	5.1	14.0	19.0
12-14 yrs	135	10.1 ± 9.5	[2.5-74.2]	7.5	3.6	4.8	16.5	33.4
15-24 yrs	139	11.7 ± 21.5	[1.9-240.0]	7.3	3.4	4.6	16.7	26.3
25-44 yrs	322	13.0 ± 37.4	[1.9-643.7]	7.7	3.6	4.5	17.9	30.0
45-64 yrs	204	13.2 ± 17.1	[0.9-130.7]	8.0	3.9	4.4	25.2	46.3
≥ 65 yrs	133	19.5 ± 73.6	[2.7-822.2]	7.4	3.4	4.1	23.4	33.6

Table F: values of the distribution parameters of the linoleic acid / α-linolenic acid ratio in the male population of the INCA study, by age group. (P: percentile)

Age group	Number	Mean ± standard deviation	[min-max]	median	5th P	10th P	90th P	95th P
3-5 yrs	132	11.1 ± 8.0	[2.6-50.1]	8.7	4.6	5.2	17.8	29.5
6-8 yrs	140	10.2 ± 8.3	[3.6-64.3]	7.7	4.4	4.9	17.8	22.6
9-11 yrs	124	15.4 ± 57.3	[3.7-635.5]	7.3	4.4	4.7	16.2	29.8
12-14 yrs	132	9.6 ± 8.7	[3.4-63.8]	7.1	4.5	4.90	16.0	23.5
15-24 yrs	114	11.2 ± 13.9	[3.0-110.1]	7.2	4.1	5.0	18.4	31.2
25-44 yrs	262	10.5 ± 11.6	[1.7-127.6]	7.7	3.8	4.6	16.9	20.3
45-64 yrs	183	11.7 ± 13.2	[1.9-123.4]	7.7	3.8	4.3	19.3	30.7
≥ 65 yrs	110	13.9 ± 28.9	[2.7-267.9]	7.1	3.7	4.4	19.2	45.1

Table G: comparison of the quantity of fish consumed based on the knowledge (where available) of the α-linolenic content in the female population by age group

Age groups	Mean quantity of fish (g/d)		Mean quantity of crustacea (g/d)	
	ω3 content known	ω3 content unknown	ω3 content known	ω3 content unknown
3-5 yrs	0.6	17.2	0.4	1.1
6-8 yrs	1.5	19.1	0.4	0.9
9-11 yrs	1.3	20.7	0.4	1.0
12-14 yrs	1.1	17.1	0.3	1.5
15-24 yrs	2.6	18.8	1.1	3.2
25-44 yrs	2.0	24.4	1.2	3.2
45-64 yrs	3.3	28.0	1.4	2.8
≥ 65 yrs	1.3	33.7	1.7	3.2

Table H: comparison of the quantity of fish consumed based on the knowledge (where available) of α-linolenic content in the male population by age group

Age groups	Mean quantity of fish (g/d)		Mean quantity of crustacea (g/d)	
	ω3 content known	ω3 content unknown	ω3 content known	ω3 content unknown
3-5 yrs	0.9	16.9	0.4	0.8
6-8 yrs	0.8	20.7	1.1	1.6
9-11 yrs	2.0	18.3	0.3	1.9
12-14 yrs	1.9	22.3	1.9	0.7
15-24 yrs	1.5	23.4	1.1	2.0
25-44 yrs	3.1	24.3	1.5	2.3
45-64 yrs	4.0	34.2	2.3	3.7
≥ 65 yrs	3.2	31.2	2.3	3.6

Table I: list of foods in the CIQUAL database for which the contents of linoleic and/or alpha-linolenic acids are available

(data from January 2002[1], 130 products)

Food	Linoleic acid (g / 100 g)	α-linolenic acid (g / 100 g)	Fat (g / 100 g)
Lamb, cutlet, grilled	0.16	0.14	16
Lamb, shoulder, roasted	0.37	0.22	24
Lamb, leg, roasted	0.22	0.17	14
Anchovy, European, raw	ND	0.04	4.5
Beaufort cheese	0.71	ND	32.7
Butter	1.16	0.46	82.5
Winkle, boiled	0.04	0.06	1.2
Puffed wheat cereal	0.54	0.04	1.3
Bœuf bourguignon	0.71	0.06	8
Beef, rump, steak, broiled	0.53	0.04	4.9
Beef, braised	0.61	0.03	12.2
Beef, sirloin steak, broiled	0.15	0.04	6.6
Soy drink, plain	ND	0.19	2.1
Blood sausage, raw	2.45	0.2	30.1
Brie cheese	0.36	0.16	27.5
Whelk, cooked, moist heat	0.01	0	1.4
Cod, raw	0	0	0.6
Cannelloni with meat	1.2	0.14	11.4
Cantal cheese	0.47	ND	30.5
Carré de l'Est cheese	0.45	ND	25.5
Bran breakfast cereal	1.44	0.1	3
Chabichou cheese	0.79	ND	29.6
Chaource cheese	0.42	ND	24
Cheddar cheese	0.45	0.23	33.5
Horse, meat, raw	ND	0.2	4.2
Comté cheese	0.68	ND	31.3
Cookies	2.4	0.12	22.9
Crab, poached	0.02	0.02	5.3
Light custard cream	ND	0.01	4.7
Custard cream	0.01	0.02	2
Whipped cream, sweetened, pressure, UHT	0.53	0.15	31.2

1 These are the data used to estimate intakes of 18:2 n-6 and 18:3 n-3 based on the data from the INCA survey (tables A to H)

Food	Linoleic acid (g / 100 g)	α-linolenic acid (g / 100 g)	Fat (g / 100 g)
Cream, raw	0.63	ND	33.5
Chocolate custard, commercial	0.08	0.02	3.9
Reduced fat pouring cream, sterilised	0.29	ND	17.3
Cream, low fat, UHT	0.59	ND	34.4
Crème fraîche	0.59	0.13	34.5
Crottin cheese	0.85	ND	31.9
Turkey, breast, meat (only), sauted	0.43	0.02	2.7
Edam cheese	0.33	0.13	26
Emmental cheese	0.63	ND	28.8
Semi-hard cheese 20-30% fidm	0.19	ND	12.3
Blue cheese	0.54	ND	29
Goat cheese, semi-dry	0.77	ND	29
Goat cheese, fresh, average	0.16	ND	6.1
Goat cheese, soft-ripened, average	0.46	ND	17.5
Goat cheese, dry, average	1.05	ND	39.4
Pyrénées cheese	0.45	ND	29.5
Processed cheese 25% fidm	0.15	0.05	8.2
Processed 45% fidm	0.38	0.21	22.7
Processed 65% fidm	0.6	ND	32.1
Processed 70% fidm	0.59	ND	31.9
Uncured cheese 30% fidm, smooth,	0.14	ND	6
Uncured cheese 40% fidm, salted	0.31	ND	13.3
Uncured cheese 70% fidm, salted, flavoured	0.79	ND	34.4
Fromage frais, 40% fidm, plain	0.17	0.02	8.3
Bonbel-Babybel ® type cheese	0.38	ND	24.8
Camembert-type cheese 75% fidm	0.69	0.1	39
Gouda cheese	0.23	ND	27.4
Goose fat	9.4	ND	99.6
Haricot bean, dried	0.81	ND	1.2
Peanut oil	30.5	0	99.9
Rapeseed oil	21.2	9.6	99.9
Maize (corn) oil	55.9	0.9	99.9
Walnut oil	56.7	12.3	99.9
Grapeseed oil	67.3	0.3	99.9
Soya oil	52.6	7.3	99.9

Food	Linoleic acid (g / 100 g)	α-linolenic acid (g / 100 g)	Fat (g / 100 g)
Sunflower seed oil	64.1	0.05	99.9
Olive oil	12.9	0.85	99.9
Vegetable oil, blended, well-balanced	47	1.2	99.9
Oyster, raw	0.02	0.01	1.6
Milk, goat's	0.11	0.03	3.7
Milk, bulk	0.07	ND	3.6
Semi-skimmed milk, pasteurised	0.03	0.01	1.6
Semi-skimmed milk, UHT	0.03	ND	1.6
Milk, whole, evaporated	0.13	ND	7.5
Milk, whole, condensed, sweetened	0.15	ND	9.1
Milk, whole, pasteurised	0.08	0.02	3.5
Milk, whole UHT	0.07	0.02	3.5
Lasagne	0.74	0.11	8.2
Reduced fat margarine 60% fat partially hydrogenated	22	1.5	55.8
Sunflower seed margarine	29.7	2	77.9
Cooking margarine	12.4	1.24	82.5
Maroilles cheese	0.41	ND	29
Light spread (38-45% fat)	3.72	0.47	38.2
Mayonnaise, soy oil	41.1	2.03	78.6
Mayonnaise, low calorie	19.7	0.94	37.8
Morbier cheese	0.43	ND	28.1
Mussels, cooked in water	0.02	0.02	3.1
Muesli	2.3	0.07	9.2
Muenster cheese	0.41	ND	28.5
Neufchâtel cheese	0.51	ND	26.8
Egg, whole, raw	1.59	0.07	10.5
Lumpfish roe, semi-conserved	0.07	0.03	6.6
Parmesan cheese	0.29	ND	27.6
Cornflakes, enriched	0.21	0.07	0.9
Small fromage frais 30% fidm, with fruit	0.07	0.01	5.5
Petit-suisse 40% fidm	0.23	ND	10.1
Petit-suisse 60% fidm	0.35	ND	18.5
Picodon cheese	0.77	ND	29.1
Pilchard in tomato sauce, canned	0.12	0.07	8.4
Pizza, tomato and cheese	0.96	0.19	10.4

Food	Linoleic acid (g / 100 g)	α-linolenic acid (g / 100 g)	Fat (g / 100 g)
Pont l'Evêque cheese	0.34	ND	24
Pork, chop, grilled	1.99	0.19	15.3
Chicken, leg, meat and skin, roasted	2	0.32	12.8
Pouligny Saint-Pierre cheese	0.75	ND	28.3
Raclette cheese	0.78	ND	28.3
Reblochon cheese	0.38	ND	26.6
Rice, parboiled, raw	1.64	0.04	1.3
Roquefort cheese	0.64	ND	31.7
Rouy cheese	0.38	ND	26.5
Lard	8.1	ND	99
Sainte-Maure cheese	0.77	ND	28.9
Saint-Nectaire cheese	0.43	ND	27.8
Saint-Paulin cheese	0.35	ND	22.7
Sardine in oil, canned, drained	2.47	0.35	13.7
Sardine, raw	ND	0.06	6.9
Sardine, tomato sauce, tinned	1.14	0.19	11.8
Frankfurter sausage	2.57	0.22	26.1
Selles-sur-Cher cheese	0.75	ND	28.4
Ground steak 10% fat, raw	0.15	0.06	10.1
Taramasalata	10.74	4.52	54
Tuna, canned in oil, drained	2.9	0.7	8.4
Tuna, canned in brine, drained	0.03	0	2.1
Tofu	ND	0.82	6.8
Tomme cheese	0.4	ND	26
Vacherin cheese	0.4	ND	27.8
Yoghurt, whole milk, flavoured	0.05	ND	3.2
Yoghurt, whole milk, with fruit	0.05	0.02	2.9
Yoghurt, non-fat, with fruit, intensive sweetener	0.01	0.01	0.3
Yoghurt, whole milk, plain	0.06	0.02	3.7

ND: not determined

Table J: Total fat, linoleic and/or alpha-linolenic acid contents of foods in the CIQUAL database, by food family

(data updated in December 2002[2], 238 products)

Food group	Food	Linoleic acid (g / 100 g)	α-linolenic acid (g / 100 g)	Fat (g / 100 g)
Butter and Cream	Butter	1.16	0.46	82.5
	Whipped cream, pressure, UHT	0.53	0.15	31.2
	Unpasteurised cream	0.63	ND	33.5
	Reduced fat pouring cream, sterilised	0.29	ND	17.3
	Pouring cream, sterilised	0.59	ND	34.4
	Crème fraîche	0.52	0.12	30.5
	Reduced fat crème fraîche	0.3	0.08	15.6
Sweet biscuits	Cookies	2.4	0.12	22.9
	Wafer cookie with chocolate filling	0.93	0.06	29.7
Non-alcoholic drinks	Soya drink, plain	ND	0.19	2.15
Cereals and pasta	Parboiled white rice, raw	1.64	0.04	1.3
Breakfast cereals	Puffed wheat breakfast cereal	0.54	0.04	1.3
	Bran breakfast cereal	1.3	0.09	2.7
	Muesli	2.3	0.07	9.2
	Cornflakes, fortified	0.21	0.07	0.9
Meat products	Black pudding, raw	2.45	0.2	30.1
	Frankfurter sausage	2.57	0.22	26.2
	Taramasalata (1)	10.74	4.52	54
Condiments and sauces	Mayonnaise with soya oil	41.1	2.03	80.3
	Mayonnaise, reduced fat	19.7	0.94	37.8
	Tapenade (olive paste)	3.35	ND	34.3
Crustacea and shellfish	Winkle, cooked	0.04	0.06	1.2
	Whelk, cooked	0.01	0	1.4
	Squid, raw	ND	0	1.1
	Crab, poached	0.02	0.02	5.3
	Prawn, raw	ND	0.77	1.1
	Oyster, raw	0.02	0.01	1.6
	Langoustine, raw	ND	0.21	0.3
	Mediterranean mussel, raw	ND	0.6	1.7
	Mussel, cooked in water	0.02	0.02	3.1
	Octopus, raw	ND	0	0.8
Milk based desserts	Custard	0.02	0.01	4.7
	Crème caramel	0.01	0.02	2
	Chocolate cream dessert, chilled cabinet	0.08	0.02	3.7
	Dairy ice cream	0.17	0.01	9.6
Miscellaneous	Tofu	ND	0.88	7.5
Starters and snacks	Pizza, tomato and cheese	0.96	0.19	10.4
Semi-soft cheeses	Cantal cheese	0.47		30.5
	Cheddar cheese	0.45	0.23	33.5
	French Edam cheese	0.33	0.13	26
	Hard cheese 20-30% fidm	0.19	ND	12.3
	Gouda cheese	0.23	ND	27.4
	Morbier cheese	0.43	ND	28.1
	Raclette cheese	0.78	ND	28.3

[2] Ciqual data, updated after estimation of the consumption of 18:2 n-6 and 18:3 n-3 based on data from the INCA survey (tables A to H).

Food group	Food	Linoleic acid (g/100 g)	α-linolenic acid (g/100 g)	Fat (g/100 g)
Semi-soft cheeses	Saint-Nectaire cheese	0.43	ND	27.8
	Saint-Paulin cheese	0.35	ND	22.7
	Tomme cheese	0.4	ND	26
Soft cheeses	Brie cheese	0.36	0.16	27.5
	Carré de l'Est cheese	0.45	ND	25.5
	Chaource cheese	0.42	ND	24
	Camembert-type cheese 75% fidm	0.69	0.1	39
	Soft ripened cheese, mould rind	0.44	ND	25
	Soft-ripened cheese, mould rind, low fat	0.22	ND	12.4
	Soft-ripened cheese, washed rind, average	0.42	ND	26.7
	Maroilles cheese	0.41	ND	29
	Munster cheese	0.41	ND	28.5
	Neufchâtel cheese	0.51	ND	26.8
	Pont l'Évêque cheese	0.34	ND	24
	Reblochon cheese	0.38	ND	26.6
	Rouy cheese	0.38	ND	26.5
	Vacherin cheese	0.4	ND	27.8
Blue cheeses	Blue cheese	0.54	ND	29
	Roquefort cheese	0.64	ND	31.7
Hard cheeses	Beaufort cheese	0.71	ND	32.7
	Comté cheese	0.68	ND	31.3
	Emmental cheese	0.63	ND	28.8
	Hard cheese	0.65	ND	30
	Parmesan cheese	0.29	ND	27.6
Goat cheeses	Chabichou cheese	0.79	ND	29.6
	Crottin cheese	0.85	ND	31.9
	Soft-ripened goat cheese, average	0.46	ND	17.5
	Goat cheese, semi-dry, average	0.77	ND	29
	Goat cheese, fresh average	0.16	ND	6.1
	Goat cheese with peppercorns	0.53	ND	20
	Goat cheese, dry. average	1.05	ND	39.4
	Picodon cheese	0.77	ND	29.1
	Pouligny Saint-Pierre cheese	0.75	ND	28.3
	Sainte-Maure cheese	0.77	ND	28.9
	Selles-sur-Cher cheese	0.75	ND	28.4
Processed cheeses	Processed cheese 25% fidm	0.15	0.05	8.2
	Processed cheese 45% fidm	0.38	0.21	22.7
	Processed cheese 65% fidm	0.6	ND	32.1
	Processed cheese 70% fidm	0.59	ND	31.9
Uncured cheese	Uncured cheese 30% fidm, smooth, plain	0.14	ND	6
	Uncured cheese 40% fidm, slightly salted	0.31	ND	13.3
	Uncured cheese 50% fidm, with fruit	0.12	ND	5.4
	Uncured cheese 50% fidm, plain	0.25	ND	11
	Uncured cheese 60% F/DM, slightly salted	0.57	ND	24.7
	Fromage frais 70% fidm, salted, with herbs	0.79	ND	34.4
	Fromage frais 40% fidm, plain	0.17	0.02	8.3
	Fromage frais 40% fidm, plain, moulded	0.17	ND	7.2
	Fromage frais 0% fidm, plain, moulded	0.002	ND	0.1
	Fromage frais 0% fidm, plain	0.001	ND	0.05
	Fromage frais 20% fidm, plain	0.06	ND	2.6

Food group	Food	Linoleic acid (g/100 g)	α-linolenic acid (g/100 g)	Fat (g/100 g)
Uncured cheese	Petit Suisse type cheese 20% fidm with fruit	0.06	ND	2.6
	Petit Suisse type cheese 20% fidm plain	0.1	ND	4.5
	Petit Suisse type cheese 30% fidm with fruit	0.07	0.01	5.5
	Petit Suisse 40% fidm	0.23	ND	10.1
	Petit-Suisse 60% fidm	0.35	ND	18.5
Milks	Whole milk, evaporated	0.13	ND	7.5
	Whole milk, sweetened, condensed	0.15	ND	9.1
	Sheep's milk	0.17	0.07	7
	Goat's milk	0.11	0.03	3.7
	Human milk	0.48	0.03	4
	Blended milk, bulk	0.07	ND	3.6
	Mare's milk	0.18	ND	1.8
	Semi-skimmed milk, pasteurised	0.03	0.01	1.6
	Semi-skimmed milk, sterilised	0.03	ND	1.6
	Semi-skimmed milk, UHT	0.03	ND	1.6
	Skimmed milk, pasteurised	0.01	0.001	0.1
	Skimmed milk, sterilised	0.002	ND	0.1
	Skimmed milk, UHT	0.002	ND	0.1
	Skimmed milk, dried	0.01	ND	0.5
	Whole milk, dried	0.52	ND	26.3
	Semi- skimmed milk, dried	0.35	ND	16
	Whole milk, raw	0.07	ND	3.6
	Whole milk, pasteurised	0.08	0.02	3.5
	Whole milk, sterilised	0.07	0.02	3.5
	Whole milk, UHT	0.07	0.02	3.5
	Semi- skimmed milk, flavoured	0.01	ND	0.6
Vegetables	Tomato paste	0.23	ND	0.6
	Haricot bean, dry	0.81	ND	1.2
Fats and oils	Shea butter, Africa	5.98	ND	99.9
	Coconut fat, hydrogenated	0	ND	99.9
	Duck fat	12	ND	99.8
	Turkey fat	21.2	ND	99.8
	Palm kernel, fat, raw	2.7	ND	99.9
	Palm kernel, fat, raw, Colombia	3	ND	99.9
	Chicken fat	19	ND	99.7
	Goose fat	9.4	ND	99.6
	Coconut fat or oil	1.02	ND	99.9
	Coconut fat or oil, raw	1.6	ND	99.9
	Coconut fat or oil, refined	1.6	ND	99.9
	Almond oil	21.9	ND	99.9
	Apricot kernel oil	27.2	ND	99.9
	Peach kernel oil	17.5	ND	99.9
	Peanut oil	30.5	0	99.9
	Peanut oil, Africa	20.2	ND	99.9
	Peanut oil, south America	36.2	ND	99.9
	Peanut oil, Chinese	34.5	ND	99.9
	Peanut oil, USA	29.4	ND	99.9
	Avocado oil	11.9	ND	99.9
	Butter oil	2.27	ND	99.6
	Borage oil	35.27	0.3	99.9

Food group	Food	Linoleic acid (g/100 g)	α-linolenic acid (g/100 g)	Fat (g/100 g)
	Safflower seed oil, >50% linolenic ac.	75.2	ND	99.9
	Safflower seed, >50% oleic acid	19	ND	99.9
	Rapeseed oil	21.2	9.6	99.9
	Rapeseed oil >5% erucic acid	12.9	ND	99.9
	Cottonseed oil	48.8	ND	99.9
	Cod liver oil	0.9	ND	99.9
	Wheatgerm oil	53.5	ND	99.9
	Maize germ oil, refined	56.7	ND	99.9
	Rye germ oil	55	ND	99.9
	Herring oil	1	ND	99.9
	Linseed oil	13.4	ND	99.9
	Lupin oil	15.4	ND	99.9
	Maize oil	55.9	0.9	99.9
	Menhaden oil	1	ND	99.9
	Hazelnut oil	15.4	ND	99.9
	Walnut oil	56.7	12.3	99.9
	Palm oil, raw, Africa	10	ND	99.9
	Palm oil, Africa	9.82	ND	99.9
	Palm oil, South America	17.6	ND	99.9
	Papaya oil	3.7	ND	99.9
	Blackcurrant seed oil	43.5	12.4	99.9
	Grapeseed oil	67.3	0.3	99.9
	Tomato seed oil	51.8	ND	99.9
Fats and oils	Pistachio oil	27.9	ND	99.9
	Castor oil	3.2	ND	99.9
	Sesame seed oil	42.1	ND	99.9
	Soya oil	52.6	7.3	99.9
	Rice oil	15.4	ND	99.9
	Sunflower seed oil	64.1	0.05	99.95
	Olive oil	12.9	0.85	99.95
	Olive oil, North African	10.7	ND	99.9
	Olive oil, European	6.5	ND	99.9
	Evening primrose oil	68.7	0.19	99.9
	Sardine oil	1.2	ND	99.9
	Vegetable oil, blended, dietetic	47	1.2	99.95
	Semi fat margarine 60% fat, partially hydrogenated	22	1.5	55.8
	Corn margarine	31.1	ND	82.5
	Sunflower seed margarine	29.7	2	73.95
	Cooking margarine	12.4	1.24	82.5
	Blended margarine	ND	ND	60
	Vegetable margarine	14.3	ND	82.5
	Low fat dairy spread	3.72	0.47	38.2
	Lard	8.1	ND	99
	Beef suet, refined	1.4	ND	99.9
	Mutton suet	5.5	ND	99.9
Eggs and egg products	Egg, whole, raw	1.59	0.07	10.5
	Bœuf bourguignon	0.71	0.06	8
Dishes	Cannelloni with meat	1.2	0.14	11.4

Food group	Food	Linoleic acid (g/100 g)	α-linolenic acid (g/100 g)	Fat (g/100 g)
	Lasagne	0.74	0.11	8.2
Fish and batrachians	Anchovy, European, raw	ND	0.04	4.5
	Bogue	ND	0	1.2
	Atlantic bonito, raw	ND	0.05	5
	Cod, raw	0	0	0.6
	Horse mackerel, raw	ND	0	1.5
	Sturgeon, raw	ND	0.05	5.8
	Chub mackerel, raw	ND	0	4
	Mackerel, raw	ND	0	14.2
	Whiting, raw	ND	0	0.8
	Mullet, raw	ND	0.01	2.2
	Caviar substitute	0.07	0.03	6.6
	Pilchard, Tomato sauce, canned	0.12	0.07	8.4
	Red mullet, raw	ND	0	2.7
	Sardine, in oil, canned, drained	2.67	0.38	14.8
	Sardine, raw	ND	0.06	6.9
	Sardine, grilled	1.2	ND	7.8
	Sardine, tomato sauce, canned (2)	1.14	0.19	11.8
	Salerna, raw	ND	0	3.7
	Sole, raw	ND	0	1.1
	Tuna, canned in oil	4.3	1	12.3
	Tuna, canned, in brine	0.03	0	2.1
Potatoes and starchy vegetables	Potato, peeled, raw	0.09	0.03	0.2
Sugars and confectionery	Cereal bar, low calorie	2.5	ND	7.2
	Frozen chocolate bar	1.11	0.06	23.2
Meat	Lamb, cutlet, grilled	0.16	0.14	16
	Lamb, shoulder, roasted	0.37	0.22	24
	Lamb, leg, roasted	0.22	0.17	14
	Beef, steak, grilled	0.53	0.04	4.9
	Beef, braised	0.6	0.02	12
	Beef, sirloin, grilled	0.15	0.04	6.6
	Horse, meat, raw	ND	0.2	4.2
	Pork, chop, grilled	1.99	0.19	15.3
	Ground steak 10% fat, raw	0.15	0.06	10.1
Poultry	Turkey, breast, meat (only), sauted	0.43	0.02	2.7
	Guinea fowl, leg	0.31	ND	1.8
	Guinea fowl, breast	0.12	ND	0.7
	Chicken, leg, skin and meat, roasted	2	0.32	12.8
Yoghurts and similar	Yoghurt, Greek style	0.18	0.05	9.2
	Low fat yoghurt, flavoured	0.03	ND	1.8
	Whole milk yoghurt, flavoured	0.05	ND	3.2
	Whole milk yoghurt, with fruit	0.05	0.02	2.9
	Non- fat yoghurt with fruit, intense sweetener	0.02	ND	0.2
	Low fat yoghurt, plain	0.03	0.007	1.1
	Whole milk yoghurt, plain	0.06	0.02	3.7
	Yoghurt, whole milk, Bulgarian-style, plain	0.06	ND	3.4

ND: not determined
(1): For taramasalata, the alpha-linolenic acid content is probably due to the use of rapeseed oil as an ingredient.
(2): The difference in the alpha-linolenic acid content from that of raw sardines may be explained by the use of the tomato sauce (which may contain oil providing alpha-linolenic acid)

Annex 2: estimate of the linoleic acid (18:2 n-6) and alpha-linolenic acid (18:3 n-3) intakes in the population of the SU.VI.MAX study (provisional data)

Figure I: distribution of linoleic acid (18:2 n-6) intakes, according to SU.VI.MAX

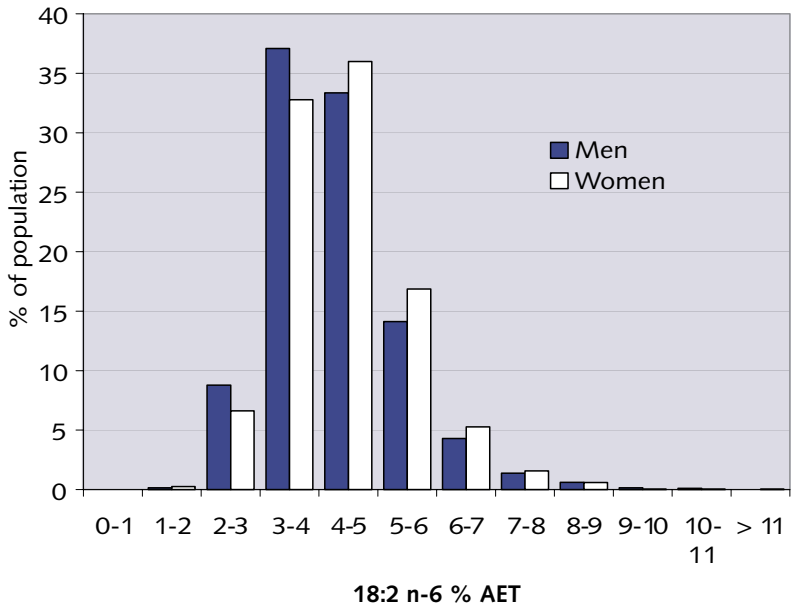


Figure II: distribution of alpha-linolenic (18:3 n-3) intake, according to SU.VI.MAX

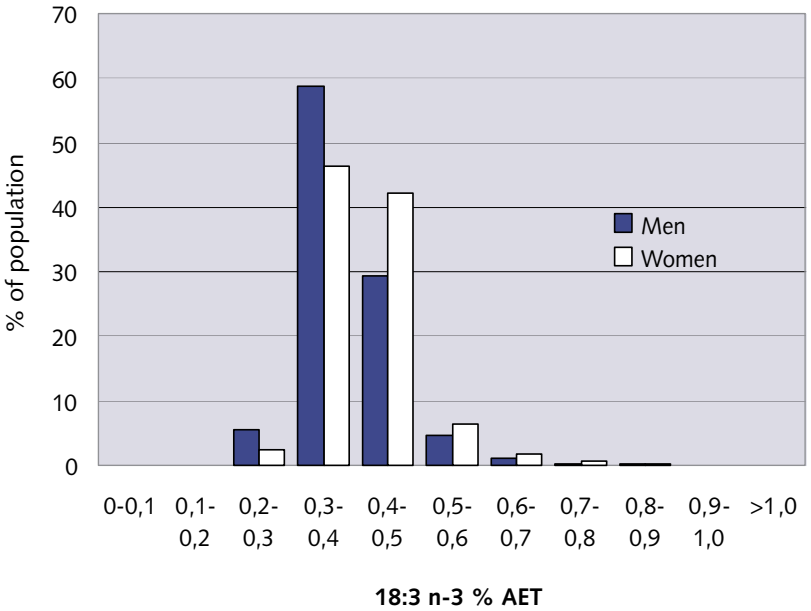


Figure III: food contribution to linoleic acid intake (for men and women), according to SU.VI.MAX

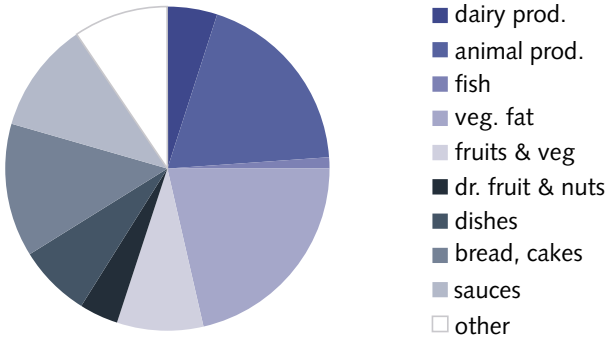


Figure IV: food contribution to alpha-linolenic acid intake (for men and women) according to SU.VI.MAX.

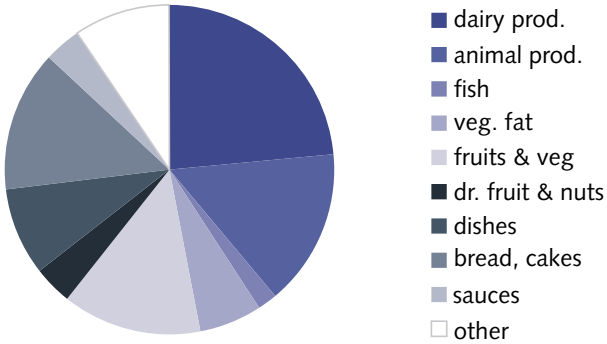
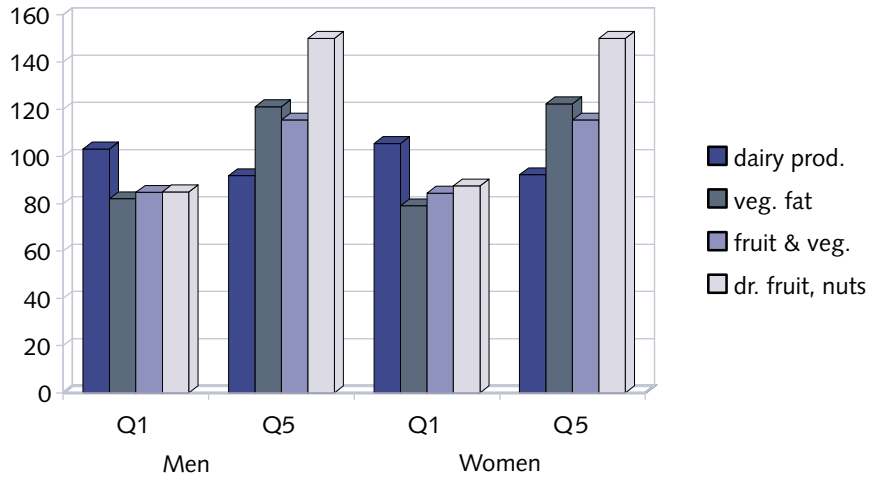


Figure V: consumption characteristics of the 1st and 5th quintiles of alpha-linolenic acid intake (as a % of mean consumption), according to SU.VI.MAX.



Annex 3: the omega-3 fatty acid composition of some foods and variability factors

The recommended intakes for omega-6 and omega-3 PUFA, evaluated in the form of the ratio of the quantity of linoleic acid (C18:2 n-6; LA) to the quantity of α-linolenic acid (C18:3 n-3; ALA), usually between 4 and 10, should now result in a ratio closer to 5 due to the desirable limitation of linoleic acid and the value of maintaining a sufficient intake of α-linolenic acid (Martin, 2001).
Examination of Table K shows that this hope is practically unachievable at the present time; a single source of dietary fat enables these conditions to be fulfilled, namely rapeseed oil.
The question takes on another aspect if omega-3 fatty acids (ω3 FA), for which seafood, fish in particular, are a very rich source, are considered globally. These provide very considerable quantities of EPA, DPA and DHA (Table L).

Special focus on the variability of the composition of fish

The data concerning the variability of the omega-3 fatty acid content of fish flesh are relatively confused.
Overall, there are different variation factors regardless of species and individual variability. These comprise the time of year in which the fish is caught, the place it is caught or the farming method, the initial preparation method, storage time and type of storage medium or preservative or the duration and intensity of freezing.
The discrepancies are extreme depending on the example used. An enrichment in alpha-linolenic acid is observed when the oil used for canned tuna is soya bean oil, with a 10% reduction in the content of EPA and DHA (Garcia-Arias *et al*, 1994). Levels of EPA are reduced by 30% when sardines and tuna are canned in olive oil (Ruiz-Roso *et al*, 1998; Medina *et al*, 2000). Smoking reduces the omega-3 fatty acid content of mackerel fillets by 30% while this content is preserved in the same fillets when marinated (Voldrich *et al*, 1991). In contrast, freezing rayfish does not cause a loss of omega-3 fatty acids (Fernandez-Reiriz *et al,* 1995). The same applies to farmed fish which maintain or even increase their content of omega-3 fatty acids (Rueda *et al*, 2001). However, frying sardines in olive oil causes a moderate loss of omega-3 fatty acids (-20 %) and in the case of hake 95% of omega-3 fatty acids are eliminated (Varela *et al*, 1990).

Table K: sources of omega-3 fatty acids with 18 carbon atoms

	C18:2/ C18:3 ratio	PUFA** /SFA	% C18:3 in FA	% C18:3 in the product (g/100 g)	Nutritional density ω3FA mg/100 kcal	% ANC ALA per 100 g
ANC ALA (adult male)*: 2 $g.d^{-1}$	5	0.615	2.5	-	90 to 100	
Human breast milk	5.7	0.21	≈ 1	-		
Fats						
Lard	9	0.23	≈ 1	≈ 1	≈ 100	≈ 50
Beef suet	1	0.1	2	2	220	100
Mutton fat	0.7	0.03	0.2	0.2	20	10
Butter	1.2	0.02	≈ 1	≈ 1	≈ 133	≈ 50
Meats						
Horse	0.57	0.87	16	0.1 to 0.8	247	5 to 40
Rabbit	4.9	0.64	4	0.02 to 0.2	200	1 to 10
Chicken	6.3	0.84	3	0.02 to 0.2	75	1 to 10
Duck	9.8	0.66	2	0.01 to 0.2	150	0.5 to 10
Oils						
Rapeseed	1.9	4.6	11	10	1100	500
Soybean	6.7	4.1	8	7	800	350
Walnut	4.4	4.6	13	12	1300	600
Linseed***	0.24	7.4	60	54	6000	2700
Olive	9	0.67	1	1	100	50
others	> 50	> 2	≤ 1	≤ 1	---	≤50

**Martin 2001.*
*** here, PUFA: 18:2 n-6 + 18:3 n-3*
**** prohibited in human food in France at the present time*

Data taken from:

- Gunstone F., Harwood J. and Padley F. (1994). *The Lipid Handbook*. 2nd edition. Chapman and Hall, London.
- Souci S.W., Fachmann W. and Kraut H. (2000*). Food composition and nutrition tables* 6th edition revised and completed. Medpharm Scientific Publishers, CRC Press, Boca Raton.
- Koletzko B., Thiel I. and Abiodum P.O. (1992). *The fatty acid composition of human milk in Europe and Africa*. J. Pediatr. 120: S62-70.

Table L: sources of omega-3 fatty acids with 20 or more carbon atoms

	ω6/ ω3(*)	PUFA/ SFA	% ω3 in the FA	% ω3 in the product (g/100 g)	ω3x10 in the product (**)	% AA(***) in the FA	% EPA in the FA	% DPA in the FA	% DHA in the FA	Nutritional density (in DHA) mg/100 kcal	% of ANC of DHA per 100 g
ANC DHA (adult male): 0.12 g/d	5	0.641	0.15	-	-	-	-	-		5.5	-
Human breast milk	9.5	0.22	1.2	0.05	0.5	0.5	0.2	0.2	0.3	9	1 à 5
Fish											
Anchovy	0.48	1.1	32	1.4	14	0.4	18	1.5	11	1400	400
Monkfish	1.12	1.86	50	0.27	52.7	3.6	8	1	34	415	150
Herring	0.7	0.39	22	1.6	16	0.4	13	1	7	687	425
Perch	0.19	1.9	43	0.8	8	7.4	11	2	26	425	400
Sardine	0.7	1.5	35	1.6	16	1.3	17	2	13	1350	460
Salmon	0.37	2.8	35	1.4	14	1.7	5	5	17	2360	570
Trout	0.35	1.3	26	0.7	7	0.5	7	1	9	690	200
Oils											
Cod liver	1.1	1.5	25	23	-	0.5	13	1	11	2500	8400
Menhaden	0.65	0.78	24	22	-	2	13	2	7	2500	5300
Tuna	0.1	1.5	42	38	-	2	6	2	30	4200	22600

NB: for fish, the data concern raw, wild fish
(*): ratio of the content of all omega-6 fatty acids to all omega-3 fatty acids
(**): omega-3 fatty acid content multiplied by the biological equivalence factor in terms of alpha-linolenic acid.
(***): AA: arachidonic acid

Annex 4: summary of the studies on omega-3 fatty acids and cardiovascular health (studies from 1990 to 2002: compilation of intervention or observational studies published in journals indexed in the Pubmed internet database)

INTERVENTION STUDIES (MORTALITY)

Study	Publication, year	Intervention	Number of subjects in intervention group x years of follow-up	RR of non-fatal MI	RR of ischaemic cardiovascular morbidity-mortality	RR of ischaemic cardiovascular mortality
Dart	Lancet 1989	Fish ≥ 2 times weekly (more than 1.8 g/d EPA)	1015 x 2	1.51 (0.76-2.21)	0.85 (0.66-1.10)	0.69 (0.51 – 0.93)
Gissi	Lancet 1999	Fish oil 850 mg EPA/ DHA (1/2) (ethyl ester)	2836 x 3.5 (+2830 with vitamin E)	1.01 (0.80 – 1.27)	0.87 (0.76 – 0.99)	0.80 (0.67 – 0.96)
Lyon Diet Heart Study	Lancet 1994	Multiple including ALA: approx. 1.15 g/d	303 x 2.2	0.30 (0.11-0.81)	0.27 (0.12 – 0.59)	0.24 (0.07 – 0.85)
Singh	Lancet 2002	Multiple including ALA: approx. 1.33 g/d	501 x 2	0.47 (0.28 – 0.79)	0.48 (0.33 – 0.71)	0.33 (0.13 – 0.86)

Abbreviations:
ALA: alpha-linolenic acid, FO: fish oil, CLO: cod liver oil, w: week, m: month, y: years
I: intervention, RR: relative risk, O: observational
MI: myocardial infarction, NIDDM: non-insulin dependent diabetes mellitus, BP: blood pressure, S: systolic, D: diastolic, TG (TAG) triglycerides
LDL: low density lipoproteins
NS: Not significant.

INTERVENTION STUDIES (INTERMEDIARY FACTORS)

Type	Population (n, characteristics)	Intake	Duration	Effects	References
I	266, general	15 ml/d FO	3 months	LDL = NS	Vognild *et al*., 1998
I	234, general	3.8 g/d EPA or 3.6g/d DHA (ethyl ester)	7 days	LDL = NS	Grimsgaard *et al*., 1997
I	68, general	6-15g/d n-3 PUFA	2.8 months	LDL = NS	Hwang *et al*., 1997
I	58, general	1.12 to 3.37 g/d FO	18 months	LDL = NS	Deslypere *et al*., 1993
I	55, general	2.28 g/d EPA +DHA (FO) + 1.68 DHA (Oil) 1.5 g/d EPA + DHA (fish)	3.6 months	LDL = NS LDL = NS LDL = NS	Agren *et al*., 1996
I	50, general	3.6 g/d EPA +DHA (FO)	4 months	LDL = increase	Adler *et al*., 1997
I I	50, general 47, general	0.91 g/d n-3 (FO, margarine) 0.91g/d EPA + DHA	3.5 months 1 months	LDL = NS LDL = NS	Marckmarman *et al*., 1997 Sorensen *et al*., 1998
I	40, general	3 g/d EPA + DHA (FO)	2 months	LDL = reduction	Morcos, 1997
I	35, general	1.5 to 1.8 g/d DHA	3 months	LDL = NS	Hamazaki *et al*., 1996
I	34, general male female	5.3 g/d EPA +DHA (CLO)	7 months	LDL = increase LDL = NS	Hansen *et al*., 1993
I	26, general	35 mg/kg FO	9 months	LDL = NS	Layne *et al*., 1996
I	24, general	0.64 g/d FO	2 months	LDL = NS	Lervang *et al*., 1993
I	24, general	3.2 g/d EPA +DHA (FO)	12 months	LDL = NS	Schmidt *et al*., 1992
I	16, general	8.8 g/d EPA + DHA (FO)	1.5 months	LDL = NS	Tsai *et al*., 1997
I	11324, MI	~ 0.9 g/d EPA + DHA ethyl ester	3.5 yrs	LDL = NS	GISSI, 1999
I	868, hyperlipo-proteinaemia	1.7 to 2.6 g/d EPA + DHA (FO)	12 months	LDL = increase	Sirtori *et al*., 1998
I	814, Coronary angioplasty	5.4 g/d n-3 FA (FO)	5 months	LDL = NS	Cairns *et al*., 1996
I	617, Coronary bypass	4.2 g/d EPA + DHA (FO)	12 months	LDL = NS	Eritsland *et al*., 1996
I	511, Coronary bypass	3.4 g/d EPA + DHA (FO)	12 months	LDL = NS	Eritsland *et al*., 1995
I	447, Coronary angioplasty	6.9 g/d EPA + DHA (FO)	6 months	LDL = NS	Leaf *et al*., 1994
I	120, moderate hypercho-lesterolaemia	2.12 g/d EPA +DHA (FO)	3 months	LDL = increase	Mori *et al*., 1994
I	59, Coronary heart disease	6 g/d EPA + DHA + DPA (FO)	2.3 years	LDL = NS	Sacks *et al*., 1995
I	57, Coronary bypass	3.4 g/d EPA + DHA (FO)	6 months	LDL = NS	Eritsland *et al*., 1994

Type	Population (n, characteristics)	Intake	Duration	Effects	References
I	28, hypertri-glyceridaemia	Omacor 4g/d	12 weeks	LDL = increase	Stalenhoef *et al.*, 2000
I	350 normotensive	3g/d n-3 (6g FO)	6 months	BP: unchanged (HDL2 increased)	Sacks *et al.*, 1994
I	224 (non-smokers 36-56 years)	4g/d EPA + DHA		BP unchanged	Grimsgaard *et al.*, 1998
I	78 hypertensive	4 g/d EPA +DHA (FO)	16 weeks	lower BP (-4S, -2D)	Toft *et al*., 1995
I	59 (overweight)	4g/d DHA 4g/d EPA	6 weeks	lower BP (-6S –3D) TA = NS	Mori *et al*., 1999
I	43 hypertensive	Omacor (85% n-3) 4g/d	4-12 weeks	lower BP (-3S, -2D)	Lungershausen *et al*., 1994
I	21 hypertensive	4.5 g/d n-3 (FO)	4-8 weeks	lower BP	Gray *et al*., 1996
I	20 hypertensive	0.12 g DHA + 0.18 g EPA/d	13 days	Major reduction in BP	Yosefy *et al*., 1996
I	20 NIDDM	FO	6 weeks	BP: unchanged	Mc Veigh, 1995
I	16 moderate hypertension	2g EPA + 1.4g DHA/d	4 months	lower BP (-6S, -5D)	Prisco *et al*., 1998
I	45	1.5 to 6g ethyl ester DHA + EPA	12 weeks	Bleeding time = unchanged	Blonk *et al*., 1990
I	12	8g n-3 (capsule)	21 days	Bleeding time = increased	Mueller *et al*., 1991
I	10	1.3 to 9g/d n-3	6 weeks	Bleeding time = increased	Schmidt *et al*., 1990
I	9	Salmon (variable consumption)	100 days	Bleeding time = unchanged	Nelson *et al*., 1991
I	6	n-3 (2% of energy)	3 weeks	Bleeding time = increased	Nordoy *et al*., 1994
I	59, coronary heart disease treated with simvastatin	Omacor (2g/d)	48 weeks	TAG = reduction (25%)	Durrington *et al*., 2001
I	28, hyper TG	Omacor 4g/d	12 weeks	TG = reduction	Stalenhoef *et al*., 2000
I	394, coronary heart disease	5.1 g/d n-3	6 months	CHD = NS	Johansen, 1999

OBSERVATIONAL STUDIES

Type	Population (n, characteristics)	Intake	Duration	Effects	References
O	76283 (women 30-55 yrs)	Questionnaire	10 years	Ischaemia = Reduction	Hu *et al*., 1999
O	52138 (smokers or non-smokers, 35-54 yrs)	CLO	10 years	Coronary disease = NS	Egeland *et al*., 2001
O	44895, general (40-75 yrs)	Fish (0 to 5 meals/week) n-3 (0.07 to 0.58g/d)	10 years	Coronary disease = unchanged	Ascherio *et al*., 1995
O	43757	Questionnaire	6 years	Infarction = reduction	Ascherio *et al*. 1996
O	21930 smokers	Fish (eq. 0.2 to 0.8 g/d n-3)	6 years	Coronary risk = Increase	Pietinen *et al*., 1997
O	21185, general	Fish (1 to 4 servings/week)	4 years	Coronary mortality and Infarction = Unchanged	Morris *et al*., 1995
O	20551, general	Fish (1 to 4 servings/week)	12 years	Fatal cardiac incident = Reduction dose dep.	Albert *et al*., 1998
O	18244 (45-64 yrs)	200 g seafood	4 years	Fatal infarction = reduction	Yuan *et al*., 2001
O	12783, general	Fish	25 years	Coronary mortality = Reduction	Kromhout *et al*., 1996
O	8006, general	Fish (0 to 1 servings/d)	23 years	Coronary mortality = Reduction	Rodriguez *et al*., 1996
O	4584 (52 +/- 14 yrs)	Questionnaire	17 years	Coronary disease = Reduction	Djoussé *et al*., 2001
O	2107 (40-55 yrs)	Fish (variable consumption)	30 days	Cardiovascular accident = Reduction	Orencia *et al*., 1996
O	1822, general, male	Fish (0 to 35 g/d)	30 years	Fatal infarction = Reduction	Daviglus *et al*., 1997
O	827, cardiac incident (arrest)	Consumption n-3	7 years	Cardiac incident = Reduction	Siscovick *et al*., 2000
O	667 men (64-84 years, Zutphen)	ALA	10 years	Coronary disease = unchanged	Oomen *et al*., 2001
O	272, general	Fish	25 years	Coronary mortality = Reduction	Kromhout *et al*., 1995
O	14916, general	Circulating EPA/DHA	12 years	Initial infarction = unchanged	Guallar *et al*., 1995
O	1449, general	Estimate of n-3 in adipose tissue		Infarction = unchanged	Guallar *et al*., 1999
O	827, general	Blood measurements (FA) and questionnaires		Initial infarction = Reduction	Siscovick *et al*., 1995
O	278	Measurement of blood parameters (n-3)	17 years	Sudden death = Reduction	Albert *et al*., 2002

Type	Population (n, characteristics)	Intake	Duration	Effects	References
O	200, general	Blood N-3 measured		Ischaemia = Reduction	Yamori *et al*., 1994
O	188, general	Plasma DHA and EPA measured	3.5 years	Coronary mortality = Reduction	Simon *et al*., 1995

META-ANALYSIS

Population (n, characteristics)	Intake	Duration	Effects	References
1354 (31 studies)	Meta-analysis	Meta-analysis	Reduction BP = dose dependent	Morris *et al*., 1993

REFERENCES

[1] Adler A.J. and Holub B.J. (1997). *Effect of garlic and fish-oil supplementation on serum lipid and lipoprotein concentrations in hypercholesterolemic men*. Am. J. Clin. Nutr. 65: 445-450.

[2] Agren J.J., Hanninen O., Julkunen A., Fogelholm L., Vidgren H., Schwab U., Pynnonen O. and Uusitupa M. (1996). *Fish diet, fish oil and docosahexaenoic acid rich oil lower fasting and postprandial plasma lipid levels*. Eur. J. Clin. Nutr. 50: 765-771.

[3] Albert C.M., Hennekens C.H., O'Donnell C.J., Ajani U.A., Carey V.J., Willett W.C., Ruskin J.N. and Manson J.E. (1998). *Fish consumption and risk of sudden cardiac death*. Jama 279: 23-28.

[4] Albert C.M., Campos H., Stampfer M.J., Ridker P.M., Manson J.E., Willett W.C. and Ma J. (2002). *Blood levels of long-chain n-3 fatty acids and the risk of sudden death*. N. Engl. J. Med. 346: 1113-1118.

[5] Ascherio A., Rimm E.B., Stampfer M.J., Giovannucci E.L. and Willett W.C. (1995). *Dietary intake of marine n-3 fatty acids, fish intake, and the risk of coronary disease among men*. N. Engl. J. Med. 332: 977-982.

[6] Ascherio A., Rimm E.B., Giovannuci E.L., Spiegelman D., Stampfer M.J. and Willet W.C. (1996). *Dietary fat and risk of coronary heart disease in men: cohort follow-up study in the United States*. B.M.J. 313:84-90.

[7] Blonk M.C., Bilo H.J., Nauta J.J., Popp-Snijders C., Mulder C. and Donker A.J. (1990). *Dose-response effects of fish-oil supplementation in healthy volunteers*. Am. J. Clin. Nutr. 52(1):120-127

[8] Burr M.L., Fehily A.M., Gilbert J.F., Rogers S., Holliday R.M., Sweetnam P. M., Elwood P.C. and Deadman N.M. (1989). *Effects of changes in fat, fish and fiber intakes on death on myocardial reinfarction: diet and reinfarction trial (DART)*. Lancet. 2(8666): 757-761.

[9] Cairns J. A., Gill J., Morton B., Roberts R., Gent M., Hirsh J., Holder D., Finnie K., Marquis J.F., Naqvi S. and Cohen E. (1996). *Fish oils and low-molecular-weight heparin for the reduction of restenosis after percutaneous transluminal coronary angioplasty*. *The EMPAR Study*. Circulation 94: 1553-1560.

[10] Daviglus M. L., Stamler J., Orencia A. J., Dyer A. R., Liu K., Greenland P., Walsh M.K., Morris D. and Shekelle R.B. (1997). *Fish consumption and the 30-year risk of fatal myocardial infarction*. N. Engl. J. Med. 336: 1046-1053.

[11] De Lorgeril M., Renaud S., Mamelle N., Salen P., Martin J.L., Monjaud I., Guidollet J., Touboul P. and Delaye J. (1994). *Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease*. Lancet 343(8911): 1454-1459.

[12] Deslypere J. P., van de Bovenkamp P., Harryvan J. L. and Katan M. B. (1993). *Stability of n-3 fatty acids in human fat tissue aspirates during storage*. Am. J. Clin. Nutr. 57: 884-888.

[13] Djoussé L. Pankow J.S., Eckfeldt J.H., Folsom A.R., Hopkins P.M., Province M.A., Hong Y. and Ellison R.C. (2001). *Relation between dietary linolenic acid and coronary artery disease in the National Heart, Lung and Blood Institute Family Heart Study*. Am. J. Clin. Nutr. 74: 612-619.

[14] Durrington P. N., Bhatnagar D., Mackness M. I., Morgan J., Julier K., Khan M. A. and France M. (2001). *An omega-3 polyunsaturated fatty acid concentrate administered for one year decreased triglycerides in simvastatin treated patients with coronary heart disease and persisting hypertriglyceridaemia*. Heart 85: 544-548.

[15] Egeland G. M., Meyer H. E., Selmer R., Tverdal A. and Vollset S. E. (2001). *Cod liver oil consumption, smoking, and coronary heart disease mortality: three counties, Norway*. Int. J. Circumpolar. Health 60: 143-149.

[16] Eritsland J., Arnesen H., Seljeflot I., Hostmark A.T. (1995) *Long-term metabolic effects of n-3 polyunsaturated fatty acids in patients with coronary artery disease.* Am. J. Clin. Nutr. 61(4): 831-836.

[17] Eritsland J., Arnesen H., Gronseth K., Fjeld N.B. and Abdelnoor M. (1996) *Effect of dietary supplementation with n-3 fatty acids on coronary artery bypass graft patency*. Am. J. Cardiol. 77(1): 31-36.

[18] Gray D. R., Gozzip C. G., Eastham J. H. and Kashyap M. L. (1996). *Fish oil as an adjuvant in the treatment of hypertension*. Pharmacotherapy 16: 295-300.

[19] Grimsgaard S., Bonaa K. H., Hansen J. B. and Nordoy A. (1997). *Highly purified eicosapentaenoic acid and docosahexaenoic acid in humans have similar triacylglycerol-lowering effects but divergent effects on serum fatty acids.* Am. J. Clin. Nutr. 66: 649-659.

[20] Grimsgaard S., Bonaa K. H., Hansen J. B. and Myhre E. S. (1998). *Effects of highly purified eicosapentaenoic acid and docosahexaenoic acid on hemodynamics in humans*. Am. J. Clin. Nutr. 68: 52-59.
[21] Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico (GISSI) (1999). *Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial*. Lancet 354(9177): 447-455.
[22] Guallar E., Hennekens C. H., Sacks F. M., Willett W. C. and Stampfer M. J. (1995). *A prospective study of plasma fish oil levels and incidence of myocardial infarction in U.S. male physicians*. J. Am. Coll. Cardiol. 25: 387-394.
[23] Guallar E., Aro A., Jimenez F. J., Martin-Moreno J. M., Salminen I., van't Veer P., Kardinaal A.F., Gomez-Aracena J., Martin B.C., Kohlmeier L., Kark J.D., Mazaev V.P., Ringstad J., Guillen J., Riemersma R.A., Huttunen J.K., Thamm M. and Kok F.J. . (1999*). Omega-3 fatty acids in adipose tissue and risk of myocardial infarction: the EURAMIC study*. Arterioscler. Thromb. Vasc. Biol. 19: 1111-1118.
[24] Hamazaki T., Sawazaki S., Asaoka E., Itomura M., Mizushima Y., Yazawa K., Kuwamori T. and Kobayashi M. (1996). *Docosahexaenoic acid-rich fish oil does not affect serum lipid concentrations of normolipidemic young adults*. J. Nutr. 126: 2784-2789.
[25] Hansen J. B., Berge L. N., Svensson B., Lyngmo V. and Nordoy A. (1993). *Effects of cod liver oil on lipids and platelets in males and females*. Eur. J. Clin. Nutr. 47: 123-131.
[26] Hu F.B., Stampfer M.J., Manson J.E., Rimm E.B., Wolk A., Colditz G.A., Hennekens C.H. and Willet W.C. (1999). *Dietary intake of alpha-linolenic acid and risk of fatal ischaemic heart disease among women*. Am. J. Clin. Nutr. 69: 890-897.
[27] Hu F. B., Manson J. E. and Willett W. C. (2001). *Types of dietary fat and risk of coronary heart disease: a critical review*. J. Am. Coll. Nutr. 20: 5-19.
[28] Hwang D. H., Chanmugam P. S., Ryan D. H., Boudreau M. D., Windhauser M. M., Tulley R. T., Brooks E.R. and Bray G.A. (1997). *Does vegetable oil attenuate the beneficial effects of fish oil in reducing risk factors for cardiovascular disease ?* Am. J. Clin. Nutr. 66: 89-96.
[29] Johansen O., Brekke M., Seljeflot I., Abdelnoor M. and Arnesen H. (1999). *N-3 fatty acids do not prevent restenosis after coronary angioplasty: results from the CART study. Coronary Angioplasty Restenosis Trial*. J. Am. Coll. Cardiol. 33: 1619-1626.
[30] Kromhout D., Feskens E. J. and Bowles C. H. (1995). *The protective effect of a small amount of fish on coronary heart disease mortality in an elderly population*. Int. J. Epidemiol. 24: 340-345.
[31] Kromhout D., Bloemberg B. P., Feskens E. J., Hertog M. G., Menotti A. and Blackburn H. (1996). *Alcohol, fish, fibre and antioxidant vitamins intake do not explain population differences in coronary heart disease mortality*. Int. J. Epidemiol. 25: 753-759.
[32] Layne K. S., Goh Y. K., Jumpsen J. A., Ryan E. A., Chow P. and Clandinin M. T. (1996). *Normal subjects consuming physiological levels of 18:3(n-3) and 20:5(n-3) from flaxseed or fish oils have characteristic differences in plasma lipid and lipoprotein fatty acid levels.* J. Nutr. 126: 2130-2140.
[33] Leaf A., Jorgensen M. B., Jacobs A. K., Cote G., Schoenfeld D. A., Scheer J., Weiner B.H., Slack J.D., Kellett M.A. and Raizner A.E... (1994). *Do fish oils prevent restenosis after coronary angioplasty ?* Circulation 90: 2248-2257.
[34] Lervang H. H., Schmidt E. B., Moller J., Svaneborg N., Varming K., Madsen P. H. and Dyerberg J. (1993). *The effect of low-dose supplementation with n-3 polyunsaturated fatty acids on some risk markers of coronary heart disease.* Scand. J. Clin. Lab. Invest. 53: 417-423.
[35] Lungershausen Y. K., Abbey M., Nestel P. J. and Howe P. R. (1994). *Reduction of blood pressure and plasma triglycerides by omega-3 fatty acids in treated hypertensives*. J. Hypertens. 12: 1041-1045.
[36] Marckmann P., Bladbjerg E.M. and Jespersen J. (1997). *Dietary fish oil (4 g daily) and cardiovascular risk markers in healthy men*. Arterioscler. Thromb. Vasc. Biol. 17(12): 3384-3391.
[37] McVeigh G.E., Brennan G.M., Cohn J.N., Finkelstein S.M., Hayes R.J. and Johnston G.D. (1994). *Fish oil improves arterial compliance in non-insulin-dependent diabetes mellitus.* Arterioscler. Thromb. 14(9):1425-1429.
[38] Morcos N.C. (1997). *Modulation of lipid profile by fish oil and garlic combination*. J. Natl. Med. Assoc. 89(10):673-678.
[39] Mori T. A., Vandongen R., Beilin L. J., Burke V., Morris J. and Ritchie J. (1994). *Effects of varying dietary fat, fish, and fish oils on blood lipids in a randomized controlled trial in men at risk of heart disease*. Am. J. Clin. Nutr. 59: 1060-1068.

[40] Mori T. A., Bao D. Q., Burke V., Puddey I. B. and Beilin L. J. (1999). *Docosahexaenoic acid but not eicosapentaenoic acid lowers ambulatory blood pressure and heart rate in humans. Hypertension* 34: 253-260.
[41] Morris M. C., Sacks F. and Rosner B. (1993). *Does fish oil lower blood pressure? A meta-analysis of controlled trials*. Circulation 88: 523-533.
[42] Morris M. C., Manson J. E., Rosner B., Buring J. E., Willett W. C. and Hennekens C. H. (1995). *Fish consumption and cardiovascular disease in the physicians' health study: a prospective study*. Am. J. Epidemiol. 142: 166-175.
[43] Mueller B. A., Talbert R. L., Tegeler C. H. and Prihoda T. J. (1991). *The bleeding time effects of a single dose of aspirin in subjects receiving omega-3 fatty acid dietary supplementation*. J. Clin. Pharmacol. 31: 185-190.
[44] Nelson G. J., Schmidt P. C. and Corash L. (1991). *The effect of a salmon diet on blood clotting, platelet aggregation and fatty acids in normal adult men.* Lipids 26: 87-96.
[45] Nordoy A., Hatcher L., Goodnight S., Fitzgerald G. A. and Conner W. E. (1994). *Effects of dietary fat content, saturated fatty acids, and fish oil on eicosanoid production and hemostatic parameters in normal men*. J. Lab. Clin. Med; 123: 914-920.
[46] Oomen C. M., Ocke M. C., Feskens E. J., Kok F. J. and Kromhout D. (2001). *Alpha-Linolenic acid intake is not beneficially associated with 10-y risk of coronary artery disease incidence: the Zutphen Elderly Study.* Am. J. Clin. Nutr. 74: 457-463.
[47] Orencia A. J., Daviglus M. L., Dyer A. R., Shekelle R. B. and Stamler J. (1996). *Fish consumption and stroke in men. 30-year findings of the Chicago Western Electric Study.* Stroke 27: 204-209.
[48] Pietinen P., Ascherio A., Korhonen P., Hartman A. M., Willett W. C., Albanes D., Virtamo J. (1997). *Intake of fatty acids and risk of coronary heart disease in a cohort of Finnish men. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study*. Am. J. Epidemiol. 145: 876-887.
[49] Prisco D., Paniccia R., Bandinelli B., Filippini M., Francalanci I., Giusti B., Giurlani L., Gensini G.F., Abbate R. and Neri Serneri G.G. (1998). *Effect of medium-term supplementation with a moderate dose of n-3 polyunsaturated fatty acids on blood pressure in mild hypertensive patients.* Thromb. Res. 91: 105-112.
[50] Rodriguez B. L., Sharp D. S., Abbott R. D., Burchfiel C. M., Masaki K., Chyou P. H. Huang B., Yano K., Curb J.D. (1996). *Fish intake may limit the increase in risk of coronary heart disease morbidity and mortality among heavy smokers. The Honolulu Heart Program*. Circulation 94: 952-956.
[51] Sacks F. M., Hebert P., Appel L. J., Borhani N. O., Applegate W. B., Cohen J. D., Cutler J.A., Kirchner K.A., Kuller L.H., Roth K.J.. (1994). *The effect of fish oil on blood pressure and high-density lipoprotein-cholesterol levels in phase I of the Trials of Hypertension Prevention. Trials of Hypertension Prevention Collaborative Research Group*. J. Hypertens. Suppl. 12: S23-31.
[52] Schmidt E. B., Varming K., Ernst E., Madsen P. and Dyerberg J. (1990). *Dose-response studies on the effect of n-3 polyunsaturated fatty acids on lipids and haemostasis*. Thromb. Haemost. 63: 1-5.
[53] Schmidt E. B., Lervang H. H., Varming K., Madsen P. and Dyerberg J. (1992). *Long-term supplementation with n-3 fatty acids, I: Effect on blood lipids, haemostasis and blood pressure*. Scand. J. Clin. Lab. Invest. 52: 221-228.
[54] Simon, J.A., M.L. Hodgkins, W.S. Browner, J.M. Neuhaus, J.T. Bernert, and S.B. Hulley. (1995). *Serum fatty acids and the risk of coronary heart disease*. Am. J. of Epidemiol. 142(5):469-476.
[55] Singh R.B., Dubnov G., Niaz M.A., Ghosh S., Singh R., Rastogi S.S., Manor O., Pella D. and Berry E.M.L. (2002). *Effect of an Indo-Mediterranean diet on progression of coronary artery disease in high risk patients (Indo-Mediterranean Diet Heart Study): a randomised single-blind trial.* Lancet 360(9344):1455-1461.
[56] Singh R. B., Niaz M. A., Sharma J. P., Kumar R., Rastogi V. and Moshiri M. (1997). *Randomized, double-blind, placebo-controlled trial of fish oil and mustard oil in patients with suspected acute myocardial infarction: the Indian experiment of infarct survival--4*. Cardiovasc. Drugs Ther. 11: 485-491.
[57] Sirtori C. R., Crepaldi G., Manzato E., Mancini M., Rivellese A., Paoletti R., Pazzucconi F., Pamparana F. and Stragliotto E.. (1998). *One-year treatment with ethyl esters of n-3 fatty acids in patients with hypertriglyceridemia and glucose intolerance: reduced triglyceridemia, total cholesterol and increased HDL-C without glycemic alterations*. Atherosclerosis 137: 419-427.
[58] Siscovick D. S., Raghunathan T. E., King I., Weinmann S., Wicklund K. G., Albright J., Bovbjerg

V, Arbogast P, Smith H, Kushi LH. (1995). *Dietary intake and cell membrane levels of long-chain n-3 polyunsaturated fatty acids and the risk of primary cardiac arrest*. JAMA 274: 1363-1367.
[59] Siscovick D.S., Raghunathan T., King I., Weinmann S., Bovbjerg V.E., Kushi L., Cobb L.A., Copass M.K., Psaty B.M., Lemaitre R., Retzlaff B. and Knopp R.H. (2000) *Dietary intake of long-chain n-3 polyunsaturated fatty acids and the risk of primary cardiac arrest*. Am. J. Clin. Nutr. 71(1 Suppl): 208S-212S.
[60] Sorensen N. S., Marckmann P., Hoy C. E., van Duyvenvoorde W. and Princen H. M. (1998). *Effect of fish-oil-enriched margarine on plasma lipids, low-density-lipoprotein particle composition, size, and susceptibility to oxidation*. Am. J. Clin. Nutr. 68: 235-241.
[61] Stalenhoef A. F., de Graaf J., Wittekoek M. E., Bredie S. J., Demacker P. N. and Kastelein J. J. (2000). *The effect of concentrated n-3 fatty acids versus gemfibrozil on plasma lipoproteins, low density lipoprotein heterogeneity and oxidizability in patients with hypertriglyceridemia*. Atherosclerosis 153: 129-138.
[62] Toft I., Bonaa K. H., Ingebretsen O. C., Nordoy A. and Jenssen T. (1995*). Effects of n-3 polyunsaturated fatty acids on glucose homeostasis and blood pressure in essential hypertension. A randomized, controlled trial*. Ann. Intern. Med. 123: 911-918.
[63] Tsai P. J. and Lu S. C. (1997). *Fish oil lowers plasma lipid concentrations and increases the susceptibility of low density lipoprotein to oxidative modification in healthy men.* J. Formos. Med. Assoc. 96: 718-726.
[64] Vognild E., Elvevoll E. O., Brox J., Olsen R. L., Barstad H., Aursand M and, Osterud B.. (1998). *Effects of dietary marine oils and olive oil on fatty acid composition, platelet membrane fluidity, platelet responses, and serum lipids in healthy humans*. Lipids 33: 427-436.
[65] Yamori Y., Nara Y., Mizushima S., Sawamura M. and Horie R. (1994). *Nutritional factors for stroke and major cardiovascular diseases: international epidemiological comparison of dietary prevention*. Health Rep. 6: 22-27.
[66] Yosefy C., Viskoper J. R., Varon D., Ilan Z., Pilpel D., Lugassy G., Schneider R., Savyon N., Adan Y. and Raz A. (1996). *Repeated fasting and refeeding with 20:5, n-3 eicosapentaenoic acid (EPA): a novel approach for rapid fatty acid exchange and its effect on blood pressure, plasma lipids and hemostasis*. J. Hum. Hypertens., 13;10 Suppl 3:S135-139.
[67] Yuan J. M., Ross R. K., Gao Y. T. and Yu M. C. (2001). *Fish and shellfish consumption in relation to death from myocardial infarction among men in Shanghai, China*. Am. J. Epidemiol. 154: 809-816.

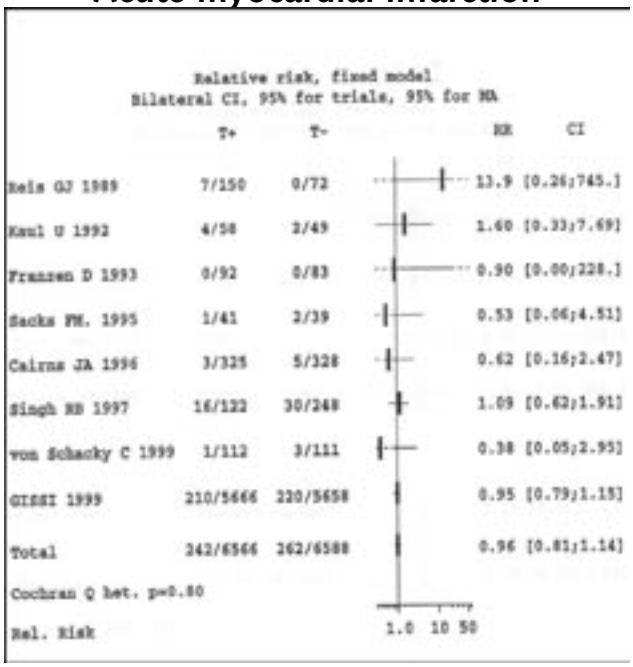
Annex 5: meta-analysis of controlled intervention studies comprising the administration of an omega-3 LC-PUFA supplement (study conducted in accordance with Cochrane criteria)

(from D. Yzebe, JP Boissel Team, University of Lyon 1, 2000)

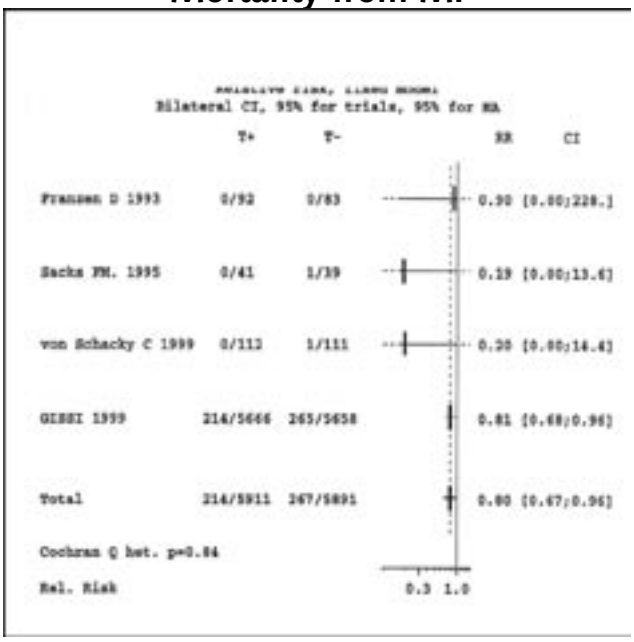
References	Selection of subjects	Randomi -sation	Double blind	Description of treatment and placebo	Proportion of patients lost from sight	Analysis in intention to treat	Overall quality of the trial
Dehmer JG et al. 1988	A	A	C	A	B	C	Unsatisfactory
Reis GJ et al.1989	A	B	B	A	A	B	Average
Nye ER et al.1990	A	B	B	A	B	C	Unsatisfactory
Kaul U. et al.1992	A	A	C	A	A	A	Unsatisfactory
Bellamy CM et al.1992	A	A	A	A	A	A	Good
Franzen D. et al 1993	A	A	A	A	A	C	Unsatisfactory
Sacks FM et al. 1995 (126)	A	A	B	A	A	A	Average
Cairns JA et al. 1996	A	B	C	A	C	C	Unsatisfactory
Singh RB et al. 1997	A	A	A	A	A	A	Good
Von Schacky C. et al. 1999	A	A	A	A	B	A	Average
GISSI trial 1999	A	A	C	A	A	A	Unsatisfactory

Quality of the studies:
A: good
B: average
C: unsatisfactory

Acute myocardial infarction



Mortality from MI



References:

- Bellamy C.M., Schofield P.M., Faragher E.B. and Ramsdale D.R. (1992). *Can supplementation of diet with omega-3 polyunsaturated fatty acids reduce coronary angioplasty restenosis rate?* Eur. Heart J. 13(12):1626-1631.
- Cairns J. A., Gill J., Morton B., Roberts R., Gent M., Hirsh J., Holder D., Finnie K., Marquis JF., Naqvi S. and Cohen E. (1996). *Fish oils and low-molecular-weight heparin for the reduction of restenosis after percutaneous transluminal coronary angioplasty. The EMPAR Study*. Circulation 94: 1553-1560.
- Dehmer G.J., Popma J.J., van den Berg E.K., Eichhorn E.J., Prewitt J.B., Campbell W.B., Jennings L, Willerson J.T. and Schmitz J.M. (1988). *Reduction in the rate of early restenosis after coronary angioplasty by a diet supplemented with n-3 fatty acids.* N. Engl. J. Med. 319(12):733-740.
- Franzen D., Schanwell M. and Oette K. (1993). *A prospective randomised and double-blind trial on the effect of fish oil in the incidence of restenosis following PTCA*. Catheter. cardiovasc. diag.; 28: 301-310.
- Grupo Italiano per la Studio della Sopravvivenza nell'Infarto miocardico (GISSI). (1999). *Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction : results of the GISSI-Prevenzione trial*. Lancet 354 (9177):447-455.
- Kaul U., Sanghvi S., Bahl V.K., Dev V. and Wasir H.S.. (1992). *Fish oil supplements for prevention of restenosis after coronary angioplasty*. Int. J. Cardiol. 35(1):87-93.
- Nye E.R., Ablett M.B.and Robertson M.C. (1990). *Effect of eicosapentaenoic acid on restenosis rate, clinical course and blood lipids in patients after percutaneaous transluminal coronary angioplasty*. Aus. NZ. J. Med. 20:549-552.
- Reis G.J., Boucher T.M., Sipperly M.E., Silverman D.I., McCabe C.H., Baim D.S., Sacks F.M., Grossman W. and Pasternak R.C. (1989). *Randomised trial of fish oil for prevention of restenosis after coronary angioplasty*. Lancet. 2(8656):177-181.
- Sacks F.M.., Stone P.H. and Gibson C.M. (1995). *Controlled trial of fish oil for regression of human coronary atherosclerosis.* J. Am. Coll. Cardiol. 25 : 1492-1498.
- Singh R. B., Niaz M. A., Sharma J. P., Kumar R., Rastogi V. and Moshiri M. (1997) *Randomized, double-blind, placebo-controlled trial of fish oil and mustard oil in patients with suspected acute myocardial infarction: the Indian experiment of infarct survival*--4. Cardiovasc Drugs Ther 11: 485-491.
- von Schacky C., Angerer P., Kothny W., Theisen K. Mudra H. (1999). *The effect of dietary omega-3 fatty acids on coronary atherosclerosis. A randomized, double-blind, placebo-controlled trial*. Ann. Intern. Med. 130: 554-562.

- Afssa opinion dated 17 January 2001, concerning assessment of the nutritional role of a regular milk fortified with omega-3 fatty acids from fish oil

- Afssa opinion dated 15 May 2002, concerning assessment of the nutritional role of a regular milk fortified with omega-3 fatty acids from fish oil

- Afssa opinion dated 28 May 2001 concerning assessment of the claims for a special seasoning oil with a guaranteed content of vitamin E and rich in omega-3 fatty acids

Maisons-Alfort, 17 January 2001

OPINION

Of the Agence française de sécurité sanitaire des aliments [French Food Safety Agency] concerning assessment the nutritional role of a regular milk fortified with omega-3 fatty acids from fish oil

The CSHPF [French Higher Council for Public Health] received a referral on 15 March 2000 from the Direction générale de la concurrence, de la consommation et de la répression des fraudes [General Directorate for Fair Trading, Consumer Affairs and Fraud Control] requesting an opinion concerning evaluation of the nutritional role of a milk for everyday consumption fortified with fish oil.

Following consultation with the combined working group composed of the CSHPF working group on "nutritional value and novel foods" and the CEDAP [Interministerial Committee for products intended for particular nutritional uses] working group on "nutritional substances" on 25 April 2000, the Food and Nutrition section of the CSHPF on 21 June 2000 and the "CES Nutrition Humaine" on 15 November 2000, the Agence française de sécurité sanitaire des aliments is issuing the following opinion:

Whereas the product is a regular milk fortified with fish oil (ROPUFA 30 n-3 Produits ROCHE) containing long chain polyunsaturated fatty acids of the n-3 series (omega-3 fatty acids): eicosapentaenoic acid (EPA: 12%) and docosahexaenoic acid (DHA: 18%); the following claim is made "contributes to healthy cardiovascular function ".

Whereas the scientific literature describes these acids as having the capacity to prevent cardiovascular disease by controlling platelet aggregation, by exercising a hypotriglyceridaemic effect, by promoting ionic exchanges in the membranes; these fatty acids have proved beneficial in other diseases such as diabetes, cancer, inflammatory disease;

Whereas omega-3 fatty acids are usually contained in small quantities in the diet of the French population; they are useful in counterbalancing the excessive intake of n-6 fatty acids which characterises the current diet;

Whereas the CSHPF issued an opinion on 11 July 2000 comprising a request for additional information;

Whereas no indication is given concerning the conservation of the product (stability of the emulsion, storage duration and temperature); no information is provided on conditions of use, in particular as regards heating (change in the composition of fatty acids, taste and resistance to heating); scientific evidence for the dose recommended by the applicant has not been supplied;

The Agence française de sécurité sanitaire des aliments:

- considers that the claim appearing on the product label "omega-3 fatty acids contribute to healthy cardiovascular function" is acceptable in view of the many scientific data on the subject;
- reiterates the request for additional information expressed by the Food and Nutrition section of the Conseil supérieur d'hygiène publique de France in the opinion issued on 11 July 2000 concerning the elements for which no information was provided, namely:
  - the conditions for the conservation of the product;
  - the conditions of use in particular as regards heating;
  - scientific substantiation of the recommended dose of 650 mg/d.

Martin HIRSCH

Maisons-Alfort, 15 May 2002

OPINION

Of the Agence française de sécurité sanitaire des aliments [French Food Safety Agency] concerning the assessment of the nutritional role of a regular milk fortified with omega-3 fatty acids from fish oil

The CSHPF [French Higher Council for Public Health] received a referral on 15 March 2000 from the Direction générale de la concurrence, de la consommation et de la répression des fraudes [General Directorate for Fair Trading, Consumer Affairs and Fraud Control] requesting an opinion concerning evaluation of the nutritional role of a milk for everyday consumption fortified with fish oil.

Following consultation with the combined CSHPF working group on "nutritional value and novel foods" and the CEDAP [Interministerial Committee for products intended for particular nutritional uses] working group on "nutritional substances" on 25 April 2000, the Food and Nutrition section of the CSHPF on 21 June 2000, the Agence française de sécurité sanitaire des aliments (Afssa) issued an initial opinion on 11 July 2000 comprising a request for additional information. Following consultation of the CES "Nutrition Humaine" on 15 November 2000, Afssa issued a second opinion on 17 January 2001.
This opinion included a request for additional information, regarding in particular:
- the conditions for the conservation of the product;
- the conditions of use in particular as regards heating;
- scientific substantiation of the recommended dose of 650 mg/d.

In a letter dated 21 November 2001 the applicant supplied the information requested.

Following consultation with the "CES Nutrition Humaine" on 19 February 2001, Afssa issued the following opinion:

Whereas the product is a regular milk fortified with fish oil containing long chain polyunsaturated fatty acids of the n-3 series (omega-3 fatty acids): eicosapentaenoic acid (EPA: 12%) and docosahexaenoic acid (DHA: 18%); the following claim is made "contributes to healthy cardiovascular function";

Whereas the applicant has provided information on the addition of antioxidants (rosemary extracts, tocopherols and vitamin C in the form of ascorbyl palmitate); these antioxidants are added to preserve the product and protect the omega-3 fatty acids; based on the results of cooking tests (milk with different best before dates and brought to the boil), it appears that heating does not destroy these fatty acids (with the exception of a 7% reduction during the cooking of a flan at 220°C for 25 min);

Whereas however, the recommended daily dose of 650 mg EPA and DHA, based on a symposium held in the United States in 1999, is not the subject of a consensus; an ad hoc working group set up by Afssa is currently reviewing the issue; in addition, the information to the public as it appears on the product label takes no account of the possible accumulation of omega-3 fatty acids from this milk and from those contained in the rest of the diet (some vegetable oils, fish, etc,);

Afssa considers that the applicant has supplied the information requested as regards storage conditions and use of the product, notably in terms of heating.
It emphasises, however, that in the current state of knowledge, the level of consumption recommended by the applicant of 650 mg EPA and DHA daily has no scientific substantiation.

Martin HIRSCH

Maisons-Alfort, 28 May 2001

OPINION

Of the Agence française de sécurité sanitaire des aliments
[French Food Safety Agency] concerning the assessment of the claims for a special seasoning oil with a guaranteed content of vitamin E and rich in omega-3 fatty acids

The Agence française de sécurité sanitaire des aliments received a referral on 4 October 2000 from the Direction générale de la concurrence, de la consommation et de la répression des fraudes [General Directorate for Fair Trading, Consumer Affairs and Fraud Control] requesting an evaluation of the claims for a special salad oil with a guaranteed content of vitamin E and rich in omega-3 fatty acids.

Following consultation of the "CES Nutrition Humaine", which met on 27 March 2001, the Agence française de sécurité sanitaire des aliments issued the following opinion:

Whereas the product is a salad oil composed of different food oils (rapeseed, walnut, grapeseed, olive, wheatgerm and fish), fortified with vitamin E; this is a special diet product intended for persons with raised cholesterol levels and/or cardiovascular risk factors; the claims "omega-3 fatty acids contribute to healthy cardiovascular function" and "included in recommendations for cholesterol-lowering diets" are being made;

Whereas polyunsaturated fatty acids of the n-3 series (omega-3 fatty acids) are provided in small quantities in the diet of the French population; the product is a vector food for omega-3 fatty acids whose consumption, according to scientific studies, contributes to the prevention of cardiovascular disease; the consumption of 20g oil per day provides 1.6g omega-3 fatty acids, a level close to the ANC for omega-3 (2 g/d);

Whereas the value of the ratio of omega-6 fatty acids / omega-3 fatty acids of the product is 4.4. (level close to that in the ANC which is 5);

Whereas the product is rich in monounsaturated fatty acids and has a low content of saturated fatty acids;

Whereas the product has no cholesterol lowering properties;

The Agence française de sécurité sanitaire des aliments is:
- satisfied with the claim «omega-3 fatty acids contribute to healthy cardiovascular function», and
- not satisfied with the claim «included in recommendations for cholesterol-lowering diets».

Martin HIRSCH

Annex 7: exploitation of compositional data to enrich the debate on threshold levels for claims concerning omega-3 fatty acids (Ciqual technical note)

1. Context

The Afssa working group on omega-3 fatty acids considered the appropriateness of claims, and notably quantitative nutrition claims such as "source of omega-3 fatty acids" or "rich in omega-3 fatty acids". One particular question was what the omega-3 content and the type of omega-3 fatty acids should be in the product when ready for use by the consumer to substantiate such claims.

2. Purpose of the document

This document presents compositional data for approximately 50 food products, generic and "fortified" with omega-3 fatty acids: oils, eggs, margarine, bread, meat, milk, fish.
Its purpose is to provide information on the following aspects:

- Content of α-linolenic acid (ALA) or docosahexaenoic acid (DHA) per 100 g or 100 ml.
- Percentage of the ANC of ALA or DHA for adult men covered by 100 g, 100 ml or 100 kcal of product. Position compared with the threshold levels of 15% (generally used for the claim "source of") or 30% of the ANC (for the claim "rich in").
- Quantity of ALA or DHA to be added to reach these levels, if necessary.
- Suitability of the nutritional composition of these foods in terms of the French nutritional recommendations on ANC. The principle adopted is that foods should, as far as possible, enable the ANC to be approached.

Table M: ANCs for fatty acids in adult men (Martin A., 2001)

In adult men	Fats[3]	Saturated fatty acids (SFA)	Monounsaturated fatty acids (MUFA)	Linoleic acid (18:2 n-6)	α-linolenic acid (18 :3 n-3)	ω6 + ω3 Long chain polyunsaturated fatty acids (LC-PUFA)	Of which DHA
g/j	81	19.5	49	10	2	0.5	0.12
% TDE (Total daily energy, or 2200 kcal/d)	33%	8%	20%	4%	0.8%	0.2%	0.05%

Several criteria, taking into account different aspects of the "quality" of fats found in foods were proposed:

- ratio of omega-6 / omega-3 fatty acids < 5 (French nutritional recommendation for the precursors),
- energy intake of fats less than 33% of total energy intake (cf. ANC),
- ratio of saturated fatty acids / total fatty acids less than 30% (clinical approach),
- cholesterol content less than 150 mg per 100 g (clinical approach).

3. Methodology

The compositional data listed in the tables below originate from different sources:

[3] all fatty acids (excluding cholesterol)

- Most have been taken from the Ciqual database which includes in particular the most recent updates on dairy products.
- When the data from Ciqual were insufficient, the values were borrowed from tables from other countries, Germany and Britain (cf. References in § 6). In particular, in the absence of recent data on the composition of fats, most values were taken from the British composition table.
- Moreover, some foods available today on the French market have particular contents of omega-3 fatty acids, either through the direct addition of ingredients providing omega-3 fatty acids (such as fish oil) or through fortification of animal feed with these oils or oleaginous ingredients (such as linseed). The compositional data for these products have also been included in the tables.

4. Data on omega-3 fatty acid content

The full tables are included in annex. They provide compositional data for some fifty food products.

- data expressed per 100g of the food: eggs, butter, margarines, yoghurts, oils, breads, meats and meat products, fish,
- data expressed per 100 ml of product: milk,
- data expressed per 100 kcal: all products.

In cases where compositional data were provided by the manufacturers, these are generally a total content of omega-3 fatty acids with no further details. The assumptions then made were as follows:

- When the diet of animals is fortified with linseed, the content of omega-3 fatty acids is attributed to α-linolenic acid (or partially attributed to this fatty acid if the corresponding generic product already contained other types of omega-3 FA, namely omega-3 LC-PUFA).
- When fish oil is used to enrich the product or the animal feed, the content of omega-3 fatty acids is attributed to DHA. This involves a simplification as these fish oils theoretically are more likely to provide a mixture of omega-3 LC-PUFA. Here again, if the corresponding generic product already contained α-linolenic acid, this content has been subtracted to estimate the DHA content.
- In cases where the two means of fortification were combined, the content of omega-3 fatty acids has been divided half into α-linolenic acid and half into DHA.

To estimate the omega 3 / omega 6 fatty acid balance, a theoretical content was calculated as follows, expressed for in "α-linolenic acid equivalent":

"α-linolenic acid equivalent = "α-linolenic acid + (10 x omega-3 LC-PUFA)

where the equivalence factor of 10 is introduced for the omega-3 LC-PUFA.

5. Some tools for identifying foods which are sources of or rich in omega-3 fatty acids and estimating their nutritional quality

Comparison of these compositional data with threshold levels expressed as ANC percentages helps to identify those foods which are likely to be considered "sources of" or "rich in" omega-3 fatty acids, whether one expresses the proportion of the ANC covered per 100g of the food (or per 100 ml) or per 100 kcal of the same food. It is important to emphasise that the reasoning applies to the ANC and not the recommended daily allowance (RDA). In effect, there is currently no RDA for these constituents. Moreover, it should be noted that the ANC used are those for ALA and DHA. Theoretically, it would therefore be more precise, as regards quantitative nutrition claims, to work on the basis of these two fatty acids only. Nevertheless, in the absence of an overall recommendation on the level of consumption of omega-3 fatty acids, it seems envisageable to

consider the claim “rich in omega-3 fatty acids” as meaning an effective richness in ALA and/or DHA.

5.1 The quantitative nutrition claims would consist of the statement “source of omega-3 fatty acids” and “rich in omega-3 fatty acids”.
For the first claim, a content of omega-3 fatty acids would be required corresponding to 15% of the ANC, per 100 g, 100 ml or 100 kcal of product. For the second, the proposed threshold is 30% of the ANC.

Table N: List of foods likely to carry a quantitative nutrition claim

Criteria	α-linolenic acid		DHA	
	Foods «sources of»	Foods «rich in»	Foods «source of»	Foods «rich in»
per 100 g	• Butter • «omega-3» butter • «omega-3» eggs • «omega-3» bread (with fish oil + linseed) • Sausage meat, pure pork • Roasted chicken, leg, meat and skin • Horse, meat, raw	• Cod liver oil • Olive oil • Rapeseed oil • Walnut oil • Wheatgerm oil • Maize (corn) oil • Soya oil • Linseed oil* • «omega-3» oil • Margarine 70%Fat • «omega-3» margarine 80%Fat • «omega-3» pork sausage • Stewed rabbit	• Lamb, leg, raw	• Cod liver oil • «omega-3» oil • «omega-3» Margarine 80% fat • «omega-3» bread (with fish oil) • «omega-3» bread (with fish oil + linseed) • Roast chicken, leg, meat and skin • Cod, raw • Tuna canned in brine
per 100 ml			• «omega-3» milk (with fish oil)	
per 100 kcal	• Wheatgerm oil • Margarine 70% fat • «omega-3» pork sausage • Rabbit, stewed • Horse, meat, raw	• Rapeseed oil • Walnut oil • Soy oil • Linseed oil • «omega-3» oil		• Cod liver oil • «omega-3» Margarine 80% fat • «omega-3» bread (with fish oil) • «omega-3» bread (with fish oil + linseed) • «omega-3» milk (with fish oil) • Cod, raw • Tuna canned in brine

** currently prohibited in human food in France.*

The reasoning for 100 kcal is in most cases stricter than that based on 100 g or 100 ml of food (except for milk with fish oil). Firstly, it "downgrades" food from "rich" to "source" and secondly it excludes the highest calorie foods from the scope of quantitative nutrition claims (e.g. butter, "omega-3" eggs, leg of lamb).

5.2 Foods rich in omega-3 fatty acids and satisfying one or more nutritional quality criteria

The following table lists the foods, either "rich in" (or "sources of") omega-3 fatty acids and which fulfil the selected nutritional criteria.
To make the table easier to read, the boxes are coloured dark blue when the criterion is satisfied, blue when it is nearly satisfied (e.g. ratio LA/ ALA equiv. = 5.1 for walnut oil, when a ratio of is recommended in France) and finally light blue when the criterion is not satisfied.

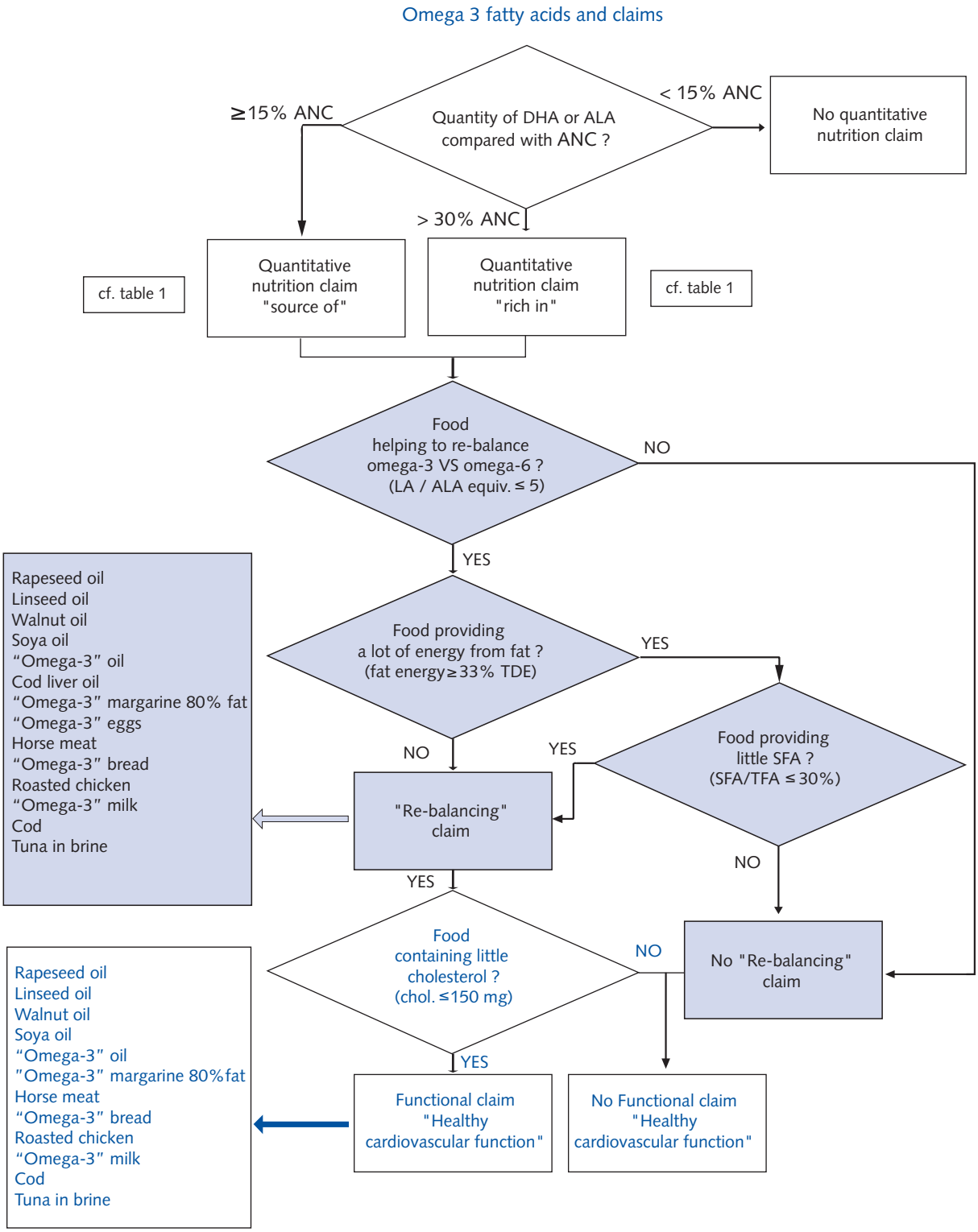
Table O: List of foods sources of or riches in omega 3 fatty acids and which meet the nutritional quality criteria selected

	LA / ALA equiv. ≤ 5 ?	Energy intake from lipids ≤ 33% TDE?	SFA/TFA ≤ 30	Cholesterol content ≤ 150 mg / 100g or 100 ml
Foods rich in ALA				
Rapeseed oil	2.1	100	7.3	0
Olive oil	10.7	100	15.9	0
Linseed oil	0.3	100	9.6	0
Walnut oil	5.1	100	10.1	0
Wheatgerm oil	10.4	100	20.6	0
Corn oil	56	100	16.1	0
Soy oil	7.1	100	17.4	0
«Omega-3» oil	3.5	100	11.1	4.5
Cod liver oil	0	100	23.5	570
Margarine 70% fat	>12	99.8	27.3	0
«Omega-3» margarine 80%fat	1.1	100	31.3	2
«Omega-3» pork sausage	0.3	73.2	45	40
Rabbit, stewed	1.6	40.4	43.4	90
Foods sources of ALA				
Butter	0.8	99.3	70.4	250
«Omega-3» butter	0.7	99.3	72.5	250
«Omega-3» eggs	3.4	64.7	28.6	380
Pork, raw sausage meat	10.5	83.3	40.7	60
Horse, meat, raw	<0.7	32.6	41.1	54
Foods rich in DHA				
Cod liver oil	0	100	23.5	570
«Omega-3» oil	3.5	100	11.1	4.5
«Omega-3» margarine 80% fat	1.1	100	31.3	2
«Omega-3» bread (with fish oil)	0.3	6.5	21.6	0
«Omega-3» bread (with fish oil + linseed)	0.1	5	23	0
Roast chicken, leg, meat and skin	2	53.8	30.5	91
«Omega-3» milk (with fish oil)	0.1	30.6	69.4	6
Cod, raw	0	8	20.6	43
Tuna canned in brine	0	12.3	35.4	60
Food source of DHA				
Lamb leg, raw	0.2	66.7	54.2	74

In addition to the quantitative nutrition claims "source of" and "rich in" already mentioned, several levels of claim could be envisaged on the basis of these criteria. In particular, the working group's discussions highlighted two possible types of claim:
- a factual claim indicating that the food could help re-balance the intake of omega-3 fatty acids,
- a functional claim explaining that omega-3 contribute to healthy cardiovascular function.

Based on the list of foods selected to illustrate this document, the application of the criteria produces the diagram below.

Diagram 1 - Decision tree illustrating the level of claim envisageable based on the nutritional quality of foods·[4]



[4]NB: - The lists of products satisfying the different levels of criteria, prepared based on the available compositional data, are for information only and are not restrictive.

- Linseed oil appears on these lists subject to its authorisation for use for human consumption (it is currently prohibited in France).

6. References

- Favier J.C., Ireland-Ripert J., Toque C. et Feinberg M. (1995). *Répertoire général des aliments, table de composition*. Inra Éditions et Tec&Doc Lavoisier, Paris.
- Food Standards Agency. (2002). *McCance & Widdowson's, The composition of food, sixth summary edition*. Royal Society of Chemistry, Cambridge.
- Ireland J., Favier J.C. et Feinberg M. (2002). *Répertoire général des aliments, tome 2, produits laitiers*. 2e édition. Inra Éditions et Tec&Doc Lavoisier, Paris..
- Martin A. coord. (2001). *Apports nutritionnels conseillés pour la population française*. 3è edition. Éditions et Tec&Doc Lavoisier, Paris.
- Martin A. coord. (2001) Nutritional Recommendations for the French Population. Sciences des aliments: vol. 24, n°4, Lavoisier, Paris.
- Ministry of Agriculture, Fisheries and Food. (1998). *Fatty acids, supplement to McCance & Widdowson's, the composition of food.*. Royal Society of Chemistry, Cambridge..
- Souci S.W., Fachmann W. and Kraut H. (2000). *Die Zusammensetzung der Lebensmittel Nahrwert-Tabellen*. Medpharm Scientific Publisher, Stuttgart.

Per 100 g of food		composition									α-linolenic acid				DHA				saturated FA		omega 6 and 3 balance		Fat ≤ 33 % TDE	cholesterol ≤ 150 mg / 100 g
		energy	fat	SFA	linoleic acid	α-linolenic acid	DHA	Total omega-3 LC-PUFA	α-linolenic acid equiv.	Cholesterol content	% ANC	source or rich	quantity to be added to reach 15% ANC	quantity to be added to reach 30% ANC	% ANC	source or rich	quantity to be added to reach 15% ANC	quantity to be added to reach 30% ANC	SFA / total FA	SFA / total FA ≤ 30 %	linolenic a. / α-linolenic acid equiv.	linolenic a. / α-linolenic acid equiv. ≤ 5		
		kCal/100 g	g/100 g	g/100 g	mg/100 g	mg/100 g	mg/100 g	mg/100 g	mg/100 g	mg/100 g	per 100 g food		in mg per 100 g food	in mg per 100 g food	per 100 g food		in mg per 100 g food	in mg per 100 g food						
Egg, whole, raw	Ciqual + McCance & Widdowson	146	10.5	3.1	1500	75	0	0	75	380	3.8	no	225	525	0.0	no	18	36	32.8%	no	20.0	no	no	no
«omega -3» eggs	Chicken fed on linseed	146	10.5	2.7	1500	435	0	0	435	380	21.8	source	0	165	0.0	no	18	36	28.6%	ok	3.4	ok	no	no
Butter	Ciqual	752	83	52.6	1410	460	0	141	1870	250	23.0	source	0	140	0.0	no	18	36	70.4%	no	0.8	ok	no	no
Butter, salted 3%	Ciqual	742	82	49	1393	460	0	139	1850	250	23.0	source	0	140	0.0	no	18	36	66.4%	no	0.8	ok	no	no
«omega -3» butter	Cattle feed fortified with linseed	743	82	53.5	1410	515	0	141	1925	250	25.8	source	0	85	0.0	no	18	36	72.5%	no	0.7	ok	no	no
Whole milk yoghurt	Ciqual	68.4	3.7	2.35	60	20	0	0	20	250	1.0	no	280	580	0.0	no	18	36	70.6%	no	3.0	ok	no	no
«omega -3» plain yoghurt	Cattle feed fortified with linseed	70	3.9	2.57	60	30	0	0	30	13	1.5	no	270	570	0.0	no	18	36	73.2%	no	2.0	ok	no	ok
Bread, baguette	Ciqual + McCance & Widdowson	271	1	0.23	326	21	0	0	21	12.8	1.1	no	279	579	0.0	no	18	36	25.6%	ok	15.5	no	ok	ok
Bread, wholemeal	Ciqual + McCance & Widdowson	234	1.8	0.35	670	50	0	0	50	0	2.5	no	250	550	0.0	no	18	36	21.6%	ok	13.4	no	ok	ok
«omega -3» bread	With fish oil	248	1.8	0.35	670	50	220	220	2250	0	2.5	no	250	550	183.3	rich	0	0	21.6%	ok	0.3	ok	ok	ok
«omega -3» bread	With wheatgerm +fish oil + linseed	252.5	1.4	0.29	498	315	315	315	3465	0	15.8	source	0	285	262.5	rich	0	0	23.0%	ok	0.1	ok	ok	ok
Cod liver oil	McCance & Widdowson	899	99.9	21.1	2600	1100	8300	20500	206100	0	55.0	rich	0	0	6916.7	rich	0	0	23.5%	ok	0.0	ok	no	ok
Olive oil	McCance & Widdowson	899	99.9	14.3	7500	700	0	0	700	570	35.0	rich	0	0	0.0	no	18	36	15.9%	ok	10.7	no	no	no
Rapeseed oil	McCance & Widdowson	899	99.9	6.6	19700	9600	0	0	9600	0	480.0	rich	0	0	0.0	no	18	36	7.3%	ok	2.1	ok	no	ok

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Per 100 g of food		composition								α-linolenic acid				DHA				saturated FA		omega 6 and 3 balance			
		energy	fat	SFA	linoleic acid	α-linolenic acid	DHA	Total omega-3 LC-PUFA	α-linolenic acid equiv.	Cholesterol content	% ANC	source or rich	quantity to be added to reach 15% ANC	quantity to be added to reach 30% ANC	% ANC	source or rich	quantity to be added to reach 15% ANC	quantity to be added to reach 30% ANC	SFA / total FA	SFA / total FA ≤ 30 %	linolenic a. / α-linolenic acid equiv.	linolenic a. / α-linolenic acid equiv. ≤ 5	Fat ≤ 33% TDE	cholesterol ≤ 150 mg / 100 g
		kCal/100 g	g/100 g	g/100 g	mg/100 g	mg/100 g	mg/100 g	mg/100 g	mg/100 g	mg/100 g	per 100 g food		in mg per 100 g food	in mg per 100 g food	per 100 g food		in mg per 100 g food	in mg per 100 g food						
Walnut oil	McCance & Widdowson	899	99.9	9.1	58400	11500	0	0	11500	0	575.0	rich	0	0	0.0	no	18	36	10.1%	ok	5.1	no	no	ok
Wheatgerm oil	McCance & Widdowson	899	99.9	18.5	55100	5300	0	0	5300	0	265.0	rich	0	0	0.0	no	18	36	20.6%	ok	10.4	no	no	ok
Corn oil	McCance & Widdowson	899	99.9	14.5	50400	900	0	0	900	0	45.0	rich	0	0	0.0	no	18	36	16.1%	ok	56.0	no	no	ok
Sunflower seed oil	McCance & Widdowson	899	99.9	12	63200	100	0	0	100	0	5.0	no	200	500	0.0	no	18	36	13.3%	ok	632.0	no	no	ok
Hazelnut oil	McCance & Widdowson	899	99.9	7.8	11100	100	0	0	100	0	5.0	no	200	500	0.0	no	18	36	8.7%	ok	111.0	no	no	ok
Soya oil	McCance & Widdowson	899	99.9	15.6	51500	7300	0	0	7300	0	365.0	rich	0	0	0.0	no	18	36	17.4%	ok	7.1	no	no	ok
Linseed oil	Ciqual + Souci + Fachman Kraut	900	100	8.6	13900	54200	0	0	54200	0	2710.0	rich	0	0	0.0	no	18	36	9.6%	ok	0.3	ok	no	ok
«omega -3» oil	Blend of fish, rapeseed walnut etc. oils	900	100	10	33000	7300	130	200	9300	0	365.0	rich	0	0	108.3	rich	0	0	11.1%	ok	3.5	ok	no	ok
Ham, cooked	Ciqual + McCance & Widdowson	135	6.5	2.3	793	65	13	52	585	4.5	3.3	no	235	535	10.8	no	5	23	39.3%	no	1.4	ok	no	ok
«omega -3» ham	Pig feed fortified with linseed	127	5.3	1.8	647	149	11	41	556	52	7.5	no	151	451	8.8	no	7	25	37.7%	no	1.2	ok	no	ok
Bacon, diced	Ciqual + McCance & Widdowson	280	23.1	9.4	2731	284	0	98	1264	42	14.2	no	16	316	0.0	no	18	36	45.2%	no	2.2	ok	no	ok
«omega -3» bacon, diced	Pig feed fortified with linseed	145	7.3	2.52	863	234	0	26	496	70	11.7	no	66	366	0.0	no	18	36	38.4%	no	1.7	ok	no	ok

Per 100 g of food			Pure pork sausage meat	«omega -3» pork sausage	Pork, round	«omega -3» pork round	Lamb, leg, raw	Rib, steak, broiled	Ground beef 20% fat, raw	Veal, round, raw	Duck, meat only, roasted	Rabbit, stewed
			Ciqual + McCance & Widdowson	Pig feed fortified with linseed	Ciqual + McCance & Widdowson	Pig feed fortified with linseed	Ciqual + McCance & Widdowson	Ciqual + Souci, Fachmann, Kraut	Ciqual + McCance & Widdowson	Ciqual + McCance & Widdowson	Ciqual + McCance & Widdowson	Ciqual + McCance & Widdowson
composition	energy	kCal/100 g	324	246	113	106	216	203	252	151	190	194
	fat	g/100 g	30	20	3.2	2	16	11.8	20.4	3	10	8.7
	SFA	g/100 g	11	8.1	1.3	0.7	7.8	5	8.6	0.95	2.7	3.4
	linoleic acid	mg/100 g	3463	2309	432	270	312	585	227	157	1154	998
	α-linolenic acid	mg/100 g	330	760	40	37	203	46	114	11	106	640
	DHA	mg/100 g	0	0	8	5	26	0	0	0	0	0
	Total omega-3 LC-PUFA	mg/100 g	0	0	32	20	117	0	0	0	0	0
	α-linolenic acid equiv.	mg/100 g	330	760	360	237	1373	46	114	11	106	640
	Cholesterol content	mg/100 g	22	60	40	65	41	74	70	69	70	120
α-linolenic acid	% ANC	per 100 g food	16.5	38.0	2.0	1.9	10.2	2.3	5.7	0.6	5.3	32.0
	source or rich		source	rich	no	no	no	no	no	no	no	rich
	quantity to be added to reach 15% ANC	in mg per 100 g food	0	0	260	263	97	254	186	289	194	0
	quantity to be added to reach 30% ANC	in mg per 100 g food	270	0	560	563	397	554	486	589	494	0
DHA	% ANC	per 100 g food	0.0	0.0	6.7	4.2	21.7	0.0	0.0	0.0	0.0	0.0
	source or rich		no	no	no	no	source	no	no	no	no	no
	quantity to be added to reach 15% ANC	in mg per 100 g food	18	18	10	13	0	18	18	18	18	18
	quantity to be added to reach 30% ANC	in mg per 100 g food	36	36	28	31	10	36	36	36	36	36
saturated FA	SFA / total FA		40.7%	45.0%	45.1%	38.9%	54.2%	47.1%	46.8%	35.2%	30.0%	43.4%
	SFA / total FA ≤ 30 %		no	no	no	no	no	no	no	no	ok	no
omega 6 and 3 balance	linolenic a. / α-linolenic acid equiv.		10.5	3.0	1.2	1.1	0.2	12.7	2.0	14.3	10.9	1.6
	linolenic a. / α-linolenic acid equiv. ≤ 5		no	ok	ok	ok	ok	no	ok	no	no	ok
Fat ≤ 33% TDE			no	no	ok	ok	no	no	no	ok	no	no
cholesterol ≤ 150 mg / 100 g			ok	ok	ok	ok	ok	ok	ok	ok	ok	ok

Per 100 g of food			Chicken, leg, meat and skin, roasted	Horse meat, raw	Margarine 70% fat	«omega-3» cooking margarine 80% fat	Cod, raw	Tuna canned in brine, drained
			Ciqual + McCance & Widdowson	Ciqual + Souci, Fachmann, Kraut	Ciqual	With fish oil	Ciqual + McCance & Widdowson	Ciqual + McCance & Widdowson
composition	energy	kCal/100 g	226	127	631	720	79	117
	fat	g/100 g	13.5	4.6	70	80	0.7	1.6
	SFA	g/100 g	3.7	1.7	17.2	22.5	0.13	0.51
	linoleic acid	mg/100 g	2130	308	26500	15000	0	0.01
	α-linolenic acid	mg/100 g	334	460	2100	1250	0	0
	DHA	mg/100 g	37	nd	nd	1250	160	370
	Total omega-3 LC-PUFA	mg/100 g	74	nd	nd	1250	170	430
	α-linolenic acid equiv.	mg/100 g	1074	nd	nd	13750	1700	4300
	Cholesterol content	mg/100 g	90	91	54	6	43	60
α-linolenic acid	% ANC	per 100 g food	16.7	23.0	105.0	62.5	0	0
	source or rich		source	source	rich	rich	no	no
	quantity to be added to reach 15% ANC	in mg per 100 g food	0	0	0	0	300	300
	quantity to be added to reach 30% ANC	in mg per 100 g food	266	140	0	0	600	600
DHA	% ANC	per 100 g food	30.8	nd	nd	1041.7	133.3	308.3
	source or rich		rich	nd	nd	rich	rich	rich
	quantity to be added to reach 15% ANC	in mg per 100 g food	0	nd	nd	0	0	0
	quantity to be added to reach 30% ANC	in mg per 100 g food	0	nd	nd	0	0	0
saturated FA	SFA / total FA		30.5%	41.1%	27.3%	31.3%	20.6%	35.4%
	SFA / total FA ≤ 30 %		no	no	ok	no	ok	no
omega 6 and 3 balance	linolenic a. / α-linolenic acid equiv.		2.0	nd	nd	1.1	0.0	0.0
	linolenic a. / α-linolenic acid equiv. ≤ 5		ok	nd	nd	ok	ok	ok
Fat ≤ 33% TDE			no	ok	no	no	ok	ok
cholesterol ≤ 150 mg / 100 g			ok	ok	ok	ok	ok	ok

Per 100 ml of food		composition									α-linolenic acid				DHA				saturated FA		omega 6 and 3 balance		Fat ≤ 33% TDE	cholesterol ≤ 150 mg / 100 g
		energy	fat	SFA	linoleic acid	α-linolenic acid	DHA	Total omega-3 LC-PUFA	α-linolenic acid equiv.	Cholesterol content	% ANC	source or rich	quantity to be added to reach 15% ANC	quantity to be added to reach 30% ANC	% ANC	source or rich	quantity to be added to reach 15% ANC	quantity to be added to reach 30% ANC	SFA / total FA	SFA / total FA ≤ 30 %	linolenic a. / α-linolenic acid equiv.	linolenic a. / α-linolenic acid equiv. ≤ 5		
		kCal/100 ml	g/100 ml	g/100 ml	mg/100 ml	mg/100 ml	mg/100 ml	mg/100 ml	mg/100 ml	mg/100 ml	per 100 ml food		in mg per 100 ml food	in mg per 100 ml food	per 100 ml food		in mg per 100 ml food	in mg per 100 ml food						
Semi-skimmed milk, UHT	Ciqual	47	1.6	1	31	10	0	0	10	6	0.5	no	290	590	0.0	no	18	36	69.4%	no	3.1	OK	OK	OK
Whole milk, UHT	Ciqual	65	3.6	2.1	72	21	0	0	21	12	1.1	no	279	579	0.0	no	18	36	64.8%	no	3.4	OK	no	OK
Skimmed milk, UHT	Ciqual	34	0.1	0.06	0	0	0	0	0	1	0.0	no	300	600	0.0	no	18	36	66.7%	no	nd	nd	OK	OK
«Omega-3» UHT semi-skimmed milk	Cattle feed fortified with linseed	46	1.55	1.1	31	13	0	0	13	6	0.7	no	287	587	0.0	no	18	36	78.9%	no	2.4	OK	OK	OK
«Omega-3» milk	With fish oil[5]	47	1.6	1	31	10	25	50	510	6	0.5	no	290	590	20.8	source	0	11	69.4%	no	0.1	OK	OK	OK
«Omega-3» milk	With fish oil	47	1.6	1	31	10	35	70	710	6	0.5	no	290	590	29.2	source	0	1	69.4%	no	0.0	OK	OK	OK

[5] According to the manufacturer's consumer department, this milk is no longer on the market.

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Per 100 kcal of food														
			Egg, whole, raw	«omega -3» eggs	Butter	Butter, salted 3%	«omega -3» butter	«omega -3» butter, salted 3%	Yoghurt, whole milk	«omega -3» plain yoghurt	Bread, baguette	Bread, wholemeal	«omega -3» bread	
			Ciqual + McCance & Widdowson	Chickens feed with linseed	Ciqual	Ciqual	Cattle feed fortified with linseed	Cattle feed fortified with linseed	Ciqual	Cattle feed fortified with linseed	Ciqual + McCance & Widdowson	Ciqual + McCance & Widdowson	Ciqual + McCance & Widdowson	with fish oil
composition	energy	kCal/100g or 100 ml	146	146	752	742	743	726	68.4	70	271	234	248	
	fat	g/100g or 100 ml	10.5	10.5	83	82	82	80	3.7	3.9	1	1.8	1.8	
	SFA	g/100g or 100 ml	3.1	2.7	52.6	49	53.5	56	2.35	2.57	0.23	0.35	0.35	
	linoleic acid	mg/100g or 100 ml	1500	1500	1410	1393	1410	1393	60	60	326	670	670	
	α-linolenic acid	mg/100g or 100 ml	75	435	460	460	515	421	20	30	21	50	50	
	DHA	mg/100g or 100 ml	0	0	0	0	0	0	0	0	0	0	220	
	Total omega-3 LC-PUFA	mg/100g or 100 ml	0	0	141	139	141	139	0	0	0	0	220	
	α-linolenic acid equiv.	mg/100g or 100 ml	75	435	1870	1850	1925	1811	20	30	21	50	2250	
	Cholesterol content	mg/100g or 100 ml	380	380	250	250	250	250	13	12.8	0	0	0	
α-linolenic acid	% ANC	per 100 kcal food	2.6	14.9	3.1	3.1	3.5	2.9	1.5	2.1	0.4	1.1	1.0	
	source or rich		no	no	no	no	no	no	no	no	no	no	no	
	quantity to be added to reach 15% ANC	in mg per 100 kcal food	249	2	239	238	231	242	271	257	292	279	280	
	quantity to be added to reach 30% ANC	in mg per 100 kcal food	549	302	539	538	531	542	571	557	592	579	580	
DHA	% ANC	per 100 kcal food	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	73.9	
	source or rich		no	no	no	no	no	no	no	no	no	no	rich	
	quantity to be added to reach 15% ANC	in mg per 100 kcal food	18	18	18	18	18	18	18	18	18	18	0	
	quantity to be added to reach 30% ANC	in mg per 100 kcal food	36	36	36	36	36	36	36	36	36	36	0	
saturated FA	SFA / total FA		20.0	3.4	0.8	0.8	0.7	0.8	3.0	2.0	15.5	13.4	0.3	
	SFA / total FA ≤ 30 %		no	ok	ok	ok	ok	ok	ok	ok	no	no	ok	
omega 6 and 3 balance	linolenic a. / α-linolenic acid equiv.		32.8%	28.6%	70.4%	66.4%	72.5%	77.8%	70.6%	73.2%	25.6%	21.6%	21.6%	
	linolenic a. / α-linolenic acid equiv. ≤ 5		no	ok	no	no	no	no	no	no	ok	ok	ok	
Fat ≤ 33% TDE			no	no	no	no	no	no	no	no	ok	ok	ok	

Per 100 kcal of food			«omega -3» bread	Cod liver oil	Olive oil	Rapeseed oil	Walnut oil	Wheatgerm oil	Corn oil	Sunflower oil	Hazelnut oil	Soy oil	Linseed oil	«omega -3» oil
			with wheatgerm + fish oil + linseed	McCance & Widdowson	McCance & Widdowson	McCance & Widdowson	McCance & Widdowson	McCance & Widdowson	McCance & Widdowson	McCance & Widdowson	McCance & Widdowson	McCance & Widdowson	Ciqual + Souci Fachman Kraut	Blendof fish, rapeseed, walnut, etc. oils,
composition	energy	kCal/100g or 100 ml	252.5	899	899	899	899	899	899	899	899	899	900	900
	fat	g/100g or 100 ml	1.4	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	100	100
	SFA	g/100g or 100 ml	0.29	21.1	14.3	6.6	9.1	18.5	14.5	12	7.8	15.6	8.6	10
	linoleic acid	mg/100g or 100 ml	498	2600	7500	19700	58400	55100	50400	63200	11100	51500	13900	33000
	α-linolenic acid	mg/100g or 100 ml	315	1100	700	9600	11500	5300	900	100	100	7300	54200	7300
	DHA	mg/100g or 100 ml	315	8300	0	0	0	0	0	0	0	0	0	130
	Total omega-3 LC-PUFA	mg/100g or 100 ml	315	20500	0	0	0	0	0	0	0	0	0	200
	α-linolenic acid equiv.	mg/100g or 100 ml	3465	206100	700	9600	11500	5300	900	100	100	7300	54200	9300
	Cholesterol content	mg/100g or 100 ml	0	570	0	0	0	0	0	0	0	0	0	4.5
α-linolenic acid	% ANC	per 100 kcal food	6.2	6.1	3.9	53.4	64.0	29.5	5.0	0.6	0.6	40.6	301.1	40.6
	source or rich		no	no	no	rich	rich	source	no	no	no	rich	rich	rich
	quantity to be added to reach 15% ANC	in mg per 100 kcal food	175	178	222	0	0	0	200	289	289	0	0	0
	quantity to be added to reach 30% ANC	in mg per 100 kcal food	475	478	522	0	0	10	500	589	589	0	0	0
DHA	% ANC	per 100 kcal food	104.0	769.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.0
	source or rich		rich	rich	no	no	no	no	no	no	no	no	no	no
	quantity to be added to reach 15% ANC	in mg per 100 kcal food	0	0	18	18	18	18	18	18	18	18	18	4
	quantity to be added to reach 30% ANC	in mg per 100 kcal food	0	0	36	36	36	36	36	36	36	36	36	22
saturated FA	SFA / total FA		0.1	0.0	10.7	2.1	5.1	10.4	56.0	632.0	111.0	7.1	0.3	3.5
	SFA / total FA ≤ 30 %		ok	ok	no	ok	no	no	no	no	no	no	ok	ok
omega 6 and 3 balance	linolenic a. / α-linolenic acid equiv.		23.0%	23.5%	15.9%	7.3%	10.1%	20.6%	16.1%	13.3%	8.7%	17.4%	9.6%	11.1%
	linolenic a. / α-linolenic acid equiv. ≤ 5		ok	ok	ok	ok	ok	ok	ok	ok	ok	ok	ok	ok
Fat ≤ 33% TDE			ok	no	no	no	no	no	no	no	no	no	no	no

Per 100 kcal of food			Ham, cooked	«omega -3» ham	Bacon, diced	«omega -3» bacon, diced	Pure pork sausage meat	«omega -3» pork sausage	Pork, round	«omega -3» pork round	Lamb, leg, raw	Rib, steak, broiled
			Cigual + McCance & Widdowson	Pig feed fortified with linseed	Cigual + McCance & Widdowson	Pig feed fortified with linseed	Cigual + McCance & Widdowson	Pig feed fortified with linseed	Cigual + McCance & Widdowson	Pig feed fortified with linseed	Cigual + McCance & Widdowson	Cigual + Souci, Fachmann , Kraut
composition	energy	kCal/100g or 100 ml	135	127	280	145	324	246	113	106	216	203
	fat	g/100g or 100 ml	6.5	5.3	23.1	7.3	30	20	3.2	2	16	11.8
	SFA	g/100g or 100 ml	2.3	1.8	9.4	2.52	11	8.1	1.3	0.7	7.8	5
	linoleic acid	mg/100g or 100 ml	793	647	2731	863	3463	239	432	270	312	585
	α-linolenic acid	mg/100g or 100 ml	65	149	284	234	330	760	40	37	203	46
	DHA	mg/100g or 100 ml	13	11	0	0	0	0	8	5	26	0
	Total omega-3 LC-PUFA	mg/100g or 100 ml	52	41	98	26	0	0	32	20	117	0
	α-linolenic acid equiv.	mg/100g or 100 ml	585	559	1264	494	330	760	360	237	1373	46
	Cholesterol content	mg/100g or 100 ml	52	42	70	22	60	40	65	41	74	70
α-linolenic acid	% ANC	per 100 kcal food	2.4	5.9	5.1	8.1	5.1	15.4	1.8	1.7	4.7	1.1
	source or rich		no	no	no	no	no	source	no	no	no	no
	quantity to be added to reach 15% ANC	in mg per 100 kcal food	252	183	199	139	198	0	265	265	206	277
	quantity to be added to reach 30% ANC	in mg per 100 kcal food	552	483	499	439	498	291	565	565	506	577
DHA	% ANC	per 100 kcal food	8.0	7.2	0.0	0.0	0.0	0.0	5.9	3.9	10.0	0.0
	source or rich		no	no	no	no	no	no	no	no	no	no
	quantity to be added to reach 15% ANC	in mg per 100 kcal food	8	9	18	18	18	18	11	13	6	18
	quantity to be added to reach 30% ANC	in mg per 100 kcal food	26	27	36	36	36	36	29	31	24	36
saturated FA	SFA / total FA		1.4	1.2	2.2	1.7	10.5	0.3	1.2	1.1	0.2	12.7
	SFA / total FA ≤ 30 %		ok	ok	ok	ok	no	ok	ok	ok	ok	no
omega 6 and 3 balance	linolenic a. / α-linolenic acid equiv.		39.3%	37.7%	45.2%	38.4%	40.7%	45.0%	45.1%	38.9%	54.2%	47.1%
	linolenic a. / α-linolenic acid equiv. ≤ 5		no	no	no	no	no	no	no	no	no	no
Fat ≤ 33% TDE			no	no	no	no	no	no	ok	ok	no	no

Per 100 kcal of food			Ground beef 20% fat, raw	Veal, round, raw	Duck, meat, roast	Rabbit, stewed	Chicken, leg, meat and skin, roast	Horse meat, raw	Semi-skimmed milk, UHT	Whole milk, UHT	Skimmed milk UHT	«Omega-3» UHT semi-skimmed milk	«Omega-3» milk
			Ciqual + McCance & Widdowson	Ciqual + McCance & Widdowson	Ciqual + McCance & Widdowson	Ciqual + McCance & Widdowson	Ciqual + McCance & Widdowson	Ciqual + Souci, Fachmann, Kraut	Ciqual	Ciqual	Ciqual	Cattle feed fortified with linseed	With fish oil
composition	energy	kCal/100g or 100 ml	252	151	190	194	226	127	47	65	34	46	47
	fat	g/100g or 100 ml	20.4	3	10	8.7	13.5	4.6	1.6	3.6	0.1	1.55	1.6
	SFA	g/100g or 100 ml	8.6	0.95	2.7	3.4	3.7	1.7	1	2.1	0.06	1.1	1
	linoleic acid	mg/100g or 100 ml	227	157	1154	998	2130	308	31	72	0	31	31
	α-linolenic acid	mg/100g or 100 ml	114	11	106	640	334	460	10	21	0	13	10
	DHA	mg/100g or 100 ml	0	0	0	0	37	nd	0	0	0	0	25
	Total omega-3 LC-PUFA	mg/100g or 100 ml	0	0	0	0	74	nd	0	0	0	0	50
	α-linolenic acid equiv.	mg/100g or 100 ml	114	11	106	640	1074	nd	10	21	0	13	510
	Cholesterol content	mg/100g or 100 ml	69	70	120	90	91	54	6	12	1	6	6
α-linolenic acid	% ANC	per 100 kcal food	2.3	0.4	2.8	16.5	7.4	18.1	1.1	1.6	0.0	1.4	1.1
	source or rich		no	no	no	source	no	source	no	no	no	no	no
	quantity to be added to reach 15% ANC	in mg per 100 kcal food	255	293	244	0	152	0	279	268	300	272	279
	quantity to be added to reach 30% ANC	in mg per 100 kcal food	555	593	544	270	452	238	579	568	600	572	579
DHA	% ANC	per 100 kcal food	0.0	0.0	0.0	0.0	13.6	nd	0.0	0.0	0.0	0.0	44.3
	source or rich		no	no	no	no	no	nd	no	no	no	no	rich
	quantity to be added to reach 15% ANC	in mg per 100 kcal food	18	18	18	18	2	nd	18	18	18	18	0
	quantity to be added to reach 30% ANC	in mg per 100 kcal food	36	36	36	36	20	nd	36	36	36	36	0
saturated FA	SFA / total FA		2.0	14.3	10.9	1.6	2.0	nd	3.1	3.4	nd	2.4	0.1
	SFA / total FA ≤ 30 %		ok	no	no	ok	ok	nd	ok	ok	nd	ok	ok
omega 6 and 3 balance	linolenic a. / α-linolenic acid equiv.		46.8%	35.2%	30.0%	43.4%	30.5%	41.1%	69.4%	64.8%	66.7%	78.9%	69.4%
	linolenic a. / α-linolenic acid equiv. ≤ 5		no	no	ok	no	no	no	no	no	no	no	no
Fat ≤ 33% TDE			no	ok	no	no	no	ok	ok	no	ok	ok	ok

Per 100 kcal of food			«Omega-3» milk	Margarine 70 % fat	«omega-3» cooking margarine 80 % fat	Cod, raw	Tuna canned in brine, drained
			With fish oil	Ciqual	With fish oil	Ciqual + McCance & Widdowson	Ciqual + McCance & Widdowson
composition	energy	kCal/100g or 100 ml	47	631	720	79	117
	fat	g/100g or 100 ml	1.6	70	80	0.7	1.6
	SFA	g/100g or 100 ml	1	17.2	22.5	0.13	0.61
	linoleic acid	mg/100g or 100 ml	31	26500	15000	0	10
	α-linolenic acid	mg/100g or 100 ml	1	2100	1250	0	0
	DHA	mg/100g or 100 ml	35	nd	1250	160	370
	Total omega-3 LC-PUFA	mg/100g or 100 ml	70	nd	1250	170	430
	α-linolenic acid equiv.	mg/100g or 100 ml	701	nd	13750	1700	4300
	Cholesterol content	mg/100g or 100 ml	6	0	2	43	60
α-linolenic acid	% ANC	per 100 kcal food	0.1	16.6	8.7	0.0	0.0
	source or rich		no	source	no	no	no
	quantity to be added to reach 15% ANC	in mg per 100 kcal food	298	0	126	300	300
	quantity to be added to reach 30% ANC	in mg per 100 kcal food	598	267	426	600	600
DHA	% ANC	per 100 kcal food	62.1	nd	144.7	168.8	263.5
	source or rich		rich	nd	rich	rich	rich
	quantity to be added to reach 15% ANC	in mg per 100 kcal food	0	nd	0	0	0
	quantity to be added to reach 30% ANC	in mg per 100 kcal food	0	nd	0	0	0
saturated FA	SFA / total FA		0.0	nd	1.1	0.0	0.0
	SFA / total FA ≤ 30 %		ok	nd	ok	ok	ok
omega 6 and 3 balance	linolenic a. / α-linolenic acid equiv.		69.4%	27.3%	31.3%	20.6%	42.4%
	linolenic a. / α-linolenic acid equiv. ≤ 5		no	ok	no	ok	no
Fat ≤ 33% TDE			ok	no	no	ok	ok

Annex 8: review of human studies on the link between omega-3 fatty acids and cancer

The working group was interested in the scientific data on the link between the intake of omega-3 fatty acids and cancer, firstly on an exploratory basis in terms of potential claims and secondly within the context of assessing the nutritional safety of an increase in intake levels.

Three types of cancer are highly influenced by the environment (including diet): breast cancer, prostate cancer and colorectal cancer (in both sexes).
Data concerning PUFA and cancer risk are very recent. Moreover, until the 1980s it was difficult to distinguish between omega-6 and omega-3 fatty acid intake, given the incomplete nature of composition tables at that time. Within this context, the use of biomarkers has grown rapidly (analysis of fatty acid levels in serum lipids, erythrocyte membranes or adipose tissue).

- *Breast cancer risk*

Data on the relationship between dietary intake of n-3 fatty acids and cancer risk diverge (Table P). As regards case-control studies, there are two studies concerning alpha-linolenic acid: one (Franceschi et al. 1996) showed a reduced risk of breast cancer with a high dietary intake of this fatty acid, the other (De Stefani et al., 1998) demonstrated an increased risk. The two studies concerning dietary intake of n-3 PUFA of marine origin showed either a reduced risk (Mannisto et al., 1999), or a non-significant reduction of the risk of breast cancer (Hursting et al., 1990). Moreover, in a cohort study (Holmes et al., 1999), no effect of n-3 PUFA of marine origin on breast cancer was demonstrated.

Regarding the studies using biomarkers (Table Q):
- Based on analysis of levels of the different fatty acids in serum phospholipids, one study (Vatten et al., 1993) showed an inverse relationship between the level of linoleic acid and the risk of breast cancer, but another study (Chajes et al., 1999) found no evidence of an effect from PUFA (n-6 or n-3) on this risk.
- The only study based on the level of fatty acids in the erythrocyte membranes (Pala et al., 2001) showed an inverse relationship between the level of DHA and the risk of breast cancer. In this study, the DHA was associated with fish consumption.
- North American studies based on analysis of adipose tissue have not generally demonstrated a link between levels of n-3 PUFA and breast cancer risk. However, one study conducted in Finland (Zhu et al., 1995) showed an inverse relationship between levels of DHA in the mammary adipose tissue and the risk of breast cancer. In addition, the Euramic study (Simonsen et al. 1998), conducted in Europe on a wide population, enabled the demonstration of an inverse relationship between the long chain n-3 PUFA / n-6 PUFA ratio and the risk of breast cancer. Another study (Klein et al., 2000), conducted in France, showed an inverse relationship between levels of alpha-linolenic acid and the appearance of benign breast tumours. However, this study had a methodology open to criticism. The study was therefore repeated, again in France (Maillard et al., 2002) with an indisputable methodology: it enabled the demonstration of an inverse relationship between levels of alpha-linolenic acid and of DHA in the adipose tissue and the risk of breast cancer. This shows, in particular, the importance of dietary intake of DHA, given the low conversion rate of alpha-linolenic acid into long chain derivatives.

Therefore an inverse relationship has been demonstrated in Europe between stores of DHA or alpha-linolenic acid in adipose tissue and the risk of breast cancer. However, to date, it has not been possible, using this approach based on nutritional epidemiology, to confirm clearly a protective effect from omega-3 fatty acids on the risk of the occurrence of this type of cancer.

Table P: number of studies on the link between estimated dietary intakes of omega-3 fatty acids and breast cancer risk.

	Reduced risk -	-, ns*	0	Increased risk +	+, ns	Total number of studies	References
Case-control studies							
Alpha-linolenic acid	1[a]			1[d]		2	[a] Franceschi et al, 1996. [d] De Stefani et al, 1998.
n-3 polyunsaturated fatty acids (of marine origin)	1[a]	1[b]				2	[a] Mannisto et al, 1999. [b] Hursting et al, 1990.
Cohort studies							
Alpha-linolenic acid						0	
n-3 polyunsaturated fatty acids (of marine origin)			1[c]			1	[c] Holmes et al, 1999.

Table Q: studies of the link between the composition in fatty acids of certain tissues and breast cancer risk

Authors **countries of the population studied**	Type of study Number of subjects	Tissue	Fatty acids	significant relationship
Vatten et al, 1993. **Norway**	Case-control 87 cases/235 controls	Serum	Saturated	none
			Monounsaturated	none
			Linoleic acid	Inverse
			n-3 polyunsaturated	None
Chajès et al, 1999 **Sweden**	Case-control 196 cases/388 controls	Serum	Saturated Stearic acid	Inverse
			Monounsaturated	none
			n-6 polyunsaturated	none
			n-3 polyunsaturated	none
Eid and Berry, 1988 **Israel**	Case-control 37 cases/27 controls	Sub-cutaneous adipose tissue	Polyunsaturated / saturated	none
London et al, 1993 **United States**	Case-control 380 cases/176 controls	Sub-cutaneous adipose tissue	Saturated	none
			Monounsaturated	none
			Total polyunsaturated	none
			Trans	none
			long chain n-3 polyunsaturated	none
Petrek et al, 1994 **United States**	Case-control	Sub-cutaneous adipose and mammary adipose tissue	Saturated	none
			Monounsaturated	None
			n-6 polyunsaturated	none
			n-3 polyunsaturated	None
Zhu et al, 1995 **Finland**	Case-control 73 cases/55 controls	Mammary adipose tissue	Saturated	None
			Monounsaturated	none
			Linoleic acid	none
			Docosahexaenoic acid 22 :6 n-3	Inverse
Bakker et al, 1997 **8 European countries + Israel,** EURAMIC Multicentre	Case-control	Sub-cutaneous adipose tissue	n-3 polyunsaturated	none
Kohlmeier et al, 1997 **5 European countries,** EURAMIC	Case-control 698 subjects	Sub-cutaneous adipose tissue	Trans fatty acids	Positive
Simonsen et al, 1998 **5 European countries**, EURAMIC	Case-control 291 cases/351 controls	Sub-cutaneous adipose tissue	Oleic acid	Inverse
Simonsen et al, 1998 **5 European countries**, EURAMIC	Case-control	Sub-cutaneous adipose tissue	Long chain n-3 PUFA/n-6 PUFA	Inverse
Klein et al, 2000. **France**	Case-control 123 cases/59 controls	Mammary adipose tissue	Saturated	none
			Monounsaturated	none
			n-6 polyunsaturated	none
			Long chain n-3 polyunsaturated	none
			Alpha-linolenic acid	Inverse
Pala et al., 2001, **Italy**	Prospective 4,022 post-menopausal women 71 cases	Erythrocyte membranes	18:0/18:1	inverse
			n-3 PUFA	none
Riboli, in course of publication	Meta-analysis	all	n-3 PUFA	No significant relationship. Does not take account of «Maillard et al.» below
Maillard et al, 2002. **France**	Case-control 241 cases/88 controls	Mammary adipose tissue	Saturated, palmitic acid (16 :0)	Inverse
			Monounsaturated, oleic acid (18 :1 n-9c)	Inverse
			n-6 polyunsaturated, linoleic acid (18 :2 n-6)	Positive
			Alpha-linolenic acid	Inverse
			Long chain n-3 polyunsaturated, docosahexaenoic acid (22 :6 n-3)	Inverse

- *Colorectal cancer risk*

This type of cancer may be more sensitive to foods with a high content of omega-3 fatty acids. Epidemiological data based on the estimation of dietary intake of omega-3 fatty acids are more in agreement on this type of cancer (in general, reduction in the risk is associated with fish consumption) (Table R).

However, the only study based on biomarkers (Bakker et al., 1997) (Table S), a multi-centre case-control study based on measurement of levels of n-3 PUFA in subcutaneous adipose tissue, did not show any significant relationship between this content and the risk of colorectal cancer.

These data support the hypothesis of the protective effect of a diet high in fish. However, it is not possible to come to a conclusion regarding a protective effect from omega-3 fatty acids against colon cancer.

Table R: Number of studies on the risk of colorectal cancer and based on the estimation of dietary intakes of omega-3 fatty acids

	Reduced risk -	-, ns*	0	Increased risk +	+, ns	Total number of studies	References
Case-control studies							
fish	3[a]					3	[a] Caygill and Hill, 1995 ; Kato et al., 1990; Franceschi et al., 1997.
n-3 PUFA/n-6 PUFA	1[a]					1	[a] Caygill and Hill, 1995.
Prospective studies							
fish	1[a] (men) 1[b] (women)		1[c]			3	[a]Giovannucci et al., 1994. [b]Willett et al., 1990. [c]Gaard et al., 1996.

Table S: Study on the risk of colorectal cancer and based on the measurement of the fatty acid content of certain tissues

Authors **countries of the population studied**	Type of study Number of subjects	Tissue	Fatty acids	significant relationship
Bakker et al., 1997 Multicentre	Case-control	Subcutaneous adipose	n-3 PUFA	none

- *Prostate cancer risk*

The few data available (Tables T and U) are favourable to a protective effect from a high consumption of n-3 PUFA of marine origin in terms of prostate cancer risk.
It should be noted that the positive relationship observed between the consumption of alpha-linolenic acid or the serum level of this fatty acid and the risk of prostate cancer, in studies conducted in the United States, could be explained by the high consumption of red meat, which is a factor of confusion.

Table T: Number of studies on the risk of prostate cancer and based on the estimation of dietary intake of omega-3 fatty acids

	Reduced risk -	-, ns*	0	Increased risk +	+, ns	Total number of studies	References
Case-control studies							
Alpha-linolenate (animal and plant sources)				1		1	De Stéfani et al., 2000.
Prospective studies							
Fish	1[a]		1[b]			2	[a]Terry et al., 2001. [b]Giovannucci et al., 1993.
Alpha-linolenate				1[a]			[a]Giovannucci et al., 1993.

Table U: Studies on the risk of prostate cancer based on measurement of the content of fatty acids in certain tissues

Authors **countries of the population studied**	Type of study Number of subjects	TISSUE	Fatty acids	significant relationship
Gann et al, 1994. **USA**	Case-control 120cases/120 controls	plasma	18:3 n-3	positive
Godley et al., 1997 **USA**	Case-control 89 cases/38 controls	Erythrocytes; adipose tissue	n-3 polyunsaturated 18:3n-3, EPA, DHA	none
Bakker et al., 1997 Multicentre	Case-control	Sub-cutaneous adipose tissue	n-3 PUFA	none
Norrish et al., 1999 **New Zealand**	Case-control 317 cases/480 controls	Erythrocyte membranes (phosphatidyl-choline)	EPA, DHA	inverse
Newcomer et al., 2001 **USA**	Case-control 67cases/156 controls	Erythrocyte membranes	18:3 n-3 n-6 PUFA	positive

- *Summary: omega-3 fatty acids and cancer*

There are no nutritional intervention data available on omega-3 fatty acids within the context of cancer prevention.

From all the observational studies conducted in humans, it appears that at present, there is no evidence indicating that fortification with omega-3 fatty acids (precursors or long chain derivatives) is beneficial as regards cancer. However, there are a number of indications suggesting that a diet high in omega-3 fatty acids is beneficial.