GR

ORIGINAL SUBMISSION

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March 6, 2012

RECEIVED
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Division of
Biotechnology and
GRAS Notice Review

Ms. Moraima J. Ramos Valle, MS
Division of Biotechnology & GRAS Notice Review (HFS-255)
Office of Food Additive Safety
Center for Food Additive Safety & Applied Nutrition
Food & Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: GRAS Notification – Canola Oil Use in Infant Formulas

Dear Ms. Ramos Valle:

On behalf of Danone Trading BV, we are submitting for FDA review the enclosed GRAS notification for Canola Oil Use in Infant Formulas. The attached documentation contains the specific information that addresses the safe human food uses for the subject notified substance as discussed in the GRAS guidance document. This notification also includes the additional information identified during the Danone February 9, 2012 telephone call with you and your colleagues, and we trust that the GRAS notice can now be filed as soon as possible.

Travel commitments precluded our being able to obtain the signature of Professor Sheila Innis of the Expert Panel prior to releasing this updated notification to you. Professor Innis has been closely involved in the subject safety review and evaluation, and she has agreed to review the modified GRAS notification as soon as her travels are completed. As you and I discussed on the telephone on March 1, 2012, we will supply the remaining panel member signature in the very near future.

If additional clarification is needed as you and your colleagues proceed with the review, please feel free to contact me *via* telephone or email.

We look forward to your feedback.

Sincerely,
(b) (6)

Robert S. McQuate, Ph.D.
CEO & Co-Founder
GRAS Associates, LLC
www.gras-associates.com

000465

Enclosure: GRAS Notification for Canola Oil Use in Infant Formulas (in triplicate)

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I. GRAS EXEMPTION CLAIM

A. Claim of Exemption from the Requirement for Premarket Approval Pursuant to Proposed 21 CFR 170.36(c)(1)[1]

Danone Trading B.V., an affiliated company of Danone Baby and Medical Nutrition B.V., has determined that canola oil (for blending with other oils to obtain the desired levels of fatty acids), meeting the specifications described below, is Generally Recognized As Safe (GRAS) for use in exempt and non-exempt infant formulas for full-term infants in accordance with Section 201(s) of the Federal Food, Drug, and Cosmetic Act. This determination was made by experts qualified by scientific training and experience; it is based on generally available and accepted scientific data, information, methods and principles, and is corroborated by the lack of adverse effects reported in countries where canola oil is permitted for use in infant formula. The evaluation accurately reflects the intended uses of the canola oil as a food ingredient (for blending with other oils to obtain the desired levels of fatty acids) in exempt and non-exempt infant formulas for term infants.

Signed:

(b) (6)

March 6, 2012

Robert S. McQuate, Ph.D.
GRAS Associates, LLC
20482 Jacklight Lane
Bend, OR 97702-3074

B. Name & Address of Notifier

Danone Trading B.V.
Schiphol Boulevard 105
1118 BG Schiphol Airport
The Netherlands

As the notifier, Danone Trading B.V. (hereinafter referred to as "Danone") accepts responsibility for the GRAS determination that has been made for canola oil as described in the subject notification; consequently, canola oil, meeting the conditions described herein, is exempt from pre-market approval requirements for use as food ingredient in exempt and non-exempt infant formulas for term infants.

[redacted]

[1] See 62 FR 18938 (17 April 1997). http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/ucm083058.htm.

C. Common Name & Identity of the Notified Substance

Canola oil is the common name for the notified substance. Canola oil is synonymous with low erucic acid rapeseed (LEAR) oil.

D. Conditions of Intended Use in Food

Canola oil is intended to be used in combination with other vegetable oils in exempt and non-exempt infant formulas for term infants. Canola oil will added at up to 31% of the fat blend such that the term infant formula provides the levels and ratio of essential fatty acids in compliance with 21 CFR 107.100 and the recommendations of the Expert Panel of the Life Sciences Research Office (LSRO) of the American Society of Nutritional Sciences (Life Science Research Office Report (LSRO) 1998).

E. Basis for the GRAS Determination

Pursuant to 21 CFR 170.30, canola oil has been determined to be GRAS on the basis of scientific procedures as discussed in the detailed description provided below.

F. Availability of Information

The data and information that serve as the basis for this GRAS notification will be available for review and copying at reasonable times at the offices of GRAS Associates, LLC, located at 20482 Jacklight Lane, Bend, OR 97702-3074 or the information will be sent to FDA upon request.

II. INTRODUCTION

A. Objective

At the request of Danone, GRAS Associates, LLC ("GA") has undertaken an independent safety evaluation of canola oil for use in exempt and non-exempt infant formulas for full-term infants. The purpose of the evaluation is to ascertain whether or not the use of canola oil at specified levels when blended with other oils to achieve desired levels of fatty acids in infant formulas can be considered to be generally recognized as safe, i.e., GRAS.

B. Foreword

Danone provided GA with a substantial body of information needed to enable the GRAS safety assessment of canola oil in combinations with other oils for incorporation into infant formulas to be undertaken. This included information from Danone suppliers on the chemical characteristics of canola oil together with the results of analyses of sample batches of canola oil supplied to Danone. Additional information received from Danone relates to the safety of canola oil in the diet that pertains to both infants and adults. The information received included reprints of toxicological studies on canola oil, including clinical studies on infants using formulas of various compositions. Danone was also asked to include any adverse reports, as well as those that supported conclusions of safety. An independent search of the scientific and regulatory literature extending through September 2011 was carried out. Included were reprints of toxicological studies on canola oil as well as clinical studies on infants who consumed infant formulas of various compositions which incorporated canola oil as part of the fat blend.

In accordance with the regulation of foods in the US, new food ingredients must undergo pre-market approval by the Food and Drug Administration (FDA) as food additives or, alternatively, the ingredients to be incorporated into foods must be determined to be GRAS. The authority to make GRAS determinations is not restricted to FDA. GRAS determinations may be provided by experts who are qualified by scientific training and experience to evaluate the safety of food and food ingredients under the intended conditions of use.[2] In 1997, FDA proposed to alter the GRAS determination process by eliminating the formal GRAS petitioning process and replacing the petitioning process with a notification procedure.[3]

Based upon the composite information, a GRAS assessment of canola oil for use in exempt and non-exempt infant formulas for term infants based on available safety information, i.e., scientific procedures, was undertaken. References that have been deemed pertinent to the objective are listed in Section IX and in Appendices B and C. A panel of experts was assembled to independently and critically review the safety of canola oil for use in infant formulas. Following a comprehensive evaluation, the Expert Panel conferred and unanimously agreed to the decision described herein.

[2] See 21 CFR 170.3(i)(3).
[3] See *Federal Register* 62 April 17, 1997, 18937; or http://www.cfsan.fda.gov/~lrd/fr970417.html.

C. Current Regulatory Status of Canola Oil

The history of the use of rapeseed oil in food in the US is well summarized in the preamble of the final rule which affirmed the GRAS status of low erucic acid rapeseed (LEAR) oil (Federal Register, 1985). Historically, rapeseed oil contained high levels of erucic acid[4] (typically 30-50% of total fatty acids), and studies in several animal species demonstrated an association between high erucic acid intakes and myocardial lipidosis. Rapeseed varieties were developed using selective breeding to transfer the low erucic acid trait, thereby allowing the production of oil with a much lower level of erucic acid. This oil was historically known as low erucic acid rapeseed (LEAR) oil but is now more commonly known today as canola oil. Internationally agreed standards require that canola oil must contain a level of erucic acid that is <2% of total fatty acids, although most of the rapeseed crop produced in the world today has a much lower level of erucic acid. The development of canola oil eventually led to its acceptance in the US as an edible oil.[5]

Current FDA regulations do not authorize the use of canola oil in infant formulas. It is also not approved in Canada and in some Middle East Countries. Dialogue with FDA scientists indicates that the original regulation excluded use in infant formulas at the request of the petitioner and that there was no apparent scientific issue on record which indicated that canola oil would not be safe for use in infant formulas. To the best of our knowledge, no prohibition exists in any other developed countries. There are no restrictions on the use of canola oil in the CODEX standard for infant formulas and formulas for special medical purposes intended for infants or in specific infant formula legislation in Europe and Australia. According to European Commission Directive 2006/141/EC of 22nd December 2006 on infant formulae and follow-on formulae, the erucic acid content of infant formulas shall not exceed 1% of total fat.[6] Similarly, the Food Standards Australia New Zealand (FSANZ) (Standard 2.1.9.) has also set a maximum limit for erucic acid in infant formula of 1% of total fatty acids.[7] These standards do not make any specific recommendations on the suitability of specific oils and fats for use in the manufacture of infant formulas, although sesame seed oil and cotton seed oil are prohibited. The levels of erucic acid in other vegetable oils suitable for and commonly used in the production of infant formulas are, if detectable, very low. The inclusion of provisions for an upper limit for the erucic acid content of infant formulas in these rules suggests that the use canola oil in infant formulas is permitted.

D. Rationale for Pursuing a GRAS Determination for the Use of Canola Oil in Infant Formulas

All infant formulas must contain fat, which provides a source of energy and of the essential fatty acids (EFA) linoleic acid (LA; C18:2 n-6) and α-linolenic acid (ALA; C18:3 n-3). EFAs must be provided in the diets of infants as they are essential for the endogenous formation of the long chain polyunsaturated fatty acids (LC-PUFA) in the n-3 and n-6 series, such as arachidonic acid (ARA; C20:4 n-6) in the n-6 series and docosahexaenoic acid (DHA: C22:6 n-3) in the n-3 series, which

[4] Common name for the monoenoic fatty acid, C22:1 n-9.
[5] See 21 CFR 184.1555 (c)(4).
[6] See http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:401:0001:0033:EN:PDF.
[7] See http://www.foodstandards.gov.au/_srcfiles/Standard_2_9_1_Infant_Formula_Products_v109.pdf.

have key roles in brain development and immune responses. There has long been considerable debate over the essentiality of dietary LC-PUFAs in infancy. Some studies suggest a higher deposition of these fatty acids in developing organs when LC-PUFA-supplemented formulas are used than when the parent EFAs alone are supplied, principally because of limited activity in the elongation/desaturation pathways in young infants. A consensus has emerged that the addition of LC-PUFA to infant formulas is beneficial, if not essential. This topic, however, is beyond the scope of this review and is not considered further. The primary focus of this assessment is on the safety and suitability of canola oil as a source of ALA in combination with a high oleic and lower linoleic acid content for use in the fat blend of infant formula.

Many infant formula manufacturers have moved away from using single vegetable oils, such as corn oil, or fats of animal origin, such as cows' milk fat, which have low concentrations of essential fatty acids, to using blends of vegetable oils. Background information on the rules and recommendations governing the fat and fatty acid composition of infant formulas is included in Section IV in detail. Danone wishes to use canola oil as a source of ALA in its infant formulas. The characteristics of canola oil which make it especially useful as a source of ALA in infant formulas are reviewed in detail in Section IV. The scientific consensus for the recommendation for ALA levels to be provided in infant formula is further discussed in Section IV.A.

E. Differences in Canola Oil from Currently Used Oils in Infant Formulas

One of the differences between canola oil and other vegetable oils commonly used in infant formulas is that canola oil contains erucic acid. In reviewing the suitability of canola oil for use in infant formulas, exposure to erucic acid needs due consideration. There are some other constituents inherently present in canola oil that also need consideration: phytosterols are present in canola oil at slightly higher levels than in other oils traditionally used in the production of infant formulas and one class of phytosterol in particular---brassicasterol---is undetectable in other vegetable oils but is present in canola oil. Long chain polyunsaturated fatty acids, because they are prone to oxidation, may influence antioxidant status in infants and also damage the components of the formula; however, this does not appear to be a concern for canola oil when used in infant formulas containing a blend of oils providing a balance of saturated and monounsaturated fatty acids and supplemented with vitamin E. These issues are addressed in discussion of the safety of the use of canola oil in infant formulas in subsequent following sections of this document.

III. INGREDIENT IDENTITY, CHEMICAL CHARACTERIZATION & PURITY & MANUFACTURING PROCESS

A. Background Information on the Composition of Canola Oil

As is the case for other food grade vegetable oils, canola oil predominantly consists of fat (99.9 g fat/100 g product), which comprises its constituent fatty acids and non-fatty acid material (unsaponifiable matter) such as phytosterols (plant sterols). In most plant oils, the 3 most common phytosterols are β-sistosterol, campesterol and sigmasterol. There are also measurable amounts of at least 9 other phytosterols in many plant oils. The most common phytosterols in canola oil are β-sistosterol, campesterol and brassicasterol.

The food grade specifications for canola oil in the US are published in the Food Chemicals Codex (FCC, 2004; see Table 1). Recently, FDA did not object to a GRAS notification for use of LEAR oil from an alternative source, *Brassica juncea* (FDA, 2000a). The oil derived from this variety contains erucic acid at a maximum level of 0.1% which is lower than that derived from the traditional sources of *Brassica napus* and *Brassica campestris.* In 2003, Health Canada[8] approved food use of LEAR oil derived from Canola-quality Brassica juncea (L.) Czern. lines PC 97-03, PC98-44 and PC98-45. The oil derived from these lines meets the specifications for canola oil as listed in the Food Chemicals Codex, 7th Edition. A full copy of FCC Specifications is attached as Appendix A. The food grade specification for canola oil which Danone intends to use in term infant formula is included in Appendix D. The canola oil specification for infant formula includes a maximum erucic acid content of 1% total fatty acids.

Table 1. Food Chemicals Codex Specification for Canola Oil

Fatty Acid	<14	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:2	20:1	22:0	22:1	24:0	24:1
Weight %	<0.1	<0.2	<6.0	<1.0	<2.5	>50	<40	<14	<1.0	<2.0	<0.5	<2.0	<0.2	<0.2
Iodine Value: 110 – 126 Acid Value: <6 Unsaponifiable matter: not more than 1.5% Peroxide Value: not more than 10 meq O_2/kg														

B. Erucic Acid Content of Commercial Sources of Canola Oil

Danone is currently supplied with canola oil by a European supplier, the canola oil is produced from a rapeseed crop that is grown in the UK and northern Europe.

[8] See http://www.hc-sc.gc.ca/fn-an/gmf-agm/appro/low_erucic-faible_erucique-eng.php.

The fatty acid composition based on batch analysis of canola oil produced by the European supplier is presented in Table 2. The specification for the canola oil intended for infant formula is included in Appendix D. The canola oil intended for infant formula has a maximum erucic acid level of 1% total fatty acids, the mean erucic acid content (<0.6% fatty acids) falls well below this maximum level. The data below shows that canola oil in compliance with the fatty acid specification included in Appendix D can be selected.

Table 2. Average Fatty Acid Composition as Percentage of Total Fatty Acids from 65 Batches of LEAR Oil (Canola Oil) Produced by the European Supplier

Fatty Acid	Iodine Value	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	C22:1
Max	119.3	7.0	0.2	2.7	66.9	22.3	10.6	4.0	1.5	1.2	1.4
Mean	115.4	4.7	0.1	1.6	61.8	19.6	9.2	0.7	1.2	0.4	0.6
Min	110.9	3.8	0.0	0.8	56.1	17.8	4.9	0.4	0.8	0.2	0.1
SD	1.9	0.5	0.1	0.3	1.6	0.8	0.7	0.5	0.1	0.1	0.3

These findings are comparable with those reported elsewhere. In 1997, the erucic acid content of 50% of the Australian canola crop was 0.3% or less of the total fatty acids. The maximum reported erucic acid level was 1.6% of the total fatty acids (FSANZ, 2003).

C. Phytosterol Content of Commercial Sources of Canola Oil

Table 3 presents information taken from the CODEX standard for the phytosterol content of canola oil and other oils typically used in infant formulas.

Table 4 presents the results of analyses carried out by the European supplier on the phytosterol content of its oils.

Table 5 includes results for the phytosterol content of six batches of canola oil from two different European suppliers of canola oil.

Table 3. CODEX Standard for Levels of Phytosterols in Crude Vegetable Oils as Percentage of Total Sterols[a]

Phytosterol	Canola Oil	Soybean Oil	Sunflower Oil	Coconut Oil
Total sterols (mg/kg)	4500-11300	1800-4500	2100-4600	400-1200
Cholesterol (%)	ND - 1.3	0.2 - 1.4	ND - 0.7	ND – 3.0
Brassicasterol (%)	5.0 - 13.0	ND - 0.3	ND - 0.4	ND – 0.3
Campesterol (%)	24.7 - 38.6	15.8 - 24.2	9.2 - 13.3	6.0 – 11.2
Stigmasterol (%)	0.2 - 1.0	14.9 - 19.1	4.5 - 9.6	11.4 – 15.6
β-sitosterol (%)	45.1 - 57.9	47.0 - 60.0	40.2 - 50.6	32.6 – 50.7
Delta5-avenasterol (%)	2.5 - 6.6	1.5 - 3.7	0.8 - 4.8	20.0 – 40.7
Delta7-stigmasterol (%)	ND - 1.3	1.4 - 5.2	13.7 - 24.6	ND – 3.0
Delta7-avenasterol (%)	ND - 0.8	1.0 - 4.6	2.2 - 6.3	ND – 3.0
Others (%)	ND - 4.2	ND - 1.8	0.5 - 6.4	ND – 3.6

[a] CODEX-STANDARD 210-1999 for Named Vegetable Oils. Adopted 1999, revised 2001,2003 and 2009. http://www.codexalimentarius.net/web/standard_list.do?lang=en.

Table 4. Levels of Phytosterols in Crude Vegetable Oils as Percentage of Total Sterols Found in Samples Produced by the European Supplier[a]

Phytosterol	Canola Oil	Soybean oil	Sunflower oil	Coconut oil
Total Sterols (mg/100 g)	480-1130	180-410	240-460	40-120
Brassicasterol (%)	10	ND[b]	ND[b]	ND[b]
Campesterol (%)	35	20	10	10
β-sitosterol (%)	48	55	60	50
Delta5-avenasterol (%)	2	3	7	25
Stigmasterol (%)	ND[b]	20	7	13
Delta7-stigmasterol (%)	ND[b]	3	7	ND[b]
Delta7-avenasterol (%)	ND[b]	3	5	ND[b]

[a] Handbook of Vegetable Oils and Fats (Karlshamns, A.B., 2002. First Edition).
[b] ND = Not Detected.

[redacted]

Table 5. Analysis of Commercial Samples of Canola Oil for Unsaponifiable Content – Weight Percentage

PHYTOSTEROL	NAN0019 190307	NAN0019 110707	NAN0019 100807	RM0147 F2265	RM0147 F51969	RM0147 F52690	AVERAGE
Total Sterol (mg/100 g)	921	929	952	939	819	858	900
Brassicasterol (%)	8.4	9.1	8.8	7.6	8.9	8.6	8.6
Campesterol (%)	31.4	33.5	30.5	30.5	33.3	34.7	32.3
β-Sitosterol (%)	44.7	46.9	45.7	45.2	45.2	45.6	45.6
Stigmasterol (%)	0.4	0.4	0.9	0.5	0.4	0.2	0.5
Cholesterol (%)	0.2	0.2	0.2	0.3	0.2	0.2	0.2
Other Sterols/stanols (%)	15.0	10.1	14.2	16.3	12.2	10.9	13.1
6 Batches of Canola oil from two different European suppliers							

The results demonstrate that the total phytosterol content for all samples is within the FCC limits of 1.5% for unsaponifiable matter. The levels of individual phytosterols in commercial samples are similar to the samples supplied to Danone as described in Table 5. The average total phytosterol content of canola oil from the analysis of 6 batches was found to be 900 mg in 100 g of oil or < 1%.

D. Manufacturing Process for Canola Oil

The manufacturing process for Low Erucic Acid Rapeseed Oil (Canola Oil) is described in Appendix E.

IV. NUTRITIONAL ASPECTS OF FATS & OILS IN INFANT FORMULAS

Infants are known to receive up to 50% of the dietary calories from fats that are present in human breast milk or in infant formulas. Over 98% of this fat is in the form of triglycerides, which contain saturated and unsaturated fatty acids that are esterified to glycerol (Giovannini et al., 1995). The fat in breast milk is also the source of the essential fatty acids. The human body is unable to synthesize the essential fatty acids such as linoleic acid (LA, C18:2) and α-linolenic acid (ALA, C18:3). Human breast milk contains LA and ALA along with other fatty acids. Observational studies suggested that breast fed infants have improved neurocognitive development compared to formula fed infants. Based on these observations, it was hypothesized that one of the contributing factors may be the availability of long chain fatty acids that are derivatives of LA and ALA as found in human milk.

A. Compositional Standards for Fat & Essential Fatty Acids in Infant Formulas

Following passage of the Infant Formula Act in 1980, a task force of the American Academy of Pediatrics (AAP) submitted revised recommendations on the nutrient content of infant formulas to the FDA in 1983 (Forbes and Woodruff, 1985). The final rule, published by the FDA in 1985, specifies concentrations of key nutrients in infant formulas. The provisions specify a minimum and maximum fat content (3.6 to 6.0 g fat/100 kcal and 30 to 54% of energy from fat) and a minimum LA content (300 mg/100 kcal and 2% of total calories) for infant formulas. Following publication of the final rule, an increased recognition of the importance of ALA in the diets of infants and the ratio of LA:ALA became evident, and standards were set accordingly by the Life Sciences Research Office (LSRO) Expert Panel (1998). The LSRO report recommended a minimum and maximum ALA content (1.75% to 4% ALA as a % of total fatty acids) and a LA:ALA ratio (between ≥6:1 and ≤16:1) for infant formulas. The LSRO also revisited the recommendations made for fat and LA in FDA's final rule, recommending that infant formulas contain between 4.4 g and 6.4 g fat/100 kcal and LA at levels between 8 and 35% of total fatty acids. Table 6 presents a summary of the recommendations from the LSRO on the fat and fatty acid compositions of infant formulas.

Also pertinent to recommendations for the fatty acid composition of infant formula, the Infant Formula Act of 1980 requires supplementation with a minimum of 0.7 International Units of vitamin E per 100 kcal (equivalent to 0.5 mg vitamin E/100 kcal) and 4 µg of vitamin K per 100 kcal of formula.[9] The LSRO recommended a minimum vitamin E content of 0.5 mg α-tocopherol equivalents (α-TE) per gram of PUFA (and maximum level of 5 mg α-TE/g PUFA), but no less than 0.5 mg α-TE/100 kcal and a minimum vitamin K content of 1µg/100 kcal (and a maximum level of 25 µg/100 kcal).

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[9] See 21 CFR 107.110. As discussed later in the document, supplementation with vitamin E and vitamin K is an important consideration.

[redacted]

Table 6. Fat & EFA Compositional Standards for Infant Formulas for Term Infants[a]

	MINIMUM	MAXIMUM	RATIO
Total Fat	4.4 g/100 kcal	6.4 g/100 kcal	NA[b]
Linoleic acid (LA; 18:2n-6)	8% of total fatty acids	35% of total fatty acids	NA[b]
α-Linolenic acid (ALA; 18:3n-3)	1.75% of total fatty acids	4% of total fatty acids	LA:ALA ≥6 & ≤16

[a] Raiten, D.J., Talbot, J.M., Waters, J.H., (Eds.), 1998. Assessment of nutrient requirements for infant formulas. J Nutr 128, 2089S-2107S.
[b] NA = Not Applicable.

B. Suitability of Canola Oil as Source of α-Linolenic Acid

Two vegetable oils---soy oil and canola oil---provide appreciable quantities of ALA that are in common use by infant formula manufacturers for European and other markets. Canola oil is included in infant formulas from a number of manufacturers including Nestle (NAN HA1™/NAN HA2™) and Danone (Cow and Gate/Milupa/SHS). Canola oil is especially useful as a source of ALA in infant formulas because it has a lower LA:ALA ratio than soy oil (approximately 9 g LA for every 1 g ALA in soy oil compared with approximately 3 g LA for every 1 g ALA in canola oil), it is a richer source of ALA, and the ALA content of canola oil is less variable than soy oil. These characteristics enable manufacturers to produce infant formulas with a more optimal overall fatty acid profile than if soy oil is used.

Other vegetable oils, such as sunflower oil and high oleic sunflower oil, used in infant formulas contain a high LA content relative to their ALA content. The use of soy oil, while providing a source of ALA, also provides an appreciable amount of LA, which in combination with the other LA-rich oils in the blend increases the LA:ALA ratio towards the upper limit recommended by LSRO of 16:1. Canola oil in combination with oils which are poor in ALA, can be used very effectively to counter the high LA levels contributed by other vegetable oils, thus allowing manufacturers to produce infant formulas that meet the recommendations for the ALA content of infant formulas but with an LA:ALA ratio closer to the lower level recommended by LSRO of 6:1. It should be noted that the recommended upper limit for the ratio of LA:ALA was intended to prevent an inappropriate combination of high LA content with low ALA content which might interfere with the formation of LC-PUFA in the n-3 series. Furthermore, the level of ALA in soy oil can be variable, making it difficult to consistently produce infant formulas that fall within the range recommended by the LSRO for ALA content which at 1.75 – 4% of total fatty acids allows only a small margin for natural variation in the composition of the ALA-source oils.

C. Fatty Acid Composition of Vegetable Oils & Breast Milk

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Table 7 compares the fatty acid composition of breast milk with the composition of oils that are typically used in infant formulas throughout the world. No single vegetable oil has a fatty acid profile that is comparable to breast milk. Given the unique fatty acid composition of breast milk fatty acid profile, it is not possible to formulate an infant formula which matches all individual fatty

[redacted]

acids in human milk by a simple blend of available oils. A typical blend of oils does achieve a fatty acid profile which matches the major fatty acid components of breast milk. The constituent oils in infant formulas make up varying proportions of fatty acids which are combined to produce a fat blend containing a balance of saturates, monounsaturates and polyunsaturates while providing appropriate levels of LA and ALA. It should be noted that there is usually an acceptable range of values for fatty acids present in oils in the manufacturer's specifications, but in estimating the amount of each fatty acid contributed by oils, a single representative figure needs to be used. The values in Table 7 are those provided by the suppliers which represent typical, average values.

Table 7. Typical Fatty Acid Composition (Percentage of Total Fatty Acids) of Breast Milk & Vegetable Oils Currently Used Worldwide in Infant Formulas[a, b]

Fatty Acids	Fractionated Coconut Oil	Sunflower Oil (High Oleic Acid)	Sunflower Oil	Palm Olein Oil	MCT Oil	Soy Oil	Canola Oil	Breast Milk
Saturates								
6:0	1.25				≤2			
8:0	57				50-65			
10:0	42.28				30-45			1.2
12:0	0.3			0.3	≤2			5.2
14:0		0.07		1				6.7
16:0		3.5	6.2	39		10.5	4.46	22.1
18:0		3.2	4.1	4		4.0	1.75	8.2
20:0		0.3	0.1			0.4	0.5	
22:0		1.1	0.6			0.3	0.4	
Monoenes								
16:1								3.3
18:1		81.2	21.7	43		22.0	60.5	36.3
20:1		0.3				0.3	1.58	0.7
22:1							0.6	0.2
N-6 Series Polyenes								
18:2		8.6	64.2	11		54.5	19.17	12.7
20:2								0.4
20:4								0.7
22:5								0.2
N-3 Series Polyenes								
18:3		0.2	0.2	0.2		6.0	9.34	0.6
20:5								0.2
22:5								0.4
22:6								0.4

[a] Manufacturers handbooks for the oils. The data for breast milk are mean values (n=12) for women 2-4 months postpartum (Innis, S. M., 1991. Essential fatty acids in growth and development. Prog Lipid Res 30, 39-103). [b] The vegetable oils included in this Table (with the exception of canola) are currently used in infant formula in North America. The Table is not an exhaustive list of fat sources for infant formula. For example cows' milk is still used and more recently single cell oils are also included in infant formula as sources of DHA and ARA.

[redacted]

V. INTENDED DIETARY USE

Danone intends to use canola oil as a source of ALA in infant formulas. It is intended that canola oil will be combined with other vegetable oils for use as the fat source in infant formulas.

A. Intended Levels of Use of Canola Oil in Infant Formulas

The addition rate of canola oil will depend on the other constituent oils added to the infant formulas in achieving the desired fatty acid profile. In the range of powdered infant formulas manufactured by Danone,[10] canola oil contributes between 17.5% and 31% of the total fatty acids. Canola oil has an ALA content of 9.34% of total fatty acids as seen in Table 7. At 17.5% of the oil blend in an infant formula, canola oil will provide 1.63% of the total fatty acids as ALA. At the higher addition rate of up to 31% of the total fatty acids, canola oil will provide 2.9% of the total fatty acids as ALA in the infant formula. Other oils (such as HO sunflower oil and sunflower oil) will contribute small quantities of ALA to the infant formula.

At the higher addition rate of canola oil (up to 31%), Danone is able to produce infant formulas that meet the LSRO recommendation for ALA as a percentage of total fatty acids (between 1.75% and 4%), while also restricting the addition rate of the LA-rich oils which would otherwise increase the LA:ALA ratio towards the upper limit of the LSRO recommendation. Given the restriction on the maximum level of ALA in infant formulas of 4% of total fatty acids recommended by the LSRO, Danone anticipates that not more than around 40% of the fat blend would comprise canola oil in ensuring compliance with this recommendation. At present Danone does not intend to use canola oil at levels higher than 31%.[11]

In assessing the consequences of the exposure of infants to canola oil-containing infant formulas, typical intakes of erucic acid, total phytosterols and brassicasterols have been estimated. These estimates have been made for a typical consumer (taken as a 6 month old infant consuming infant formula as a sole source of nutrition) and an extreme consumer (taken as a younger infant aged 3 months who requires additional energy to support catch-up growth and whose intakes, because of the lower body weight, are greater per unit of weight than an older infant). In the range of infant formulas manufactured by the notifier, canola oil contributes between 17.5% and 31% of the total fatty acids, and, therefore, likely intakes of erucic acid, total phytosterols and brassicasterols for typical and extreme consumers have been calculated for both formula compositions. A fat content of 5.1 g/100 kcal has been used in these calculations since this level is representative of the infant formulas manufactured by Danone for the North American market. All estimates of energy requirements are for boys and are taken from the Institute of Medicine, National Academies of Sciences, 2002/2005. US Dietary Reference Intakes, Table 5-16.

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[10] Danone currently markets only powdered formulas in North America.

[11] The 31% limit proposed is related to formulation convenience and not because of any safety concern. At 40% canola, this would contribute 3.7% of total fatty acids as ALA and Danone prefers to set lower target percentages of ALA to stay comfortably below the maximum allowable level of 4% as other oils will have minor contributions of ALA.

Given that it is unlikely that there will be any other appreciable sources of erucic acid and brassicasterol in the diets of infants consuming only infant formula, the estimates of intakes in Tables 8 and 9 for erucic acid and brassicasterol represent probable total intakes. However, the other oils used in the manufacture of infant formulas will contribute to total phytosterol intakes (see Table 4), and thus total intakes for phytosterols are likely to be slightly higher. A discussion on the safety of erucic acid and phytosterols intakes arising from canola oil-containing infant formulas is specifically included in Section VI.

In the intake assessment described below, a typical consumer is considered to be a 6 month old infant with a daily energy requirement of 645 kcal/day. Based on a fat content of 5.1 g fat/100 kcal formula, the 6 month old infant will consume 32.9 g fat daily. Thus, for the typical consumer, the daily intake of canola oil will range between 5.7 and 10.2 g/day, when the infant formula content of canola oil (as a fat blend) ranges between 17.5 and 31%, respectively.

The extreme consumer is taken as an infant born with a birth weight on the 50th percentile, subsequently experiencing growth failure and on the 3rd centile for weight at 3 months of age and having increased energy requirements to facilitate catch-up growth. Calculations are based on a 3 month infant weighing 4.7 kg (Reference Lengths and Weights for Boys 1-35 months, Institute of Medicine, National Academies of Science 2002/2005, US Dietary Intakes Table 5-6 with a daily energy requirement of 115 kcal/kg (Nutritional Requirements for Children in Health and Disease, 2000). Again based on a fat content of 5.1 g/100 kcal infant formula, the daily fat intake of the extreme consumer is 27.5 g fat per day. Therefore, for the extreme consumer, the daily canola intake will be 4.8 or 8.5 g, respectively, for an infant formula containing 17.5 or 31%of the fat blend as canola.

These calculations are based on assumptions that closely agree with estimates of energy intake done by other researchers. For example, Fomon (1993) indicated that the 95th percentile energy intake in male infants between 56 and 113 days is 113.9 kcal/kg/day and for male infants between 8 and 12 weeks that the 90th percentile for energy intake in male infants is 115.6 kcal/kg. This also suggests that the above consideration of "extreme consumer" will represent the 95th percentile intake of the formula by infant.

B. Intakes of Erucic Acid & Phytosterols by a Typical Consumer

Current WHO infant feeding guidelines recommend that infants are breast fed, or if not breast feeding, receive infant formula, as sole source of nutrition, until the infant is 6 months of age. Therefore, a typical consumer is taken as a 6 month infant consuming formula as sole source of nutrition. Intakes of erucic acid, phytosterols and brassicasterol by a 6 month old infant weighing 7.9 kg with a typical energy requirement of 645 kcal/day and consuming infant formula as a sole source of nutrition are summarized in Table 8.

C. Intakes of Erucic Acid & Phytosterols by an Extreme Consumer

The youngest infants, because of their low body weights, taking infant formula as a sole source of nutrition will have the highest energy requirements per kilogram body weight. The recommended energy intake for a 1 month old infant is 472 kcal/day, which is equivalent to 107 kcal/kg body

weight (bw)/day for an infant of a median weight of 4.4 kg. This compares with the requirements of a 3 month old infant, whose energy intake of 645 kcal/day is equivalent to 82 kcal/kg bw/day.

Table 8. Erucic Acid, Phytosterol & Brassicasterol Intake from Infant Formula by Typical Consumer[a]

	ERUCIC ACID		TOTAL PHYTOSTEROLS		BRASSICASTEROL	
	MG/DAY	MG/KG BW/DAY	MG/DAY	MG/KG BW/DAY	MG/DAY	MG/KG BW/DAY
Infant formula in which 17.5% of the total fatty acids is provided by canola oil	34	4.3	52	6.5	4.5	0.6
Infant formula in which 31% of the total fatty acids is provided by canola oil	61	7.7	91	11.5	7.7	1.0

[a] Infant consuming infant formulas as a sole source of nutrition, weighing 7.9 kg and requiring 645 kcal/day. Formula contains 5.1 g fat/100 kcal. Infant formula in which 17.5% of the total fatty acids are provided by canola oil contains 5.3 mg erucic acid/100 kcal (assumes erucic acid in canola oil at 0.6% of total fatty acids), 8.0 mg phytosterols/100kcal (assumes 900 mg/100 g canola oil) and 0.7 mg brassicasterol/100 kcal (assumes 77 mg/100 g canola oil). Infant formula in which 31% of the total fatty acids are provided by canola oil contains 9.4 mg erucic acid/100 kcal, 14.1 mg phytosterols/100 kcal and 1.2 mg brassicasterol/ 100 kcal.

Within this subpopulation of young infants, some may have energy requirements that are above the norm, for example, those requiring catch-up growth to correct faltering growth. This group represents the most extreme consumer. As mentioned above, intakes by a 3 month old underweight infant born with a birth weight on the 50th percentile, subsequently experiencing growth failure and on the 3rd centile for weight at 3 months of age and having increased energy requirements to facilitate catch-up growth have been included to illustrate the highest intakes per kg bw likely by any infant. For purposes of this assessment, calculations are based on a 3 month infant weighing 4.7 kg (Reference Lengths and Weights for Boys 1-35 months, Institute of Medicine, National Academies of Science 2002/2005, US Dietary Intakes Table 5-6 with a daily energy requirement of 115 kcal/kg (Nutritional Requirements for Children in Health and Disease, 2000)). This recommended intake compares with the mean energy intake of 95 kcal/kg bw/day for a normal weight 3 month old infant. An energy intake of 115 kcal/kg bw/day compares with quoted energy needs for catch-up growth (113 to 123 kcal/kg bw/day) in malnourished children under controlled conditions in a clinical setting (Institute of Medicine, National Academies of Sciences, 2002/2005. US Dietary Reference Intakes, Table 5-32).

[redacted]

Table 9. Erucic Acid, Phytosterol & Brassicasterol Intake from Infant Formulas by Extreme Consumers[a]

	ERUCIC ACID		TOTAL PHYTOSTEROLS		BRASSICASTEROL	
	MG/DAY	MG/KG BW/DAY	MG/DAY	MG/KG BW/DAY	MG/DAY	MG/KG BW/DAY
NORMAL WEIGHT 1 MONTH OLD INFANT REQUIRING 470KCAL/DAY						
Infant formula in which 17.5% of the total fatty acids is provided by canola oil	24.9	5.7	37.6	8.5	3.3	0.8
Infant formula in which 31% of the total fatty acids is provided by canola oil	44.2	10.0	66.3	14.1	5.6	1.3
UNDERWEIGHT 3 MONTH OLD INFANT REQUIRING 540KCAL/DAY						
Infant formula in which 17.5% of the total fatty acids is provided by *canola oil*	28.6	6.1	43.2	9.2	3.8	0.8
Infant formula in which 31% of the total fatty acids is provided by canola oil	50.8	10.8	76.1	16.2	6.5	1.4

[a] Infant consuming infant formulas as a sole source of nutrition, weighing 4.4 kg (median for 1 month old infant) and 4.7 kg (infant born on 50% centile but dropped to 3rd centile at 3 months due to growth failure) and requiring 107 kcal/kg bw/d (for 1 month old infant with a median weight) and 115kcal/kg bw/d (for 3 month old infant crossed from higher birth weight centile to3rd centile for weight). Formula contains 5.1g fat/100 kcal. Infant formula in which 17.5% of the total fatty acids are provided by canola oil contains 5.3 mg erucic acid/100kcal (assumes erucic acid in canola oil at 0.6% of total fatty acids), 8.0 mg phytosterols/100 kcal (assumes 900 mg/100 g canola oil) and 0.7 mg brassicasterol/100 kcal (assumes 77 mg/100 g canola oil). Infant formula in which 31% of the total fatty acids are provided by canola oil contains 9.4 mg erucic acid/100 kcal, 14.1 mg phytosterols/100 kcal and 1.2 mg brassicasterol/100 kcal.

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VI. REVIEW OF SAFETY DATA ON CANOLA OIL

A. Safety Related Information from Canola Oil Regulation

The GRAS status of canola oil was established in 1985 based on the scientific evidence available at that time. Since then considerable research has surfaced on the safety and nutrition of canola oil. As described below---as well as more fully in Appendix B---a number of studies exist which outline the history of the use of canola oil as a human food.

Borg (1975) reviewed the toxicology studies on traditional rapeseed oil with higher levels of erucic acid. Many of the oil samples used in these studies contained between 30-50% erucic acid. Effects on a number of organs as well as a growth depressing effect were reported. The primary effect of concern was cardiotoxicity. As noted by Borg, the primary effect observed in several species is fatty infiltration of heart muscle followed by fibrosis of the heart muscle. Borg noted that both Sweden and Canada, two countries where rapeseed oil had a long history of use in the diet, took action to limit the amount of erucic acid. Interestingly, there is no epidemiological evidence that indicates an increase in cardiovascular disease in countries where rapeseed or other oils high in erucic acid content are commonly used (Grice and Heggtveit,1983; Svaar, 1982).

The interpretation of many of the studies on erucic acid and rapeseed oil are confounded by the high levels of fat calories administered as well as by unbalanced diets where rapeseed oil was the sole source of dietary fat in various species where optimum dietary composition may not be known. One nutritional study demonstrated that longevity in rats with reasonable levels of dietary rapeseed oil was comparable to several other oils and was superior to butter fat (Thomasson, 1955).

Regarding the safety of erucic acid and its role in human nutrition, FDA (1985) determined that erucic acid is not toxic when it is a component of canola oil at a level of not more than 2% of the total fatty acids. The agency noted that erucic acid is metabolized by mammals, albeit less rapidly than some fatty acids and that it is synthesized in some mammalian species. Additionally, several animal and human studies also demonstrate that canola oil containing up to 2% erucic acid is as safe as other vegetable oils for human consumption. When considering whether canola oil containing a small amount of erucic acid can be safely consumed by humans, the agency considered that the levels of erucic acid in canola oil does not represent a safety concern. Additionally, the agency also concluded that the nutritional and physiological properties of canola oil in humans have been adequately investigated, and the available data demonstrate that canola oil containing 2% or less erucic acid is safe. Consequently, there is no need for additional research to support its safety.

B. Studies Impacting Safety Assessment of Infant Formulas Containing Canola Oil

Many studies have been carried out on canola oil-containing feeds which are relevant to the question of whether canola oil should be considered to be safe for use in infant formula. The majority of these studies have been conducted since 1985, and a comprehensive compilation of

published papers is included in Appendix B. The main findings from these studies are summarized and discussed below.

There are two categories of studies that have been conducted that are particularly relevant when considering the safety of canola oil-containing infant formulas:

1. Studies Investigating the Effects of Canola Oil-Containing Feeds in Animal Models

Several studies have been carried out investigating the effects of the inclusion of canola oil in the feeds of newborn piglets, an animal model known to be more comparable to human infants in physiology and fatty acid metabolism than rat models (Innis, 1993). The aims of these studies were to investigate the effects of canola oil in milk replacer diets on piglet growth, organ vitamin E stores, platelets, red blood cells, lipid composition and cardiac lipid deposition. These studies are discussed in Section VI.C.

2. Studies Investigating the Effects of Feeding Infant Formulas Incorporating Canola Oil in Human Infants

There have been several studies investigating the consequences of feeding varying compositions of infant formulas to infants. Of particular interest in the context of this assessment are those studies that included infant formulas that incorporated canola oil as part of the oil blend. In these studies, canola oil has been added to one or more of the test infant formulas as a source of ALA at levels of between 3% and 40% of total fatty acids. These studies were not primarily designed to test the safety of canola oil but rather to investigate nutritional or other possible beneficial endpoints. Many of these studies followed growth and development but did not investigate other safety endpoints. Other outcomes may also be reported which are specific to the expected effects of the formula's composition, such as the effects of supplementing the formula with particular quantities of specific fatty acids on infants' neurological development. Physical growth is a valuable component of the clinical evaluation of an infant formula as it is an indicator of its overall nutritional quality. In these clinical studies, the absence of adverse effects was noted during the course of the study, and many of the studies included a schedule of follow-up visits in later life to confirm continued normal growth and development. While these studies did not include other more in-depth endpoints for safety, these studies are reviewed because they are the best available human studies addressing the safety of canola oil in human infants. In addition, the safety of canola oil in human infants is also supported by a safe history of use in infant formula for many years outside of North America. These studies are discussed in Section VI.D.

In addition to the above studies, there are several other types of investigations with canola oil. However, given the experimental model used in these studies and its applicability to humans, these studies are of minimal significance from the present safety assessment perspective. These studies include toxicology testing in rats and investigations elucidating the mechanism by which various PUFA-rich oils influence blood cell membrane structure and function, platelet function, erythrocyte fragility and vitamin E requirements. Only tentative extrapolation of the results from studies using the rat as a model is possible since fatty acid metabolism in rats differs from humans, and neonatal pigs are a superior animal model. Indeed, Grice and Heggtveit (1983), in reviewing earlier studies in rats, commented that the etiology of the cardiotoxicity observed in rats is most

likely based on nutritional and metabolic considerations rather than being toxicological in nature because rats cannot utilize high levels of vegetable oil. These studies are discussed in Section VI.E.

C. Studies Investigating Effects of Canola Oil-Containing Feeds in Neonatal Pig Model

Several studies have been conducted in the neonatal pig model using sow milk replacement formulas containing rapeseed oil of both high erucic acid (HEAR) and low erucic acid (LEAR), canola versions combined with other oils. The design and results of these studies are summarized in Table 10 and are described more fully in Appendix B, section 1. The studies outlined in Table 10 were designed to identify and investigate possible adverse effects of a canola oil- enriched diet. The effects of canola oil on vitamin E, cardiac lipolysis and platelets, along with its significance from a safety perspective of the current GRAS assessment are discussed more fully below.

Table 10. Studies in Neonatal Pig Model

STUDY	ANIMAL MODEL	DOSING REGIMEN/DURATION	SUMMARY
Sauer et al., 1997	Neonatal pigs	Piglets on milk replacer diets with the following lipid composition: 1. 98% canola/2 % HEAR[b] 2. 100% soybean 3. Olive 4. HOAS[a] 5. HOAS[a], soy, linseed 6. 60% canola/40% coconut	Several experiments were done to study the effect of different diets on the vitamin E levels in heart and liver. Vitamin E depletion in these tissues was greater in the canola/ HEAR[b] than other diets when the formulas contained low vitamin E supplementation. Liver pathology was also evident with this diet. At higher supplementation levels, all diets tested had greater tissue levels of vitamin E than sow reared controls. At higher supplementation levels there was no liver pathology with any diet tested.
Kramer et al., 1990	Neonatal pigs	7 groups of piglets were fed diets with erucic acid levels ranging from 0.8 to 42.9% of total fatty acids for 16 days.	The study examined the incidence of cardiac lipidosis in piglets before weaning with various milk replacer diets. Varying dietary levels of erucic acid were achieved by using various blends of rapeseed oil. The incidence and severity of lipidosis generally increased with erucic concentration. In one experiment with 4.7% erucic acid, lipidosis incidence and severity was comparable to a group receiving soybean oil. Author concluded that there were no adverse nutritional or myocardial effects at 750 mg/kg bw/d.

STUDY	ANIMAL MODEL	DOSING REGIMEN/DURATION	SUMMARY
Kramer et al., 1994	Neonatal pigs	Several groups of piglets were raised on various milk replacement diets incorporating various vegetable oils (soy, canola, HOAS,[a] olive and various mixtures of these, some of which included coconut) as the sole source of lipids.	The study examined the effect of diet on platelet number, platelet size and clotting time and cell membrane composition. Sow reared controls were used in some experiments. Delays in normal platelet count rises after birth and other related changes in hematological factors and platelet membrane composition were observed with diets high in linolenic acid, including canola oil. The differences were generally reduced with age over the four week observation period. Effects were mitigated by mixtures of unsaturated and saturated oils. The author concluded that there was no increased bleeding risk when canola oil is used in a balanced lipid formula.
Kramer et al., 1998	Neonatal pigs	Several groups of piglets were raised on various milk replacement diets using various vegetable oils (soy, canola, HEAR[b], and a canola/cocoa mixture) as the sole source of lipids.	The study examined the effect of diet on platelet number, platelet size and clotting time and cell membrane composition. Sow reared controls were used. Observations were similar to Kramer et al., 1994. There were changes in platelet membrane sphingomyelin lipid composition, particularly increases in 24:1n-9 content and 24:1n-9/24:0 ratio with diets containing canola or HEAR[b], but not HOAS[a] or soy.
nnis, Dyer, 1999	Neonatal pigs	Several groups of piglets were raised on various milk replacement diets using either canola oil, a balanced blend of various oils with canola, a canola mimic blend, soy, or a balanced blend with soy as the sole source of lipids for 10 or 18 days.	The study examined the effect of diet on growth, platelet number, platelet distribution width (PDW), mean platelet volume (MPV) and plasma lipid composition. Sow reared controls were not used. Body weight, weight gain, heart, and liver weight were not different after 10 or 18 days among any of the diets. Platelet counts were lower, and PDW and MPV were higher due to diets with 100% canola or soy as opposed to oil blends. Plasma lipid composition was found to vary with each diet.
Green, Innis, 2000	Neonatal pigs	Several groups of piglets were raised on various milk replacement diets using either canola oil, a balanced blend of various oils with canola, a canola mimic blend, soy, or a balanced blend with soy as the sole source of lipids for 10 or 18 days.	The study examined the effect of diet on heart triglyceride accumulation and heart lipid composition. Sow reared controls were used. There were no changes in total heart triglyceride, phospholipid or cholesterol concentration with diet. Fatty acid composition of heart lipids varied with diet. Erucic acid content of heart lipids were increased on diets containing canola oil. There was no evidence of cardiac triglyceride accumulation with these diets.

[a] HOAS = High oleic acid sunflower oil. [b] HEAR = High erucic acid rapeseed oil.

[redacted]

The remaining studies listed in Table 11 all included formulas that incorporated canola oil in the oil blend at rates of around 20% of total fatty acids. Although the main objectives of these studies did not include the investigation of the consequences of using canola oil as a source of ALA, the investigators in all of these studies collected data on growth at frequent intervals during the conduct of the study, in some cases following up growth and other outcomes long after feeding with the study formulas had ceased. No adverse effects of formula assignment or differences in growth between groups were reported in any of the papers.

2. Studies Investigating Infant Formulas Incorporating Canola Oil in Preterm Infants

Table 12 summarizes the findings from studies in pre-term infants in which at least one of the formulas contained canola oil. In the studies by Billeaud et al. (1997) and Gibson et al. (1998), the authors investigated the effects of altering the LA:ALA ratio of infant formulas by increasing the ALA content using canola oil as the exclusive source of ALA. No effects of formula assignment on growth were observed in either of the studies. As part of the study by Boehm et al. (2002) on the effects of feeding a prebiotic infant formula in which both of the formulas contained canola oil, no effect on growth was observed.

Table 11. Clinical Studies Investigating Infant Formulas Incorporating Canola Oil in Term Infants

INFANTS RECEIVING CANOLA OIL/FEEDING DURATION	CANOLA OIL CONTENT OF INFANT FORMULA (AS A PERCENTAGE OF TOTAL FATTY ACIDS)	PRIMARY STUDY OBJECTIVES AND MAIN FINDINGS
Jensen, et al., 1996. Biochemical effects of dietary linoleic acid/α linolenic acid ratio in term infants.		
80 infants; 20 in each group. Feeding duration: 120 days	Formula 1: canola oil at 3% of total fatty acids; Formula 2: canola oil at 10% of total fatty acids; Formula 3: canola oil at 20% of total fatty acids; Formula 4: canola oil at 40% of total fatty acids.	Objective: To investigate the effect of different levels of ALA and LA:ALA ratios in infant formula on plasma lipid and erythrocyte membrane lipid composition. Findings: Infants fed formula with the highest canola oil content had the highest docosahexaenoic acid (DHA) levels in erythrocyte phospholipids after 120 days feeding. No differences were found in growth parameters or formula intake between the 4 groups.
Jensen, et al., 1997. Effect of dietary linoleic/α-linolenic acid ratio on growth and visual function of term infants.		

INFANTS RECEIVING CANOLA OIL/FEEDING DURATION	CANOLA OIL CONTENT OF INFANT FORMULA (AS A PERCENTAGE OF TOTAL FATTY ACIDS)	PRIMARY STUDY OBJECTIVES AND MAIN FINDINGS
As Jensen, et al., 1996 – same cohort of infants. Control group of 19 breastfed infants included. Included a follow up at 240 days.	As Jensen et al, 1996 – same cohort of infants.	Objective: To determine the effects of different ratios of LA:ALA on the growth and visual function of full-term infants. Findings: Transient visual evoked responses (VERs) were measured at 120 and 240 days of age. As a control, growth and VER were also measured in a group of 19 breastfed infants at 120 and 240 days of age. There was no difference in VER between groups but the mean body weight of the infants who received the lowest LA:ALA ratio was significantly less at 120 days (but not at 21, 60 or 240 days) than those receiving the highest LA:ALA ratio, although when compared with age-matched breastfed control infants, the difference was small.
Makrides, et al., 2000. A randomized trial of different ratios of linoleic to α-linolenic acid in the diet of term infants: effects on visual function and growth.		
37 infants in the group receiving Formula 2. Control group of 85 breastfed infants included. Feeding duration: 34 weeks	Formula 1 (10:1 LA:ALA): contains no canola oil. Soy oil at 23% of total fatty acids; Formula 2 (5:1 LA:ALA): canola oil at 29% of total fatty acids combined with soy oil at 11% total fatty acids.	Objective: To compare the fatty acid status, visual evoked potential (VEP) acuity and growth of term infants fed formulas containing an LA:ALA ratio of 10:1 or 5:1 with those of a breastfed reference cohort. Findings: Infants fed the canola oil containing formula had greater DHA concentrations in plasma and erythrocyte phospholipids than did infants fed formula 1, although DHA status in infants fed formula 1 was poorer than breastfed infants. Growth (rate of gain in weight, length and head circumference) was not significantly different between the 2 formula-fed groups. The authors concluded that lowering the LA:ALA ratio in formula from 10:1 to 5:1 by using canola oil resulted in a modest increase in plasma DHA concentrations but had no effect on the growth rate or VEP acuity. No adverse events were reported in infants fed either formula.
Von Berg, et al., 2003. The effect of hydrolyzed cow's milk formula for allergy prevention in the first year of life: The German Infant Nutrition Intervention Study, a randomized double blind trial		
1115 infants in the groups receiving Formulas 1 (n=556) and 3 (n=559). 557 infants (Formula 1: 286; Formula 2: 271) completing 12 month follow up. Feeding duration: 12 months.	Formula 1 (cow's milk formula/CMF): canola oil at 25% of total fatty acids; Formula 2 (partially hydrolyzed whey formula/pHF-W): contains no canola oil; Formula 3 (extensively hydrolyzed whey formula/eHF-W): canola oil at 25% of total fatty acids; Formula 4 (extensively hydrolyzed casein formula): contains no canola oil.	Objective: To prospectively investigate whether hypoallergenic infant formulas influence the manifestation of atopic disease in infants with a hereditary predisposition to allergic disease. Infants were followed up at 1, 4, 8 and 12 months. Findings: eHF-C formula compared with CMF significantly reduced the incidence of allergic manifestations. The incidence of atopic dermatitis was significantly reduced by the eHF-C or partially hydrolyzed whey formula. The authors concluded that dietary intervention with a hydrolyzed formula may play a role in preventing allergic diseases in the first year of life. There were no differences between the formula groups in the growth of infants.

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INFANTS RECEIVING CANOLA OIL/FEEDING DURATION	CANOLA OIL CONTENT OF INFANT FORMULA (AS A PERCENTAGE OF TOTAL FATTY ACIDS)	PRIMARY STUDY OBJECTIVES AND MAIN FINDINGS
Von Berg, et al., 2007. Certain hydrolyzed formulas reduce the incidence of atopic dermatitis but not that of asthma: Three year results of the German Nutritional Intervention Study		
As Von Berg, et al., 2003 – same cohort of infants included a follow-up to 3 years of age	As Von Berg, et al., 2003 – same cohort of infants	Objective: To prospectively investigate whether hypoallergenic infant formulas influence the manifestation of atopic disease in infants with a hereditary predisposition to allergic disease. Infants were followed up at 3 years. Findings: At the 3 year follow up, none of the formulas reduced the incidence of asthma, but the eHF-C formula significantly reduced atopic dermatitis.
Rzehak, et al., 2011. Growth of infant fed formula rich in canola oil (low erucic acid rapeseed oil).		
60 infants in the groups receiving Formulas 1 (n=34) and 3 (n=26). Feeding duration: 16 weeks.	As Von Berg et al, 2003 – same cohort of infants	Objective: To investigate whether infant formulas containing canola oil support normal growth in infants by re-analyzing the weight and length data collected from infants exclusively formula fed between birth and 16 weeks recruited to the cohort of infants who took part in the GINI study. Findings: Absolute and standardized weight and length measures did not differ between the formula groups, either within each of the measurement periods (4-6 wks, 3-4 mo, 6-7 mo) or longitudinally over the whole time period.
Bakker-Zierikzee, et. al., 2005. Effects of infant formula containing a mixture of galacto- and fructo-oligosaccharides or viable Bifidobacterium animalis on the intestinal microflora during the first four months of life.		
57 infants; 19 per group. Control group of 63 breastfed infants included. Feeding: birth to 4 months.	Formula 1 (standard formula): canola oil at 19% of total fatty acids Formula 2 (standard formula supplemented with 6 g/l of GOS/FOS (prebiotic)): canola oil at 19% of total fatty acids Formula 3 (standard formula containing 6.0 x 10^{10} viable Bb-12 cells/L (probiotic)): canola oil at 19% of total fatty acids.	Objective: to investigate the effect of standard, prebiotic and probiotic infant formulas on the composition and metabolic activity of intestinal microflora of term infants during the first four months of life. Findings: the metabolic activity of the intestinal microflora in infants that were fed the prebiotic formula was most similar to the infants in the breastfed control group. Infants had a higher fecal acetate ratio, lactate concentration, and pH after 16 weeks than did infants fed the probiotic formula. No differences were reported in anthropometric measures between the formula groups.
Bakker-Zierikzee, et al., 2006. Faecal SIgA secretion in infants fed on pre- or probiotic infant formula		

INFANTS RECEIVING CANOLA OIL/FEEDING DURATION	CANOLA OIL CONTENT OF INFANT FORMULA (AS A PERCENTAGE OF TOTAL FATTY ACIDS)	PRIMARY STUDY OBJECTIVES AND MAIN FINDINGS
As Bakker-Zierikzee, et al., 2005 – same cohort of infants included a follow up at 32 weeks.	As Bakker-Zierikzee, et al., 2005 – same cohort of infants	Objective: Tao investigate the effect of standard, prebiotic and probiotic infant formulas on fecal SIgA levels of term infants. Findings: SIgA concentrations were highest in the breast fed infants compared to the standard formula fed infants throughout the study. The infants that were fed the prebiotic formula had fecal SIgA concentrations that were higher than the infants fed the standard formula. There were no differences between the group that was fed the probiotic formula and the standard formula. The authors concluded that formula fed infants may benefit from formulas containing a prebiotic blend because of the tendency to increase fecal SIgA secretion.
Kennedy, et al., 1999. Double-blind, randomized trial of a synthetic triaceylglycerol in formula-fed term infants: effect on stool biochemistry, stool characteristics, and bone mineralization		
203 infants, 100 and 103 per group. Control group of 120 breastfed infants included. Feeding duration: 12 weeks.	Formula 1 (standard formula): canola oil at 19% of total fatty acids Formula 2 (standard formula with high palmityl sn-2): canola oil at 19% of total fatty acids	Objective: To measure the effect of the experimental formula on stool characteristics and bone mineralization. Findings: Infants receiving the experimental formula had higher whole body bone mineral content, softer stools and a lower proportion of stool soap fatty acids than did the infants fed the standard formula. No differences between the formula groups were found in growth at any of the time points.
Bouwstra, et al., 2005. Long-chain polyunsaturated fatty acids and neurological developmental outcome at 18 months in health term infants.		
315 infants, 169 and 146 per group Control group of 159 breastfed infants included. Feeding duration: 2 months	Formula 1 (standard formula): canola oil at 19% of total fatty acids Formula 2 (LC-PUFA supplemented formula): canola oil at 19% of total fatty acids.	Objective: To investigate whether the beneficial effect of LC-PUFA supplementation on neurological development observed in this cohort of infants at 3 mo of age persists at 18 months of age. Findings: The groups did not show significant differences in neurological condition or psychomotor and mental development indices at 18 months of age.

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Table 12. Clinical Studies Investigating Infant Formulas Incorporating Canola Oil in Pre-Term Infants

INFANTS RECEIVING CANOLA OIL/FEEDING DURATION	CANOLA OIL CONTENT OF INFANT FORMULA (AS A PERCENTAGE OF TOTAL FATTY ACIDS)	PRIMARY STUDY OBJECTIVES AND MAIN FINDINGS
Billeaud ,et al., 1997. Effects of preterm infant formula supplementation with α-linolenic acid with a linoleate/α-linolenate ratio of 6: a multicentric study.		
63 infants, 31 and 32 per group. Control group of 120 breastfed infants included. Feeding duration: 37 weeks	Formula 1 (low α-linolenic acid formula/LLF): canola oil at 5.5% of total fatty acids Formula 2: high α-linolenic formula/HLF): canola oil at 19.5% of total fatty acids	Objective: To investigate the effects of formulas supplemented with ALA on plasma and RBC fatty acids. Findings: ALA supplementation in the HLF group maintained RBC membrane DHA values within the confidence intervals of those obtained in the breastfed group. Such was not the case for the LLF group. No differences were found between the groups in growth.
Gibson, et al., 1998. Effect of lowering the dietary linoleic acid to α-linolenic acid ratio through the use of canola oil on visual and growth outcomes of term infants. Based on abstract only.		
28 term and preterm infants in group receiving formula 2. Control group of 44 breastfed infants included. Feeding duration: 34 weeks	Formula 1 (10:1 LA:ALA): contains no canola oil Formula 2: (5:1 LA:ALA): canola oil at 30% of total fatty acids	Objective: To investigate the effect of an LA:ALA ratio of 5:1 on growth, fatty acid status and visual acuity. Findings: Infants in the group fed the 5:1 formula had greater plasma phospholipid DHA levels than infants fed the 10:1 formula, although the phospholipid DHA levels of infants in the 5:1 formula group was approximately 50% that of breastfed infants. No effect on formula assignment and growth was observed, although infants in the 5:1 group were consistently larger for each growth parameter (weight, length and head circumference) than the 10:1 group. The authors noted however that the 5:1 group had been larger at birth.
Boehm, et al., 2002. Supplementation of a bovine milk formula with an oligosaccharide mixture increases counts of faecal bifidobacteria in preterm infants.		
30 infants, 15 in each group. Control group of 12 infants fed fortified breast milk included. Feeding duration: 28 days.	Formula 1 (standard formula): canola oil at 21% of total fatty acids Formula 2 (standard formula supplemented with 10 g/l of GOS/FOS (prebiotic)): canola oil at 21% of total fatty acids	Objective: To investigate the effects of a prebiotic infant formula on faecal flora and stool characteristics of preterm infants Findings: Supplementing preterm infant formula with a prebiotic blend results in bifidobacteria counts in the upper range of those found in the breastfed controls. Stool characteristics (frequency and consistency) of infants in the prebiotic formula group were also similar to infants fed breast milk. There was no effect of the different formulas on the incidence of side effects (e.g. crying, regurgitation, vomiting) or on weight gain or length gain.

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E. Other Studies Investigating the Effects of Feeds Incorporating Canola Oil

1. Clinical Studies in Infants in which the Canola Oil Content is Unknown

In addition to the above-described specific studies conducted with infant formulas containing canola oil, several published studies reported results of PUFA-enriched oil blends, as part of formulas, fed to infants. Although the use of canola oil in these blends is not made clear in the body of the publications, these studies are described to provide a complete review of the literature on the consequences of PUFA-enriched oil blends fed to infants. A summary of these studies is presented in Appendix B, section 3. These studies nonetheless provide information on the consequences of feeding PUFA-enriched formulas to infants.

2. Effects Observed in Spontaneously Hypertensive Rats

Many studies have been done with canola oil in spontaneously hypertensive rats that are derived from Wistar Kyoto (WKY) rats. Details of these studies are included in Appendix B, section 4. Canola oil had several detrimental cardiovascular effects when compared to soybean oil in normal WKY rats (Naito et. al., 2000a). WKY rats are more sensitive to phytosterol toxicity compared to other strains of rats (Ratanyake, 2000b). These effects seen in the normal WKY rats may be exacerbated in spontaneously hypertensive variants, and such effects have been investigated in several studies. Only one of these studies focused on weanling rats (Tatematsu et al., 2004) where effects seemed to result from phytosterols and other factors.

3. Studies Elucidating the Mechanism by which Various PUFA-Rich Oils Influence Blood Cell Membrane Structure & Function, Platelet Function, Erythrocyte Fragility & Antioxidant Nutrient Requirements

There have been many studies in animal models and humans that have sought to elucidate the relationship between PUFA intakes and blood cell structure and function and the effects on antioxidant nutrient status. Details of these studies are included in Appendix B, sections 5, 6, 7 and 8. It is evident that there is a significant relationship between dietary fatty acids and blood cell structure and function, the conclusions drawn from many of these studies refer to the effects of the fatty acid composition of the constituent oils (e.g., high PUFA levels in the oil blend) on outcomes rather than on the effects of the oils per se.

It is known that newborn infants are at greater risk of oxidative stress because of an imbalance between high oxidant loads and immature antioxidant defenses. The studies described in Appendix B, section 8 investigating the relationship between PUFA intakes and vitamin E status broadly concur with the studies in neonatal pigs which demonstrated that ingesting canola oil as the sole dietary lipid source depletes vitamin E stores, and this effect is ameliorated by using canola oil in combination with saturated oils and vitamin E supplementation. The LSRO report (1998) stated that there is no evidence to suggest that high PUFA intakes by infants lead to vitamin E deficiency.

F. Safety Aspects of Erucic Acid in Canola Oil

1. General Information Related to Erucic Acid

The majority of exposure to erucic acid comes from canola oil, which has virtually replaced traditional high erucic acid varieties of rapeseed oil worldwide. The concern over human health related to dietary erucic acid arises from animal studies that have demonstrated an association with high erucic acid intakes and myocardial lipidosis and heart lesions. There is no evidence that dietary erucic acid has these effects in humans. However, it is appropriate in the context of this GRAS assessment that the risk of exposure to erucic acid should be assessed.

2. Evaluation of Safe Intakes of Erucic Acid by Official Bodies

Food Standards Australia New Zealand (FSANZ, 2003) carried out a toxicological review and risk assessment of erucic acid in food. Having reviewed the toxicology data available from animal studies, FSANZ established a No Observed Adverse Effect Level (NOEL) of 750 mg/kg bw/day. This level was based on toxicology data from studies in piglets since there were no adequate human data on which to base it. By applying an uncertainty factor of 100 to the NOEL, a tolerable level for human exposure of 7.5 mg/kg/day was established. This is regarded as a provisional tolerable daily intake (PTDI) for erucic acid.

The current Codex Standard for Infant Formulas, EU Infant Formula legislation and the Australian Food Standard on Infant Formula all include an upper limit for the erucic acid content of infant formula at 1% of total fatty acids or total fat.

3. Safety of Intakes of Erucic Acid Resulting from Exposure to Infant Formula Containing Canola Oil

The study performed by Kramer and colleagues (1990) in the neonatal piglet model allowed the identification of the main potential adverse effect linked to the consumption of high levels of erucic acid, which is cardiac lipidosis (deposition of triglycerides in cardiac tissue). The neonate animals were fed for 16 days with diets containing various levels of erucic acid (0.8% up to 42.9% of total fatty acids). As cardiac lipidosis appears rapidly after only short period of intakes, the data of this short-term exposure study allowed the identification of a NOEL at 750 mg/kg bw/day (diet containing rapeseed oil at 4.9% erucic acid); at this level the effects were similar to the control diet containing soybean oil. The LOEL was determined to be at 900 mg/kg bw/day based on increased cardiac lipidosis at this level. The absence of cardiac lipidosis and the absence of adverse effects on growth were subsequently confirmed in the study performed by Green and Innis (2000) on the neonatal piglet model with formula containing 100% or 26% of canola oil (0.5% erucic acid in the canola oil). There were some elements indicating the deposition of triglycerides within cardiac tissue is anticipated to be of low long-term concern as this phenomenon seems reversible. Indeed, it could be demonstrated in weanling rats that the fatty accumulation appears to decrease with time up to disappearance even if feeding is continued with rapeseed oil (Abdellatif and Vles, 1970). The accumulation of fat diminished more quickly if erucic acid is completely removed from the diet (Kitts, 1996). However, no data specifically linked to adverse effects of erucic acid on the accumulation of heart triglycerides could be retrieved from the trials performed in infants.

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As reported in Section III, the erucic acid content of canola oil is typically 0.6% (i.e., 600 mg/100 g). Considering that canola oil is intended to be used between 17.5% and 31% in infant formulas manufactured by Danone, the resulting levels of erucic acid contributed by the canola oil fraction will be at 5.3 mg and 8.8 mg per 100 kcal formula, respectively. Intakes of erucic acid from formulas are presented on Tables 8 and 9. Intakes by typical consumers of formula with the highest levels of canola oil (31% of total fatty acids) are around the PTDI (intakes of 7.7 mg/kg bw/day compared to a PTDI of 7.5 mg/kg bw/day; see Table 8). Intakes for extreme consumers are likely to be marginally higher than the PTDI (intakes of up to 10.8 mg/kg bw/day; see Table 9). Additionally, epidemiological evidence does not indicate a causal link between cardiac lipidosis and erucic acid intake (through consumption of canola oil) in humans. In addition there is a long history of safe use of canola oil as a component of the fat blend of infant formulas. The anticipated erucic acid level in formulas containing up to 31% of canola oil is in line with recommendations of the European Society for Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) which indicated that erucic acid should represent at maximum 1% of total fat (in the present case this would be equivalent to up to 51 mg) (ESPGHAN, 2005). The canola oil used by Danone will have a maximum erucic acid content of <1 % total fatty acids is typically well below the FCC specification for erucic acid content (<2%), and this will ensure the production of infant formulas with erucic acid levels that fall well below legislative limits for erucic acid content in infant formulas (maximum 1% total fatty acids) set by CODEX, the EU and FSANZ.

G. Safety Aspects of Plant Sterols in Canola Oil

1. General Information Related to Phytosterols

Phytosterols (plant sterols) present in oil seed belongs to a class of compounds composed primarily of β-sitosterol, sigmasterol, and campesterol with lesser amounts of brassicasterol, delta-5-avenasterol and several corresponding stanols. These phytosterols appear as free sterols, esterified forms with fatty acids and phenolic acids, or conjugated with glycosides. Natural occurrence of phytosterols is reported in many fruits, vegetables, nuts, seeds, cereals and legumes. Crude food oils derived from soy, corn, sunflower, canola, palm, and other crops may contain 100-500 mg phytosterol/100 g of oil. Available evidence from multiple studies indicates that daily dietary intake of phytosterols ranges from 78 mg/day in the general population to 344 mg/day in lacto-ovo vegetarians (Nair, 1984).

There is a long history of use of vegetable oils as a fat source in infant formulas (Huisman et al., 1996; Wong et al., 1993) and, thus, infant formulas, with or without canola oil, have long been a source of phytosterols in the diets of infants. Huisman et al. (1996) investigated differences in---among other constituents---phytosterol concentrations between breast milk and 10 commercially available infant formulas (of which 3 were from North America: Enfamil, Mead Johnson, Canada; SMA, Wyeth, USA; S-26, Wyeth, USA). While phytosterols were undetectable in breast milk, all of the infant formulas were found to contain varying concentrations of phytosterols.

2. Evaluation of Safe Intakes of Phytosterols by Official Bodies

Phytosterols and their esters have been the subject of multiple GRAS notifications to the FDA for use as ingredients in a variety of foods (FDA 2000a, b, c, 2001, 2002, and 2006). Based on information from FDA's GRAS Notice Inventory[12] website as of January, 2011, the agency has received 11 notices on phytosterols and all these notices received "no questions" letters from the agency. In these notices, biological data, metabolism, and safety of phytosterols and its esters have been extensively presented and discussed. The most recent review and response from the FDA occurred in 2010. In GRAS notification 000061(FDA 2001), the cumulative intake of phytosterols from its addition to various foods was estimated to be as high as 10.6 g/person/day. FDA did not question the safety at this intake level. Food uses cited in the GRAS notifications include margarine spreads and yogurt which can be consumed by infants as they progress to solid food. The European Food Safety Agency (EFSA) has also approved rapeseed oil enriched with phytosterols (EFSA 2005).

The WHO/FAO Joint Expert Committee on Food Additives (JECFA) has critically evaluated the safety of phytosterols and concluded that dietary exposure to phytosterols and phytostanols would typically be within the ADI range of 0-40 mg/kg bw/d.

3. Safety of Intakes of Phytosterols Resulting from Exposure to Infant Formula Containing Canola Oil

3.1 Safety of Total Phytosterol Content of Infant Formulas

Limited data are available from dedicated studies related to the effects of plant sterols in infants (or neonatal animals). It has been suggested by one laboratory that phytosterols absorption can be higher in infancy and childhood, based on observation of accumulated plant sterols in the aortic tissues of vegetable oil formula-fed infants and in hypercholesterolemic children on a low-cholesterol, phytosterol-rich diet (Mellies et al., 1976a, b). It has been shown that the consumption of phytosterol-rich diet (i.e., infant formulas-containing vegetable oils) was able to increase the plasma level of the different phytosterols. The observed increase of phytosterols (campesterol, stigmasterol and beta-sitosterol) plasma levels was about 3- to 5-fold (normal 2 mg/100 mL up to 9 mg/100 mL) (Mellies et al., 1976b). In terms of accumulation of phytosterols, however, existing data indicate that there is no evidence for selective accumulation of phytosterols in diseased tissue (i.e., atheroma). According to Jones (2007), cholesterol and phytosterols have similar properties, and both types of sterols would tend to accumulate similarly across body tissues.

Additional information on the safety of plant sterols in younger populations than adults comes from studies performed in children. Recently, no adverse effects and good tolerance were observed in child subjects with familial hypercholesterolemia at a daily oral intake as high as 2 g/day for 3 months through consumption of plant sterols-enriched foods (Guardamagna et al., 2011). In a randomized prospective study aiming at reducing exposure of young children to known environmental atherosclerosis risk factors, 81 children were recruited to a double-blind crossover study at 6 years of age (Tammi et al., 2000). Each day, 20 g of the child's dietary fat intake were

[12] Accessible at: http://www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=grasListing&displayAll=true.

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replaced with plant stanol ester margarine (leading to a mean daily intake of 1.5 g of plant stanols) or control margarine for 3 months with a wash-out period of 6 weeks. It could be concluded from the study results that plant stanol ester margarine had beneficial effects on cholesterol parameters without adverse clinical effects in healthy children already consuming a low saturated-fat, low-cholesterol diet, but decreased the serum β-carotene to low-density lipoprotein cholesterol ratio. The authors also indicated that the serum sitostanol concentration after three months of treatment was only about 1/6000 of the concentration of serum cholesterol even though a 10-fold greater amount of sitostanol than cholesterol was ingested, showing a very low absorption of those types of plant sterols even in the young gastrointestinal tract. In another study in children with severe familial hypercholesterolemia, no liver and gallbladder abnormalities (sonography examination) were reported after daily intakes of both sitosterol (6 g/day for 3 months) followed by sitostanol (1.5 g/day for 7 months) through capsule feeding. In addition, plant sterol administration did not cause any change in serum sitosterol concentrations, therefore indicating the absence or a very low absorption of these plant sterols (Becker et al., 1993).

As reported in Section III, the phytosterol content of canola oil is reported to be around 0.9% (i.e., 900 mg/100 g). Considering that canola oil is intended to be used between 17.5% and 31% in infant formulas manufactured by Danone, the resulting levels of phytosterols contributed by the canola oil fraction will be at 8.0 mg and 14.1 mg per 100 kcal formula, respectively. Intakes of phytosterols from the canola oil fraction of the formulas are presented in Tables 8 and 9. Intakes by typical consumers of formula with the highest levels of canola oil (31% of total fatty acids) fall well below the JECFA ADI (intakes of 11.5 mg/kg bw/day compared to the ADI of 40 mg/kg bw/day; see Table 8). Similarly, intakes for extreme consumers also fall well below the ADI (intakes of up to 16.2 mg/kg bw/day compared to the ADI of 40 mg/kg bw/day; see Table 9). Even allowing for additional phytosterol exposure because of the presence of other oils in the formulas, intakes of phytosterols are likely to fall well below the ADI for typical and extreme consumers. Intakes of phytosterols arising from the addition of canola oil to infant formulas do not raise any safety concerns.

3.2 Safety of the Brassicasterol Content of Infant Formulas

As with other phytosterols, the chemical structure of brassicasterol is based on the cholesterol molecule with a methyl group on the lateral chain. Brassicasterol belongs to the phytosterol subgroup 4-desmethyl sterols which are the most frequently encountered group of phytosterols. Other 4-desmethyl sterols include sitosterol, stigmasterol and campesterol. Only limited data are available where brassicasterol as part of the global phytosterol blend was tested either in animal or human clinical studies. Most studies investigating the beneficial or potential adverse effects of phytosterols are based on commercial blends of phytosterols containing a maximum level of 3% phytosterols. Some animal and human studies (outlined below) have indicated lack of adverse effects with higher intakes of brassicasterol above 3%.

Absorbability of plant sterols appears to be dependent on the structure of the side chain present. To illustrate this, Batta et al. (2005) have reported in a rat experimental model a low plasmatic and biliary incorporation of brassicasterol and stigmasterol indicating a weaker absorption of those sterols (Wistar and WKY strains) fed for 4 weeks with a diet containing 1% of phytosterols (6.3% of brassicasterol and 15.6% stigmasterol among total phytosterols). This poor absorption may be

linked to a common feature of those 4-desmethyls sterols that have both a double bond at C22. Schroder et al. (2009) investigated the impact of sterols derived from canola oil in an experimental model of hyperlipidemic rabbits fed with cholesterol (heterozygous Watanabe heritable hyperlipidaemic rabbits, Hh-WHHL). The animals were daily fed with a diet enriched with 100 mg/kg bw/day of plant sterols containing around 10% of brassicasterol (i.e., 10 mg/kg bw/day). From a general perspective, the exposure to a plant sterol blend with high levels of brassicasterol was well tolerated given that it did not reveal any adverse effects on clinical appearance, body weight, feed intake and weight gain. In the animals, besides a positive effect on atherosclerotic process in the thoracic aorta, the plasma concentration percentage of brassicasterol remained low in the cranial aorta, indicating a low uptake of brassicasterol when fed as part of other plant sterols. Moreover, the authors reported a low concentration of brassicasterol within the aortic tissue which indicates a slower clearance from the blood and a lower incorporation into tissues compared to other sterols. In their study on Golden Syrian hamsters, Demonty and colleagues (2005) investigated the effect of non-esterified plant sterol mix containing 5.12% of brassicasterol among the global phytosterol content. The animals were fed daily over 5 weeks with non-esterified plant sterols at 1% in the diet, thus providing a daily phytosterol dose of approximately 900 mg/kg bw/day (~46 mg/kg bw/day of brassicasterol). The authors reported no adverse effects on body weight or food consumption, no modification of plasma triglycerides levels, and no effect on red blood cells fragility. In other studies, it has been reported that a plant sterol ester mixture containing 6.4% of brassicasterol administered to Dunkin Hartley guinea pig through the diet (total phytosterol dosage 0.5 or 1 g/kg bw/day for 4 weeks, equivalent to ~32 or 64 mg/kg bw/day of brassicasterol), resulted in no modification of body weight and food consumption (Brufau et al., 2006, 2007). Administration of soybean non-esterified sterols containing approximately 4% of brassicasterol to Mongolian Gerbils (total phytosterols dosage 1 g/kg bw/day for 4 weeks, ~40 mg/kg bw/day of brassicasterol), did not induce adverse effects on body weight (Hayes et al., 2004).

Some specific information on the general safety and tolerance related to brassicasterol can be retrieved from data available only in adults. In a double-blind controlled study, adult subjects (n=16) with moderate hypercholesterolemia were exposed on a daily basis with the consumption of capsules containing phytosterols esters with 4% brassicasterol (phytosterols dosage 1.3 g/day, ~52 mg/day of brassicasterol) for 4 weeks. The study was not designed for the identification of adverse effects, but the treatment was well tolerated by the participants, and no subject stopped the trial due to adverse effects (Acuff et al., 2007). De Jong et al. (2008, 2009) reported results of a double-blind, placebo-controlled study in which adults (n=41) who had been taking statins for at least 6 months together with other medications, such as beta-blockers and angiotensin converting enzyme inhibitors, took 2.5 g/day of plant phytosterols of which 125 mg/day was brassicasterol. The adverse events were not recorded in the study, but apparently no changes in hematological parameters were observed. In a double-blind randomized controlled trial, adult subjects (n=17) consumed 3 daily portions of mayonnaise providing 1.2 g/day of plant sterol esters (5% brassicasterol equivalent to ~60 mg/day). No changes between groups were reported in hematology, blood clinical biochemistry, serum electrolytes or glucose/fat metabolism-related factors. Moreover, no differences in abdominal symptoms incidence were reported between the different groups (Saito et al., 2006).

As reported in Section III, the brassicasterol content of canola oil is reported to be around 0.077% (i.e., 77 mg/100g). Considering that canola oil is intended to be used between 17.5% and 31% in infant formulas manufactured by Danone, the resulting levels of brassicasterol contributed by the canola oil fraction will be at 0.7 mg and 1.2 mg per 100 kcal formula, respectively. Intakes of brassicasterol from the canola oil fraction of the formulas are presented on Tables 8 and 9. Intakes by typical consumers of formula with the highest levels of canola oil (31% of total fatty acids) will be around 1.0 mg/kg bw/day (see Table 8), and intakes for extreme consumers will be around 1.4 mg/kg bw/day (see Table 9). Given that brassicasterol in other oils typically used in infant formulas has not been detected, exposure to canola oil is likely to constitute the only source of brassicasterol in the diets of formula fed infants. At present, there is no ADI for brassicasterol for comparison purposes. However, the anticipated daily intakes are significantly lower (up to 20-fold lower) than the brassicasterol levels that were not associated with adverse effects in human trials and animal studies.

VII. DISCUSSION OF REVIEWED INFORMATION & GRAS CRITERIA

A. GRAS Criteria

FDA defines "safe" or "safety" as it applies to food ingredients as:

> "...reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use. It is impossible in the present state of scientific knowledge to establish with complete certainty the absolute harmlessness of the use of any substance."[13]

Amplification is provided in that the determination of safety is to include probable consumption of the substance in question, the cumulative effect of the substance, and appropriate safety factors. It is FDA's operational definition of safety that serves as the framework against which this evaluation is provided.

Furthermore, in discussing GRAS criteria, FDA notes that

> "...General recognition of safety requires common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food."[14]

FDA discusses in more detail what is meant by the requirement of general knowledge and acceptance of pertinent information within the scientific community, i.e., the so-called "common knowledge element," in terms of the two following components:[15]

- Data and information relied upon to establish safety must be generally available, and this is most commonly established by utilizing published, peer-reviewed scientific journals; and

- There must be a basis to conclude that there is consensus (but not unanimity) among qualified scientists about the safety of the substance for its intended use, and this is established by relying upon secondary scientific literature such as published review articles, textbooks, or compendia, or by obtaining opinions of expert panels or opinions from authoritative bodies, such as the National Academy of Sciences.

The apparent imprecision of the terms "appreciable," "at the time," and "reasonable certainty" demonstrates that the FDA recognizes the impossibility of providing absolute safety, in this or any other area (Lu, 1988; Renwick, 1990).

As noted below, the safety assessment to ascertain GRAS status for canola oil as part of a blend with other oils for uses in infant formulas with the defined use levels meets FDA criteria for

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13 See 21 CFR 170.3(i).
14 See 21 CFR 170.30(a).
15 See footnote 3.

[redacted]

reasonable certainty of no harm by considering both the technical and common knowledge elements.

B. Summary & Discussion of Canola Oil Use in Infant Formulas

1. Background

Canola oil, which is produced from varieties of rapeseed which have been bred to contain much lower levels of erucic acid than traditional varieties, has been safely used in the US since 1985 and is considered to be GRAS for general foodstuffs. The GRAS regulation indicates that the oil from new varieties of rapeseed that contained less than 2% erucic acid would be safe for common use in food. Subsequent to its GRAS status, an FCC specification was published retaining the 2% erucic acid limit, and millions of pounds are consumed each year in the US. The use of canola oil in infant formula is not explicitly approved in North America, although this prohibition does not appear to be based on any evidence suggesting that canola oil may not be safe for use in infant formulas. Canola oil is widely used in the production of infant formulas in many other countries where the only restriction is that the erucic acid content of the finished infant formulas should not exceed 1% of total fatty acids or total fat.

The original concern with rapeseed oil was the presence of high levels of the fatty acid, erucic acid, which has been shown in animal studies to be associated with fatty infiltration of the myocardium. Efforts were made to produce rapeseed oil with lower erucic acid content, and this led to the development of canola oil. It is interesting to note that epidemiology evidence from countries where intakes of erucic acid-rich oils are particularly high did not identify a causal link between erucic acid and cardiac lipidosis in humans (Grice and Heggtveit, 1983; Svaar, 1982).

The exclusion of canola oil from infant formulas in North America seems to have persisted because of the perceived expense of challenging it and lack of incentives in the North American infant formula industry. However, with the development of canola oil, there have been many studies conducted by various research camps that can be looked upon retrospectively as supporting the safety of canola oil in infant formulas. These studies include those in neonatal animal models investigating the effects of canola oil-enriched diets on growth, antioxidant nutrient status, blood cell integrity and cardiac lipid deposition. In addition, many studies have also been carried out in humans involving more than 900 infants consuming infant formulas containing canola oil at up to 40% of total fatty acids (Rzehak et al., 2011). The large majority of these studies were not designed as primary safety studies, and many were conducted in Europe where canola oil is a normal constituent of infant formula. Others studied the effects and benefits of varying fatty acid composition of the formulas.

2. Rationale for Pursuing GRAS Determination for Use of Canola Oil in Infant Formulas

After more than 35 years of the publication of 21 CFR 184.1555 by FDA, Danone believes that the GRAS status of canola oil can be extended to infant formulas. It would provide a useful source of ALA in its infant formulas. The Panel noted that canola oil has a higher ALA content with a lower LA content than soy oil, thereby facilitating the production of infant formulas with a more optimal

overall EFA profile than if soy oil is used. Canola oil is a preferred source of ALA to soy oil in countries where there are no restrictions on its use in infant formula. Indeed, Danone has marketed infant formulas in many countries for many years with various levels of canola oil. There has been no pattern of complaints that are attributed to canola oil (Rzehak et al., 2011).

3. Studies Investigating the Effects of Feeding Canola Oil in Animal Models & Human Infants

Canola oil differs primarily from other vegetable oils commonly used in infant formulas in that it is a source of erucic acid. Given the history of canola oil development and in consideration of the suitability of canola oil for use in infant formulas, it is important to critically evaluate the exposure to erucic acid and its safety. Other constituents inherently present in canola oil also need due safety considerations. For example, phytosterols are present in canola oil at slightly higher levels than other oils traditionally used in the production of infant formulas, and one class of phytosterols in particular---brassicasterol---is undetectable in other vegetable oils but is present in canola oil. Unsaturated fatty acids, because they are prone to oxidation, may influence antioxidant status in infants and, thus, the effect of a canola oil-enriched infant formula on antioxidant nutrient status of infants should be scrutinized. The Panel considered these and other points as part of the safety assessment in the use of canola oil in infant formulas.

3.1. Erucic Acid

The acceptable level of erucic acid in canola oil is stipulated in the FCC specification at <2% of total fatty acids. Legislative requirements laid down by CODEX, the EU and FSANZ require that infant formulas contains erucic acid at <1% of total fatty acids. The erucic acid contents of batches of canola oil supplied to Danone are typically around 0.6% of total fatty acids. At the highest concentration of canola oil in an infant formula manufactured by Danone, the erucic acid content should be around 0.2% of total fatty acids, a level well below the limit for infant formulas of 1% of total fatty acids. total fatty acids. The canola oil used by Danone for manufacture of infant formula contains a maximum erucic acid content of 1% total fatty acids (see Appendix D). The Panel noted that canola oil used by Danone is typically well below the FCC specification for erucic acid content and that this will ensure the production of infant formulas with erucic acid levels that fall well below legislative limits for infant formulas set by CODEX, the EU and FSANZ.

The Panel notes that intakes of erucic acid by some groups of consumers based on the composition of their diet may, in some instances, exceed the PTDI set by FSANZ of 7.5 mg/kg bw/day. However, the Panel noted that epidemiological evidence from countries where intakes of erucic acid-rich oils are particularly high does not indicate a causal link between cardiac lipidosis and erucic acid intake (through consumption of canola oil) in humans (Grice and Heggtveit, 1983; Svaar, 1982). In addition, there is a long history of safe use of canola oil as a component of the fat blend of infant formulas. Additionally, the available evidence from experimental animal and human studies did not reveal adverse effects of canola oil at the intended use levels. The Panel concludes that intakes of erucic acid from infant formulas containing canola oil are unlikely to present a risk to health.

3.2. Total Phytosterols & Brassicasterols

Phytosterols have been shown to have beneficial effects in humans. Multiple GRAS notifications to FDA to add these constituents to foodstuffs for their cholesterol-lowering properties have not been questioned. The Panel noted the ubiquitous nature of phytosterols in vegetable oils used in the production of infant formulas. The phytosterol content of batches of canola oil supplied to Danone are typical of the levels stipulated in the CODEX standard. The Panel noted that the intakes by typical and extreme consumers of formula containing the highest levels of canola oil (31% of total fatty acids) fall well below the JECFA ADI of 40 mg/kg bw/day, even allowing for additional phytosterol exposure because of the presence of other oils in the formulas. Given that brassicasterol in other oils typically used in infant formulas has not been detected, exposure to canola oil is likely to constitute the only source of brassicasterol in the diets of formula fed infants. At the present time, no specific ADI is available for brassicasterol for comparison purposes, however, the anticipated daily intakes are significantly lower (up to 20-fold lower) than the brassicasterol levels that were not associated with adverse effects in human trials and animal studies. The available evidence suggests that low levels of intake of total phytosterols and brassicasterol from infant formulas containing canola oil are likely to be safe.

3.3. Antioxidant Nutrient Status

The Panel has considered the outcomes from studies investigating the consequences of PUFA-enriched diets on antioxidant nutrient status. Danone intends to use canola oil with other oils to provide infant formulas with a balance of saturates, monounsaturates and polyunsaturates, supplemented with a range of other nutrients including vitamins E and K. The Panel noted that, provided infant formulas are supplemented with antioxidant nutrients at the levels recommended by expert bodies, there is no cause for concern for the antioxidant status of infants consuming infant formulas containing canola oil.

C. Common Knowledge Elements Needed for GRAS Conclusions

The first essential element of common knowledge that is required for a GRAS determination is the general availability of the key information on which the GRAS conclusion is based. This aspect has been fulfilled since the overwhelming majority of the studies and data relied upon in this assessment have been published in the scientific literature. These include:

- Several published studies in the neonatal pig model summarized in Table 10 which predict a low likelihood of adverse effects in humans at the levels expected to be consumed by infants;
- As summarized in Table 11, there are three particular published clinical studies in term infants where the levels of various concentrations of canola oil were investigated:
    - Two publications by Jensen et al, (1996, 1997), where formulas containing canola oil at varying concentrations were fed to infants to investigate the consequences of increasing the ALA content of infant formulas on biochemical markers of fatty acid status and on physiological outcomes, such as visual acuity.

[redacted]

- A study carried out by Makrides et al. (2000), in which 2 formulas with LA:ALA ratios of 10:1 (soy oil-containing formula at 23% of total fatty acids) and 5:1 (canola oil-containing formula at 29% of total fatty acids) were compared, found that growth rates in both formula groups were similar.

- Table 11 also summarizes several other published clinical studies in term infants where canola oil was known to be part of the basal diet.
- Several published clinical studies in term infants summarized in Appendix B, section 3 where the fatty acid composition of the diets was similar to that provided by canola oil.

The second critical aspect of fulfilling the common knowledge criteria for GRAS determinations is through the establishment of consensus within the scientific community that is knowledgeable in the subject area. As cited in Tables 11 and 12 in this document, an extensive number of published clinical studies by several groups of investigators have been conducted with formula preparations containing canola without adverse effects. In addition, the Panel believes that consensus regarding the safe use of defined uses of canola oil in infant formulas has been established at the use levels noted above is largely demonstrated by the fact that major manufacturers of infant formulas outside of North America supported by competent scientific staff are extensively using canola oil in infant formula preparations. In addition, there are several key decisions made by regulatory agencies which support a consensus opinion of the safety of canola oil in infant formulas. Most notably these include:

- FSANZ review of canola oil and the assignment of a provisional tolerable daily intake of 7.5 mg/kg/day intake for erucic acid;
- The Codex Alimentarius standard for infant formulas and formulas for special medical purposes intended for infants which does not preclude the use of canola oil.

D. Summary Assessment by Expert Panel

In addition to fulfilling the requisite common knowledge elements required for GRAS conclusions, the Panel concurs that there is sufficient scientific evidence to conclude that canola oil (as a blend with other oils to obtain the desired levels of fatty acids) is generally recognized as safe (GRAS) for use in infant formulas for term infants. The Panel recognizes the importance of the safety of ingredients used in infant formulas as infants rely on formulas as a sole source of food for many months early in life. In arriving at a view on the likelihood of the use of canola oil in infant formulas, the Panel considered the current uses of canola oil in infant formulas in other countries, the outcomes from animal studies, and clinical studies in human infants carried out using infant formulas that incorporate canola oil.

Canola oil is used safely and extensively in infant formulas throughout the world. The Panel notes that the studies carried out in the neonatal pig model support the safety of canola oil as an oil in infant formulas if used within a blend of oils, containing nutritionally balanced amounts of saturates, monounsaturates and polyunsaturates and supplemented with adequate amounts of vitamin E. A considerable number of clinical studies in infants have been conducted with canola oil---or blends of other dietary oils---and no significant adverse effects were noted during the studies or were

reported to the investigators after the studies were completed. The Panel agrees that there is no compelling reason to exclude the use of canola oil from infant formulas. The estimated dietary intake of erucic acid from formulas at the intended use levels of canola oil will be reasonably consistent with the provisional tolerable daily intake for erucic acid assigned by FSANZ (2003) and the supplementary human use levels of canola oil as cited above. Similarly, the Panel agrees that the resulting intake of phytosterol and brassicasterol from the uses of canola oil does not raise any safety concerns, and the identical usage of canola oil in the designated infant formulas is considered to be safe.

The Panel also notes that none of the studies in human infants suggested that the canola oil-containing formulas were inferior to formulas that did not contain canola oil. No adverse effects were reported from the studies or in follow up investigations long after feeding had stopped. The Panel accepts that these studies provide a considerable body of evidence suggesting no harm from the use of canola oil as one of the oils in infant formulas. The Panel is satisfied that canola oil is no less safe for use in infant formula than other PUFA-rich oils.

Finally, the Panel has taken into consideration the fact that infant formulas containing canola oil are consumed during the earliest stages of growth and development for infants and children. It is also recognized that the dietary needs evolve while such development occurs. The use of canola oil will facilitate achieving desired levels of essential fatty acids in infant formulas. In summary, the Panel agrees that the available information does not indicate any safety concerns from the limited uses of canola oil as a blend with other oils in infant formula.

VIII. CONCLUSIONS

The members of the Expert Panel consisted of: Richard C. Kraska, Ph.D. (Chair), DABT, Madhusudan G., Soni, Ph.D., Robert S. McQuate, Ph.D., Stanley T. Omaye, Ph.D., Walter H. Glinsmann, MD, and Sheila M. Innis, Ph.D., RDN. Each has an extensive technical background in the evaluation of the safety of food ingredients.[16]

> The Panel concludes that the use of canola oil produced in compliance with current Good Manufacturing Practices requirements and which meets Food Chemicals Codex specifications is generally recognized as safe (GRAS) when used as a component of the fat blend in infant formulas for term infants, with a typical intended use level of up to 31% of the fat blend, such that the fat blend provides a nutritionally balanced blend of essential fatty acids, saturated, monounsaturated and polyunsaturated fatty acids in line with dietary recommendations for fatty acid intakes for infants.

(b) (6)

Richard C. Kraska, Ph.D., DABT - Chair

(b) (6)

Madhusudan G. Soni, Ph.D., FACN, FATS

(b) (6)

Robert S. McQuate, Ph.D.

(b) (6)

Stanley T. Omaye, Ph.D., DATS

(b) (6)

Walter H. Glinsmann, M.D.

Sheila M. Innis, Ph.D., RDN

March 6, 2012

[16] The detailed educational and professional credentials for some of the individuals serving on the Expert Panel can be found on the GRAS Associates website at www.gras-associates.com. Dr. Kraska served as Chair of the Panel. Drs. Kraska and McQuate worked on GRAS and food additive safety issues within FDA's GRAS Review Branch earlier in their careers and subsequently continued working within this area in the private sector. Dr. Omaye is a professor of nutrition at the University of Nevada, Reno and has published extensively on the nutritional and toxicological aspects of food ingredients. Dr. Soni's credentials can be accessed at http://www.soniassociates.net. Dr. Glinsmann has extensive experience in clinical nutrition and food safety that includes service with FDA's Division of Nutrition where he addressed a broad range of food and human nutrition topics. Dr. Innis is currently Director, Nutrition & Metabolism Research Program, CFRI; and Professor, Department of Pediatrics, University of British Columbia. Dr. Innis' credentials can be accessed at http://www.cfri.ca/our_research/researches/search_researchers/researcher_detail.asp?ID=61.

IX. REFERENCES

Abdellatif, A.M., Vles, R.O., 1970. Pathological Effects of Dietary rapeseed oil in Rats. Nutr Metabol 12(5): 285-295.

Acuff, R.V., Cai, D.J., Dong, Z.P., Bell, D., 2007. The lipid lowering effect of plant sterol ester capsules in hypercholesterolemic subjects. Lipids Health Dis. Apr 9, 6:11.

Allman, M.A., Pena, M.M., Pang, D., 1995. Supplementation with flaxseed oil versus sunflower seed oil in healthy young men consuming a low fat diet: effects on platelet composition and function. Eur J Clin Nutr 149:169-178.

Badawy, I.H., Atta, B., Ahmed, W.M., 1994. Biochemical and toxicological studies on the effect of high and low erucic acid rapeseed oil in rats. Die Nahrung 38:402-411.

Bakker-Zierikzee, A.M., Alles, M.S., Knol, J., Kok, F.J., Tolboom, J.J.M., Bindels, J.G., 2005. Effects of infant formula containing a mixture of galacto- and fructo-oligosaccharides or viable *Bifidobacteriumanimalis* on the intestinal microflora during the first four months of life. Br J Nutr 94:783-790.

Bakker-Zierikzee, A.M., Tol, E.A., Kroes, H., Alles, M.S., Kok. F.J., Bindels, J.G., 2006. Faecal SIgA secretion in infants fed on pre- or probiotic infant formula. Pediatr Allergy Immunol 17:134-140.

Barradas, M.A., Christofides, J.A., Jeremy, J.Y., Mikhailidis, M.D., Fry, D.E., Dandona, P., 1990. The effect of olive oil Supplementation on human platelet function, serum cholesterol-related variables and plasma fibrinogen concentrations: a pilot study. Nutr Res 10:403-411.

Batta, A.K., Xu, G., Bollineni, J.S., Shefer, S., Salen, G., 2005. Effect of high plant sterol-enriched diet and cholesterol absorption inhibitor, SCH 58235, on plant sterol absorption and plasma concentrations in hypercholesterolemic wild-type Kyoto rats. Metabolism 54(1) :38-46.

Becker, M., Staab, D., Bergmann, K., 1993. Treatment of Severe Familial Hypercholesterolemia in Childhood with Sitosterol and Sitostanol. J Ped 122(2):292-296.

Berra, B., Montorfano, G., Adorni, L., Zoppi, S., Mazzali, P., Rapelli, S., 1998. Serum lipids and lipidic composition of red blood cell membranes after diet with sunflower oil at normal or high content of oleic acid. Rivista Italiana delle Sostanze Grasse 75:127-132.

Billeaud, C., Bougle, D., Sarda, P., Combe, N., Mazette, S., Babin, F., Entressangles, B., Descomps, B., Nouvelot, A, Mendy, F., 1997. Effects of preterm infant formula supplementation with alpha-linolenic acid with a linoleate/alpha linolenate ratio of 6:1: A multicentric study. Eur J Clin Nutr 51:520-526.

Bing, O.H.L., Contrad, C.H., Boluyt, M.O., Robinson, M.T., Brooks, W.W., 2002. Studies of prevention, treatment and mechanisms of heart failure in the aging spontaneously hypertensive rat. Heart Fail Rev 7:71-88.

Boehm, G., Moro, G., Muller, D.M., Muller, H., Minoli, I., 1995. Fecal cholesterol excretion in preterm infants fed breast milk or formula with different cholesterol contents. Acta Paediatr 84(3):240-244.

Boehm, G., Lidestri, M., Casetta, P., Jelinek, J., Negretti, F., Stahl, B., Marini, A., 2002. Supplementation of a bovine milk formula with an oligosaccharide mixture increases counts of fecal bifidobacteria in preterm infants. Arch Dis Child 86:F178-181.

Boehm, G., Fanaro, S., Jelinek, J, Stahl, B., Marini, A., 2003. Prebiotic concept for infant nutrition. Acta Paediatr Suppl 441:64-67.

[redacted]

Borg, K., 1975. Physiopathological effects of rapeseed oil: a review. Acta Medica Scandinavica Supplementum 587:5-13.

Bouwstra, H., Dijck-Brouwer, D.A.J., Boehm, G., Boersma, E.R., Muskiet, F.A.J., Hadders-Algra, M., 2005. Long-chain polyunsaturated fatty acids and neurological developmental outcome at 18 months in healthy term infants. Acta Paediatr 94:26-32.

Brufau, G., Canela, M.A., Rafecas, M., 2006. A high-saturated fat diet enriched with phytosterol and pectin affects the fatty acid profile in guinea pigs. Lipids Feb 41(2):159-68.

Brufau, G., Canela, M.A., Rafecas, M., 2007. Phytosterols, but not pectin, added to a high-saturated-fat diet modify saturated fatty acid excretion in relation to chain length. J Nutr Biochem. Sep18(9):580-6.

Bruckner, G., 1986. Fats, their potential isomers, and platelet function. J Med Technol 3:24-28.

Butcher, L.A., O'Dea, K., Sinclair, A.J., Parkin, J.D., Smith, I.L., Blombery, P., 1990. The effects of very low fat diets enriched with fish or kangaroo meat on cold-induced vasoconstriction and platelet function. Prostag Leukotr Ess 39:221-226.

Carlson, S.E., Rhodes, P.G., Rao, V.S., Goldgar, D.E., 1987. Effect of fish oil supplementation on the n-3 fatty acid content of red blood cell membranes in preterm infants. Pediatr Res 21:507-510.

Chi, M.S., Ray, R.L., Williams, D.C., Tuig, M.V., Galbreath, K., 1997. Effects of dietary fat on blood pressure and plasma lipids in spontaneously hypertensive rats. Nutr Res 19:917-925.

Clark, C., Radostits, O., Petrie, L., Allen, A.,2001. Toxic effects in dairy cattle following the ingestion of a large volume of canola oil. Can Vet J 42:721-723.

CODEX-STANDARD 210-1999 for Named Vegetable Oils. Adopted 1999, revised 2001, 2003 and 2009. http://www.codexalimentarius.net/web/standard_list.do?lang=en.

de Jong, A., Plat, J., Lutjohann, D., Mensink, R.P., 2008. Effects of long-term plant sterol or stanol ester consumption on lipid and lipoprotein metabolism in subjects on statin treatment. Br J Nutr, 2008 Nov, 100(5):937-41. Erratum in: Br J Nutr, 2009 Dec, 102(11):1701-2.

Demonty, I., Ebine, N., Jia, X., Jones, P.J., 2005. Fish oil fatty acid esters of phytosterols alter plasma lipids but not red blood cell Fragilityin hamsters. Lipids Jul 40(7):695-702.

Desci, T., Koletzko, B., 1995. Growth, fatty acid composition of plasma lipid classes, and plasma retinol and a-tocopherol concentrations in full-term infants fed formula enriched with w-6 and w-3 long-chain polyunsaturated fatty acids. Acta Paediatr 84:725-32.

Duthie, I.F., Barlow, S.M., Ashby, R., Tesh, J.M., Whitney, J.C., Sauders, A., Chapman, E., Norum, K., R., Svaar, H., Opstvedt, J., 1989. Feeding of partially hydrogenated fish oils to rats in comparison with partially hydrogenated soybean oil and refined rapeseed oil: a combined chronic oral toxicity and carcinogenicity study combined with in utero phase. Acta Med Scand 726:1-89.

EFSA, (European Food Safety Authority), 2005. Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to rapeseed oil high in unsaponifiable matter as a novel food ingredient (Request N° EFSA-Q-2004-013) (adopted on 6 December 2005) The EFSA Journal 304:1-11.

Erman, F., Suleyman, A., Demir, Y., Akcay, F., Bakan, E., 2006. Determination of saturated and unsaturated fatty acids amount in leukocyte membranes from subjects fed with solid and fluid oils. J Biochem Mol Biol 39:516-521.

ESPGHAN International Expert Group. Koletzko, B., Baker, S., Cleghorn, G., Neto, U.F., Gopalan, S., Hernell, O., Hock, Q.S., Jirapinyo, P., Lonnerdal, B., Pencharz, P., Pzyrembel, H., Ramirez-Mayans, J., Shamir, R., Turck, D., Yamashiro, Y., Zong-Yi, D., 2005. Global Standard for the Composition of infant formula: recommendations of an ESPGHAN Co-Ordinated International Group. J Pediatric GAstroenterol Nutr 41 (5):584-599.

Fanaro, S., Jelinek, J., Stahl, B., Boehm, G., Kock, R., Vigi, V., 2005. Acidic oligosaccharides from pectin hydrolysate as new component for infant formulae: effect on intestinal flora, stool characteristics, and pH. J Pediatr Gastroenterol Nutr 41: 136-90.

Farwer, S.R., Der Boer, B.C.J., Haddeman, E., Kivits, G.A.A., Wiersma, A., Danse, B.H.J.C., 1994. The vitamin E nutritional status of rats fed on diets high in fish oil, linseed oil or sunflower seed oil.Brit J Nutr 72:127-145

FDA, 1985. Food and Drug Administration, Final Rule. Direct Food Substances Affirmed as Generally Recognized as Safe; Low Erucic Acid Rapeseed Oil. Federal Register 50 FR 3745-3756, January 28, 1985.

FDA, (Food and Drug Administration), 2000a. Agency response letter to GRAS Notice GRN 000033. Available at http://www.fda.gov/Food/FoodIngredientsPackaging/GeneralRecognizedasSafeGRAS/GRASListings/ucm154145.html.

FDA (Food and Drug Administration), 2000ab. Agency response letter to GRAS Notice GRN 000048. Available at http://www.cfsan.fda.gov/~rdb/opa-g048.html.

FDA (Food and Drug Administration), 2000c. Agency response letter to GRAS Notice GRN 000048. Available at http://www.cfsan.fda.gov/~rdb/opa-g039.html.

FDA (Food and Drug Administration), 2000d. Agency response letter to GRAS Notice GRN 000053. Available at http://www.cfsan.fda.gov/~rdb/opa-g053.html.

FDA (Food and Drug Administration), 2001. Agency response letter to GRAS Notice GRN 000061. Available at http://www.cfsan.fda.gov/~rdb/opa-g061.html.

FDA (Food and Drug Administration), 2002. Agency response letter to GRAS Notice GRN 000112. Available at http://www.cfsan.fda.gov/~rdb/opa-g112.html.

FDA (Food and Drug Administration), 2006. Agency response letter to GRAS Notice GRN 000181. Available at http://www.cfsan.fda.gov/~rdb/opa-g181.html.

Federal Register, 1985. Low Erucic Acid Rapeseed Oil; Final rule. Federal Register 50:3745-3755.

Food Chemicals Codex, 2004. National Academy Press, Fifth Edition, Washington, D.C.

Fomon, S.J., 1993. Energy intake by normal infants. In Nutrition of Normal Infants. Mosby, St.Louis, MO. p. 104- 111.

Forbes, G.B., Woodruff, C.W., (Eds), 1985. Pediatric Nutrition Handbook. 2nd Ed. Elk Grove Village, IL. AmericanAcademy of Pediatrics.

Forsyth, J.S., Willatts, P., Agostoni, C., Bissenden, J., Casaer, P., Boehm, G., 2003. Long chain polyunsaturated fatty acid supplementation in infant formula and blood pressure in later childhood: follow up of a randomised controlled trial. BMJ 326:953.

[redacted]

Freese, R., 2001. Low erucic rapeseed oil and platelet function. Eur. J. Lipid Sci. Technol 103:483–489

FSANZ (Food Standards Australia New Zealand), 2003. Erucic Acid in Food: A Toxicological Review and Risk Assessment. Technical Report Series No. 21.

Garrido, A., Garrido, F., Guerra, R., Valenzuela, A., 1989. Ingestion of high doses of fish oil increases the susceptibility of cellular membranes to the induction of oxidative stress. Lipids 24:833-835.

Garrido, A., Garate, M., Campos, R., Villa, A., Nieto, S., Valenzuela, A., 1993. Increased susceptibility of cellular membranes to the induction of oxidative stress after ingestion of high doses of fish oil: effect of aging and protective action of dl-a tocopherol supplementation. J Nutr Biochem 4:118-122.

Gibney, M.J., Bolton-Smith, C., 1988. The effect of a dietary supplement of n-3 polyunsaturated fat on platelet lipid composition, platelet function and platelet plasma membrane fluidity in healthy volunteers. Brit J Nutr, 60:5-12.

Gibson, R.A., Makrides, M., Neumafi, M.A., Jeifrey, B., Lien, E., 1998. Effect of lowering the dietary linoleic acid (LA) to alpha-linolenic acid (ALA) ratio through the use of canola oil on visual and growth outcomes of term infants. Proceedings of the Nutrition Society of Australia 22:289.

Giovannini, M., Riva, E., Agostoni, C., 1995. Fatty acids in pediatric nutrition. Pediatr Clin North Am. 42(4):861-877.

Green, T.J., Innis, S.M., 2000. Low erucic acid canola oil does not induce heart triglyceride accumulation in Neonatal pigs fed formula. Lipids 35:607-612.

Grice, H.C., Heggtveit, H.A., 1983. The relevance to humans of myocardial lesions induced in rats by marine and rapeseed oil. In: Kramer, J.K.G., Sauer, F.D., Pigden, W.J. (eds). High and low erucic acid rapeseed oils. Production, usage, chemistry and toxicological examination. Academic Press, Toronto, Canada, 558.

Guardamagna, O., Abello, F., Baracco, V., Federici, G., Bertucci, P., Mozzi, A., Mannucci, L., Gnasso, A., Cortese, C., 2011. Primary hyperlipidemias in children: effect of plant sterol supplementation on plasma lipids and markers of cholesterol synthesis and absorption. Acta Diabetol 2011 Jun, 48(2):127-33.

Harmsen, H.J., Wildeboer-Veloo, A.C., Raangs, G.C., Wagendorp, A.A., Klijn, N., Bindels, J.G., Welling, G.W., 2000. Analysis of intestinal flora development in breastfed and formula-fed infants by using molecular identification and detection methods. J Pediatr Gastroenterol Nutr 30:61-67.

Hayes KC, Pronczuk A, Perlman D., 2004. Nonesterified phytosterols dissolved and recrystallized in oil reduce plasma cholesterol in gerbils and humans. J Nutr. Jun 134(6):1395-9.

Henderson, R.A., Jensen, R.G., Lammi-Keefe, C.J., Ferris, A.M., Dardick, K.R. Effect of fish oil on the fatty acid composition of human milk and maternal and infant erythrocytes. Lipids 27:863-869.

Huang, M., Naito, Y., Watanre, S., Kobayashi, T., Kanai, H., Nagai, H., Okuyama, H., 1996. Effect of rapeseed and dietary oils on the mean survival time of stroke-prone spontaneously hypertensive rats. Biol Pharm Bull 19:554-557.

Innis, S.M., 1991. Essential fatty acids in growth and development. ProgLipid Res 30:39-103.

Innis, S.M., 1993. The colostrum-deprived piglet as a model for study of infant lipid nutrition. J Nutr 123:386-390.

Innis, S.M., Dyer, R.A., 1999. Dietary canola oil alters haematological indices and blood lipids in neonatal piglets fed formula. J Nutr 29:1261-1268.

Institute of Medicine of the National Academies, 2002/2005. Food and Nutrition Board. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids Choleseterol, Protein and Amino Acids. National Academies Press Washington.

Jacobs, N.J., Van Zoeren-Grobben, D., Drejer, G.F., Bindels, J.G., Berger, H.M., 1996. Influence of long chain unsaturated fatty acids in formula feeds on lipidperoxidation and antioxidants in preterm infants. Pediatr Res 40:680-686.

Jensen, C.L., Chen, H., Fraley, J.K., Anderson, R.E., Heird, W.C., 1996. Biochemical effects of dietary linoleic/alpha linolenic acid ratio in term infants. Lipids 31:107-113.

Jensen, C.L., Prager, T.C., Fraley, J.K., Chen, H., Anderson, R.E., Heird, W.C., 1997. Effect of dietary linoleic/alpha linolenic acid ratio on growth and visual function of term infants. J Pediatr 131:200-209.

Jones, P.J., 2007. Ingrestion of Phytosterosl is not Potentially Hazardous. Letters to the Editor: J Nutr 137(11):2485.
Karlshamns, A.B., 2002. Handbook of Vegetable Oils and Fats. ISBN 91-631-2210-3.

Kitts, D., 1996. Toxicity and Safety of Fats and Oils. Bailey's Industrial Oil and Fat Products 1:215-280.

Kennedy, K., Fewtrell, M.S., Morley, R., Abbott, R., Quinlan, P.T., Wells, J.C.K., Bindels, J.G., Lucas, A., 1999. Double-blind, randomized trial of a synthetic triacylglycerol in formula-fed term infants: effect on stool biochemistry, stool characteristics, and bone mineralization. Am J Clin Nutr 70:920-927.

Kitts, D., 1996. Toxicity and Safety of Fats and Oils. Bailey's Industrial Oil and Fat Products 1:215-280.

Knapp, H.R., Reilly, I.A.G., Alessandrini, P., Fitzgerald, G.A., 1986. In vivo indexes of platelet and vascular function during fish oil administration in patients with atherosclerosis. N Engl J Med 314:937-942.

Kramer, J.K., Farnworth, E.R., Johnston, K.M., Wolynetz, M.S., Modler, H.W., Sauer, F.D., 1990. Myocardial changes in newborn piglets fed sow milk or milk-replacer diets containing different levels of erucic acid. Lipids 25:729-737.

Kramer, J.K., Sauer, F.D., Farnworth, E.R., Wolynetz, M.S., Jones, G., Rock, G.A., 1994. Hematological and lipid changes in newborn piglets fed milk-replacer diets containing vegetable oils with different levels of n3 fatty acids. Lipids 29:859-868.

Kramer, J.K., Sauer, F.D., Farnworth, E.R., Stevenson, D., Rock, G.A., 1998. Hematological and lipid changes in newborn piglets fed milk-replacer diets. Lipids 33:1-10.

Leibovitz, B.E., Hu, M., Tappel, A.L., 1990. Lipid peroxidation in rat tissue slices: effect of dietary vitamin E, corn oil-lard and menhaden oil. Lipids 25:125-129.

Life Science Research Office (LSRO) Expert Panel Report. Raiten, D.J., Talbot, J.M., Waters, J.H., (Eds.), 1998. Assessment of nutrient requirements for infant formulas. J Nutr 128:2089S-2107S.

Lu, F.C., 1988. Acceptable daily intake: inception, evolution and application. Regul Toxicol Pharmacol 8:45-60.

Makrides, M., Neumann, M.A., Jeffrey, B., Lien, E.L., Gibson, R.A., 2000. A randomized trial of different ratios of linoleic to α-linolenic acid in the diet of term infants: effect on visual function and growth. Am J Clin Nutr 71:120-129.

Mawatari, S., Ohnishi, Y., Kaji, Y., Maruyama, T., Murakami, K., Tsutsui, K., Fujino, T., 2003. High-cholesterol diets induce changes in lipid composition of rat erythrocyte membrane including decrease in cholesterol, increase in α-tocopherol and changes in fatty acids of phospholipids. Biosci Biotechnol Biochem. 67:1457-1464.

[redacted]

Mellies, M.J., Ishikawa, T.T., Glueck, C.J., Bove, K., Morrison, J., 1976a. Phytosterols in aortic tissue in adults and infants. J Lab Clin Med. 88(6):914-921.

Mellies, M., Glueck, C.J., Sweeney, C., Fallat, R.W., Tsang, R.C., Ishikawa, T.T., 1976b. Plasma and dietary phytosterols in children. Pediatrics 57(1):60-67.

Miyazaki, M., Huang, M., Watanabe, S., Kobayashi, T., Okuyama, H., 1998. Early mortality\ effect of partially hydrogenated vegetable oils in stroke-prone spontaneously hypertensive rats (SHRSP). Nutr Res 18:1049-1056.

Mori, T.A., Beilin, L.J., Burke, V., Morris, J., Ritchie, J., 1997. Interactions between dietary fat, fish, and fish oils and their effects on platelet function in man at risk of cardiovascular disease. Arterioscl Throm Vas 17:279-286.

Muriana, F.J.G., Villar, J., Ruiz-Gutierrez, V., 1997a. Intake of olive oil can modulate the transbilayer of human erythrocyte membrane cholesterol. Cell Mol Life Sci 53:496-500.

Muriana, F.J.G., Ruiz-Gutierrez, V., Guerrero, A., Mantilla, C., Leon-Camacho, M., Villar, J., 1997b. Olive oil normalizes the altered distribution of membrane cholesterol and Na+-Li+ countertransport activity in erythrocyte of hypertensive patients. Nutr Biochem 8:205-210.

Nair, P.P., Turjman, N., Kessie, G., Calkins, B., Goodman, G.T., Davidovitz, H., Nimmagadda, G., 1984. Diet, nutrition intake, and metabolism in populations at high and low risk for colon cancer. Dietary cholesterol, beta-sitosterol, and stigmasterol. Am J Clin Nutr 40(4 Suppl):927-30.

Naito, Y., Kasama, K., Yoshida, H., Ohara, N., 2000a. Thirteen-week dietary intake of rapeseed oil or soybean oil as the only dietary fat in Wistar Kyoto rats - Change in blood pressure. Food Chem Toxicol 38:811-816.

Naito, Y., Konishi, C., Ohara, N., 2000b. Blood coagulation and osmolar tolerance of erythrocytes in stroke-prone spontaneously hypertensive rats given rapeseed oil or soybean oil as the only dietary fat. Toxicol Lett 116:209-215.

Naito, Y., Yoshida, H., Nagata, T., Tanaka, A., Ono, H., Ohara, N., 2000c. Dietary intake of rapeseed oil or soybean oil as the only fat nutrient in spontaneously hypertensive rats and Wistar Kyoto rats - Blood pressure and pathophysiology. Toxicology 146:197-208.

Naito, Y., Nagata, T., Takano, Y., Nagatsu, T.,Ohara, N., 2003. Rapeseed oil ingestion and exacerbation of hypertension-related conditions in stroke prone spontaneously hypertensive rats. Toxicology 187:205-216.

Nutritional Requirements for Children in Helath and Disease Great Ormond Street Hospital for Children NHS Trust, 2000. London, U.K.

Ogawa, H., Yamamoto, K., Kamisako, T., Meguro, T., 2003. Phytosterol additives increase blood pressure and promote stroke onset in salt-loaded stroke-prone spontaneously hypertensive rats. Clin Exp Pharmacol P 30:919-924.

Periago, J.L., Suarez, M.D., Pita, M.L., 1990. Effect of dietary olive oil, corn oil and medium-chain triglycerides on the lipid composition of rat red blood cell membranes. J Nutr 120 :986-994.

Picaud, J.P., Boucher, .P, Lapillone, A., Berthouze, M., Delvin, E., Boehm, G., Claris, O., Laborie, S., Reygroellet, B., Papillonne, H., Clorieux, F.H., Salle, B.L., 2002. Infleunce of dietary cholesterol on vitamin D metabolism in formula fed preterm infants. J Pediatr Gastroenterol Nutr 35:180-184.

Ping, W., Chunrong, W., Jian, Z., Wenxun, F., 1993. Effect of teaseed oil on blood lipids and platelet function in animals. Acta Nutrimenta Sinica 15:377-384.

Raiten, D.J., Talbot, J.M., Waters, J.H., 1(Eds.), 1998. Life Science Research Office (LSRO) Expert Panel Report. Assessment of nutrient requirements for infant formulas. J Nutr 128:2089S-2107S.

Rand, M.L., Hennissen, A.A.H.M., Hornstra, G., 1988. Effects of dietary palm oil on arterial thrombosis, platelet responses and platelet membrane fluidity in rats. Lipids 23:1019-1023.

Ratnayake, W.M.N., Plouffe, L., Hollywood, R., L'Abbe, M.R., Hidiroglou, N., Sarwar, G., Mueller, R., 2000a. Influence of sources of dietary oils on the life-span of stroke-prone spontaneously hypertensive rats. Lipids 35:409-420.

Renwick, A.G., 1990. Acceptable daily intake and the regulation of intense sweeteners. Food Addit Contam 7, 463-75.

Rigo, J., Boehm, G., Georgi, G., Jelinek, J., Nyambugabo, K., Sawatzki, G., Studzinski, F., 2001. An infant formula free of glycomacropeptide prevents hyperthreoninemia in formula-fed preterm infants. J Pediatr Gastroenterol Nutr. 32(2):127-130. Erratum in: J Pediatr Gastroenterol Nutr 32, 620.

Ruiz-Gutierrez, V., Muriana, F.J.G., Guerrero, A., Villar, J., 1997. Olive oil and high-oleic sunflower oil on human plasma and erythrocyte membrane lipids. Nutr Res 17:1391-1399.

Rzehak, P., Koletzko, S., Koletzko, B., Sausenthaler, S., Reinhardt, D., Grübl, A., Bauer, C.P., Krämer, U., Bollrath, C., von Berg, A., Berdel, D., Wichmann, H.E., Heinrich, J., 2011. The GINI Study Group: Growth of infants fed formula rich in canola oil (low erucic acid rapeseed oil). Clinical Nutrition 30:339-345.

Saito S, Takeshita M, Tomonobu K, Kudo N, Shiiba D, Hase T, Tokimitsu I, Yasukawa T., 2006. Dose-dependent cholesterol-loweringeffect of a mayonnaise-type product with a main component of diacylglycerol-containing plant sterol esters. Nutrition Feb 22(2):174-8.

Sauer, F.D., Farnworth, E.R., Belanger, J.M.R., Kramer, J.K.G., Miller, R.B., Yamashiro, S., 1997. Additional vitamin E required in milk-replacer diets that contain canola oil. Nutr Res 17:259-269.

Schroder M, Fricke C, Pilegaard K, Poulsen M, Wester I, Lutjohann D, Mortensen A., 2009. Effect of rapeseed oil-derived Plantsterol and stanol esters on atherosclerosis parameters in cholesterol-challenged heterozygous Watanabe Heritablehyperlipidaemic rabbits. Br J Nutr. Dec 102(12):1740-51.

Svaar, H., 1982. The long term heart lesion phenomenon in animals and humans. In: Barlow, S.M., Stansby, M.E. (eds). Nutritional evaluation of long-chain fatty acids in fish oil. Academic Press, New York, 180-181.

Tammi, A., Rönnemaa, T., Gylling, H., Rask-Nissilä, L., Viikari, J., Tuominen, J., Pulkki, K., Simell, O., 2000. Plant stanol ester margarine lowers serum total and low-density lipoprotein cholesterol concentrations of healthy children: the STRIP project. Special Turku Coronary Risk Factors Intervention Project. J Pediatr 136(4):503-10.

Tatematsu, K., Fuma, S.Y., Nagase, T., Ichikawa, Y., Fujii, Y., Okuyama, H., 2004. Factors other than phytosterols in some vegetable oils affect the survival of SHRSP rats. Food Chem Toxicol 42:1443-1451.

Thomasson, H.J., 1955. The Biological Value of Oils and Fats III. The Longevity of Rats Fed Rapeseed Oil- or Butterfat-containing Diets. J Nutr 57:17-27.

Vicario, I.M., Malkova, D., Luna, E.K., Johnson, I.T., 1998. Olive oil supplementation in healthy adults: effects in cell membrane fatty acid composition and platelet function. Ann Nutr Metab 42:60-169.

Vajreswari, A., Srinivasa Rao, P, Kaplay, S.S., Tulpule, P.G., 1983. Erythrocyte membrane in rats fed high erucic acid-containing mustard oil: osmotic fragility, lipid composition, and (Na^+, K^+)-and (Ca^{2+}, Mg^{2+})-ATPases. Biochem Med 29:74-84.

Vongild, E., Elvevoll, E.O., Brox, J., Olsen, R.L., Barstad, H., Aursand, M., 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.

Von Berg, A., Koletzko, S., Grub,.A., Fillpiak-Pittroff, B., Wichmann, H-.E., Bauer, C.P., Reinhardt, D., Berdel, D., 2003. The effect of hydrolysed cow's milk formula for allergy prevention in the first year of life: The German Infant Nutritional Intervention Study, a randomised double-blind trial. J Allergy Clin Immunol 111: 533-540.

Von Berg, A., Koletzko, S., Filipak-Pittroff, B., Laubereau, B., Grubl A, Wichmann, H-.E., Bauer, C-.P., Reinhardt, D., Berdel, D., 2007. Certain hydrolysed formulas reduce the incidence of Atopic dermatitis but not that of asthma: Three year results of the German Infant Nutritional Intervention Study. J Allergy Clin Immunol 119:718-725.

Winocour, P.D., Kinlough-Rathbone, R.L., Morazain, R., Mustard, J.F., 1987. Effect of the amount and type of dietary fat on platelet function, platelet survival and response to continuous aortic injury in rats. Atherosclerosis 168:221-230.

Wong, W.W., Hachey, D.L., Insull, W., Opekun, A.R., Klein, P.D., 1993. Effect of dietary cholesterol on cholesterol synthesis in breast-fed and formula-fed infants. J Lipid Res 34(8):1403-11.

APPENDIX A

Food Chemicals Codex Specifications on Canola Oil

Pages 517-519 have been removed in accordance with copyright laws. The removed reference is:
2011 The United States Pharmacopeial Convention p. 162-4

APPENDIX B

Summary of Studies of Interest on Canola Oil

1. Toxicology Studies in Animal Models Related to Infants

Sauer et al. (1997) studied whether additional vitamin E is required in milk-replacer diets that contain canola oil when fed to neonatal piglets. A total of 72 piglets were assigned to be sow-fed or to be fed one of 6 milk-replacer diets with different oil mixtures at birth: 1) canola oil (mixture of canola oil and high erucic acid rapeseed (HEAR) oil in a ratio of 98:2); 2) soybean oil; 3) olive oil; 4) high oleic acid sunflower (HOAS) oil; 5) mixture of HOAS, soybean oil and linseed oil in a ratio of 53:31:14; 6) canola oil and coconut oil blend at a ratio of 60:40. In Experiment 1, piglets were either sow-fed or fed either canola oil, soybean oil, olive oil, canola/coconut oil replacer with 16 mg tocopherol (vitamin E)/kg milk powder for 48 days. In Experiment 2, pigs were fed either canola oil or soybean oil replacer with 16 mg tocopherol/kg milk powder or canola oil with 65 mg tocopherol/kg milk powder for 28 days. In Experiment 3, pigs either sow-fed or were fed replacer with either canola oil, soybean oil, HOAS or oil mixture with 93 mg tocopherol/kg milk powder for 28 days. To summarize all 3 experiments, pigs that were fed canola oil milk-replacer diets had low vitamin E levels in their tissues. The vitamin E deficiency manifested acutely, causing death, or mildly, with microscopic evidence of hepatocyte dissociation. In Experiment 1, 5 of the 6 pigs that were fed canola oil replacer diets died before day 28 of the experiment. The post-mortem exam confirmed low vitamin E levels in the liver and heart tissues in this group. When vitamin E was added to the other milk-replacer diets, even in mixtures containing canola oil, the piglets grew well and showed no adverse symptoms. The authors concluded that the increased vitamin E requirements in canola oil supplemented formulas needs additional study.

Kramer et al. (1990) studied the myocardial effects of different levels of erucic acid in milk-replacer diets of newborn piglets from birth to two weeks of age. The growth, histological changes and lipid changes of the heart were compared with sow-reared piglets. In Experiment I, piglets from five litters were sampled at birth and an average weight male and female were sacrificed for histological analysis, while the remaining piglets were returned to the sow. In Experiment II, six litters were selected based on numbers of male and females and two litters were randomly assigned to one of three diets: sow milk, canola oil replacer with 0.8% erucic acid, or high erucic acid rapeseed (HEAR) oil (42.9% erucic acid). In Experiment III, piglets from six litters were used and were fed either replacer with 100% soybean oil or replacer with rapeseed oil (4.7% erucic acid). In Experiment IV, piglets from four litters were fed either sow milk or replacer with rapeseed oil containing 7.0, 11.7 or 20.7% erucic acid. In Experiment V, piglets from three litters were split among groups that were fed replacer with rapeseed oil containing either 2.3 or 11.7% erucic acid. There were no significant differences in growth or nutrient digestibility due to any treatment. At the completion of each experiment, myocardial lipidosis was determined by Oil Red O staining of the heart tissue. Milk-replacer diets that contained soybean oil or rapeseed oil mixtures with up to 5% erucic acid showed trace myocardial lipidosis. Piglets that were fed 7-42.9% erucic acid in rapeseed oil mixtures showed definite lipidosis. The only significant cardiac lipid increase was seen in triacylglycerol (TAG) levels in piglets fed replacer containing rapeseed oil with 42.9% erucic acid. The authors concluded that no adverse nutritional and myocardial effects occur in piglets fed milk-replacer with 25% oil containing 5% erucic acid (a consumption of 750 mg erucic acid/kg body weight/day).

Kramer et al. (1994) studied the effects of linolenic acid (18:3 n-3) from vegetable oils on bleeding times and platelet counts in newborn piglets. The piglets that were fed milk-replacer diets containing various vegetable oils with different levels of 18:3 n-3 were compared to sow-reared piglets for 28 days. At one day of age, the piglets were fed every two hours to equal an intake of solid matter of 7% of body weight/day. The piglets were housed individually, arranged in banks of six and were fed by a common reservoir of milk-replacer. The milk-replacer diets either contained soybean oil, high oleic acid sunflower (HOAS) oil, a mixture of canola oil and high erucic acid rapeseed (HEAR) oil (98:2 ratio), or a mixture of HOAS oil, soybean oil and linseed oil (55:31:14 ratio). All piglets that were fed replacer diets showed normal growth. The bleeding times of the piglets on milk-replacer diets increased significantly with age. Piglets that were fed the canola oil milk-replacer had a significant and consistent delay in the rise of platelet counts. To test whether this effect was due to the erucic acid in canola oil, another study was conducted where piglets were fed milk-replacer that contained a mixture of oils that had a similar composition to canola oil, but without the erucic acid. These piglets showed a similar delay in the rise of platelet counts to piglets fed canola oil. A subsequent experiment showed that adding saturated fatty acids in the form of coconut oil to canola oil (2:3 ratio) resulted in no delay in the rise of platelet counts. Levels of saturated fatty acids in the blood lipids were lower in canola oil-fed and HOAS oil-fed piglets than soybean oil-fed or sow-fed piglets. The authors concluded that milk-replacer diets containing oils rich in 18:3 n-3 do not pose a hematological risk in newborn piglets, and that a platelet decrease can be reversed by the addition of saturated fatty acids.

Kramer et al. (1998) studied the hematological platelet and lipid changes associated with feeding newborn piglets milk-replacer diets containing canola oil until four weeks of age. Piglets were either left with the sow or fed milk-replacer diets containing soybean oil, canola oil (98:2 mixture to give a final oil of 2% erucic acid), a canola oil/cocoa butter mixture (52:4:4:40 of canola oil, HEAR oil, linseed oil, and cocoa butter), or high erucic acid rapeseed (HEAR) oil (54:46 mixture of canola oil and HEAR oil to give a final oil of 20% erucic acid). The canola oil/cocoa butter mixture was included to determine if the long-chain fatty acids in cocoa butter would attenuate the canola oil effect on platelets. The piglets fed milk-replacer diets gained significantly more weight over 4 weeks than piglets fed by the sow. Platelet counts were decreased and platelet size was increased in piglets fed the canola-oil replacer compared to the sow-reared group. After four weeks, the bleeding times were significantly higher in the HEAR oil and canola oil groups. The additions of cocoa butter to canola oil enriched the long-chain fatty acids 16:0 and 18:0. The group that was fed replacer containing cocoa butter mixed with canola oil had a significantly higher platelet count than canola oil replacer alone. The HEAR-oil fed group had significantly lower platelet counts throughout the four-week study. The authors reported that the observed effects on platelets may be related to fatty acid composition changes in platelet membrane lipids. In the canola oil and HEAR oil replacer groups, 24:1 n-9 and 24:1 n-9/24:0 ratios in platelet sphingomyelin were significantly increased. The authors concluded that milk-replacer containing canola oil results in significant hematological changes.

Innis and Dyer (1999) investigated the effects of canola oil on growth, blood lipids and platelet characteristics in piglets. The following treatment formulas were comprised of 51% energy from fat: 1) canola oil alone (100% canola oil; 0.5% erucic acid [22:1n-9]); 2) canola oil blend (26% canola oil , 16% sunflower oil, 42 % palm oil, 4 % high-oleic sunflower oil, and 12% coconut oil); 3) canola oil mimic (29% soybean oil , 59% high-oleic sunflower oil and 12% flax oil); 4) soybean oil alone (100% soy oil); 4) soybean oil blend (26% soy, 48% palm oil, 14% high-oleic sunflower oil, and 12% coconut oil). Male piglets were assigned to one of the five treatment groups, 10 piglets each, and were bottle-fed from birth until day 10 (4 piglets/group) or day 18 (6 piglets/group). Blood samples were drawn from the vena cava at 5, 10 and 15 days of age. There were no differences among the groups at any age in body weight, formula intake or liver weight. The heart weight of piglets fed 100% canola oil formula was significantly higher than piglets fed the canola oil mimic formula. The authors attribute this difference to a non-significant but proportional body weight difference between these two groups. Piglets fed formula with 100% or blended canola oil had lower platelet counts than piglets fed soybean oil or canola oil mimic formulas. Platelet counts were lower, while platelet volume and distribution were higher in the groups fed 100% canola oil or soybean oil formulas compared to the blended oil formulas. In addition, piglets fed formula with 100% canola oil or the canola oil blend had significantly higher red blood cell counts and hematocrit at 5 days of age than piglets fed formula with 100% soybean oils. However, at 18 days the red blood cell count was lower in the group fed 100% canola oil compared to the group fed 100% soybean oil. In piglets fed canola oil compared to soybean oil, there were no significant differences in total or HDL cholesterol or phospholipids, but the levels of free cholesterol were significantly higher in piglets fed canola oil compared to soybean oil at 10 and 18 days of age. The authors concluded that canola oil as a source of formula fat affects the normal developmental increase of platelets and the developing hematological system in piglets.

Green and Innis (2000) investigated the effect of low erucic acid canola oil on the heart triglyceride accumulation in neonatal pigs. The following treatment formulas were comprised of 50% energy from fat: 1) canola oil alone (100% canola oil; 0.5% erucic acid [22:1 n-9]); 2) canola oil blend (26% canola oil , 16% sunflower oil, 42 % palm oil, 4% high-oleic sunflower oil, and 12% coconut oil); 3) canola oil mimic (29% soybean oil, 59% high-oleic sunflower oil, and 12% flax oil); 4) soybean oil alone (100% soy oil); 4) soybean oil blend (26% soy, 48% palm oil, 14% high-oleic sunflower oil, and 12% coconut oil). The canola oil mimic was designed to give a similar composition as canola oil, but without the inclusion of erucic acid. Formulas with soybean oil were used for comparison due to this oil being currently used as a source of unsaturated fatty acids in many infant formulas and the canola oil and soybean oil blend were used to resemble infant formulas that include C16:0 and 18:1 at levels similar to those in human breast milk. Male piglets were assigned to one of 5 formula groups and were bottle-fed from birth until day 10 (4-6 piglets/group) or 18 (6 piglets/group), after which they were sacrificed for heart biochemical and histological analysis. There were no differences in formula intake, body weight, liver weight or kidney weight among the groups. The mean heart weight of the piglets fed canola oil (100%) formula was higher at 18 days than those fed the canola oil mimic formula. There were no differences in heart triglyceride, phospholipid and cholesterol concentrations among the groups at 10 or 18 days of age. The authors reported a modest accumulation of erucic acid linked with feeding canola oil formula that was not associated with heart triglyceride accumulation at 10 or 18 days.

Silva et al. (2006) investigated the effects of four is energetic diets of differing fat composition on blood lipid profile and adiposity in young rats. Diets containing partially hydrogenated vegetable oil (6%), palm oil (5%), canola oil (6%), and soy oil (7%) were fed to lactating rats during the 21 days of lactation, and then fed to young Wistar male rats following weaning until the 45th day of life. The oil content for all oils was adjusted 7% by addition of soy oil. *In vivo* lipogenesis rate, lipid content, relative level of FA,and the activity of lipoprotein lipase enzyme were measured in adipose tissue. Body weight of partially hydrogenated vegetable oil and palm oil groups was significantly higher compared to canola oil and soy oil from day 14 of lactation to day 45, despite the lower food intake in the partially hydrogenated vegetable oil group. Partially hydrogenated vegetable oil and palm oil groups revealed higher lipogenesis rate and lipid content in the adipose tissue compared canola oil and soy oil groups. Similarly carcass fat content was significantly higher in partially hydrogenated vegetable oil and palm oil groups than in canola oil and soy oil groups. The results of this study show that the kind of fatty acid in the dietary lipid offered early in life can affect lipid metabolism and adiposity. No biologically significant differences between canola oil and soy oil fed groups were noted.

2. Clinical Studies in Infants

Studies with Canola Oil

Jensen et al. (1996) studied the effects of four ratios of linoleic acid (LA) to α-linolenic acid (LNA) in infant formula on the fatty acid patterns of plasma and erythrocyte phospholipid fractions and plasma lipid fractions in term infants. Eighty infants (gestational age 39.6 ± 1.7 weeks) were randomly assigned to receive formula that differed in LNA content from birth to 120 days of age. The four formulas contained 16% of fat as LA and either 0.4% LNA in formula 1, 0.95% LNA in formula 2, 1.7 % LNA in formula 3, or 3.2% LNA in formula 4 (LA:LNA of 44, 18, 10 and 5, respectively). The oils included in each formula were as follows: Formula 1: 3% canola oil, 15% safflower oil, 26% high-oleic sunflower oil, 30% palm stearin oil, 26% coconut oil; Formula 2: 10% canola oil, 13% safflower oil, 22% high-oleic sunflower oil, 30% palm stearin oil, 25% coconut oil; Formula 3: 20% canola oil, 10% safflower oil, 15% high-oleic sunflower oil, 30% palm stearin oil, 25% coconut oil; Formula 4: 40% canola oil, 5% safflower oil, 30% palm stearin oil, 25% coconut oil. Blood samples were collected shortly after birth and at 21, 60 and 120 days of age. There were no differences in growth parameters or formula intake among the four groups throughout the study. The content of n-3 fatty acids was higher in both plasma fractions in infants that were fed the formula with the highest level of LNA. The AA content of plasma lipids was higher in infants that received the lowest LNA formula, which was only half of what has been reported in breastfed infants. At 120 days of age, the infants that were fed formula with the highest LNA content had the highest DHA levels in erythrocyte phospholipid fraction. In this fraction, the AA content did not differ at any time. The authors concluded that the LA:LNA ratios in these four formulas do not result in DHA and AA levels that are comparable to breastfed infants, but it is clear that the ratio of LA:LNA plays an important role in achieving these levels.

Billeaud et al. (1997) studied the effects of infant formula supplementation with α-linolenic acid on plasma and red blood cell fatty acids on premature infants. Thirty-one infants (gestational age ~32 weeks) received formula that was supplemented with high α-linolenic acid (from rapeseed oil in an18:2n-6/18:3 n-3 ratio near 6/1). Thirty-two infants (gestational age ~32 weeks) received formula that was supplemented with a low α-linolenic acid (18:2 n-6/18:3 n-3 = 22/1). A third group of 25 infants that received only human milk was included in the study. At day 2, 15 and week 37 measurements of crown-heel length, head circumference and body weight was taken. A blood sample was drawn at these times to determine total plasma cholesterol, triglyceride, cholesterol ester, phospholipid and red blood cell phosphotidylethanolamine levels. In all three groups, weight, length and head circumference were the same throughout the study. The high α-linolenic acid group demonstrated biological values and DHA levels that were comparable to those of the human milk-fed group. The authors indicated that the effects of supplementation with high α-linolenic acid from low erucic acid rapeseed (LEAR) oil are more beneficial than low α-linolenic acid supplementation in formula.

Jensen et al. (1997) studied the effects of different levels of linoleic acid (LA) to α-linolenic acid (ALA) on the growth and visual function of full-term infants. Eighty infants (gestational age 39.6 ± 1.7 weeks) were randomly assigned to receive one of four formulas that differed in ALA content from birth to four months of age. The formulas contained 16% of total fatty acids as LA and either 0.4% in formula 1, 1.0% in formula 2, 1.7% in formula 3 or 3.2% in formula 4 of fatty acids as ALA (a LA:ALA ratio of 44, 18.2, 9.7 and 4.8, respectively). The fatty acid pattern of plasma phospholipids was determined after birth and at 21, 60 and 120 days of age. Growth measurements were taken at these same times as well as at 240 days of age. Transient visual evoked responses (VER) were measured at 120 and 240 days of age. Growth and VER were also measured in a group of 19 breastfed

infants at 120 and 240 days of age. The mean weight of infants that were fed formula with the 3.2% ALA was 760 grams less at 120 days of age than the mean weight of infants that were fed formula with 0.4% ALA. In addition, the group that was fed formula with 3.2% ALA had higher plasma concentrations of DHA and lower concentrations of AA at 21, 60 and 120 days of age. There were no differences in VER among formula groups or the breastfed group. The authors suggested that the lower LA:ALA ratios in infant formulas may affect infant growth, and should not be adopted until this effect is studied further. It was postulated that the canola oil or other impurity that is used to lower the LA:ALA ratio may be responsible for this growth effect.

Kennedy et al. (1999) studied the effects of a synthetic triacylglycerol in formula fed infants on stool characteristics and bone mineralization. One hundred and three healthy full-term infants were selected to receive either standard formula, and 100 infants were selected to receive formula containing 50% *sn*-2 palmitate for 12 weeks. Both formulas were supplied by Nutricia (Cuijk, Netherlands) and contained 20% canola oil.[17] A reference group of 100 breastfed infants was included. At 6 and 12 weeks of the study, radial and whole-body bone mineral content and stool frequency, volume, consistency and fatty acid content were measured. The infants that received the *sn*-2 palmitate formula had higher whole-body bone mineral content, softer stools and a lower level of stool fatty acids than the infants that received the standard formula. The breastfed infants demonstrated similar results to the infants that received *sn*-2 palmitate formula. The authors concluded that these similarities warrant further investigation.

Makrides et al. (2000) & Gibson et al (1998) compared the effects of infant formula that contained either a 10:1 or 5:1 ratio of linoleic (LA) to α-linolenic acid (ALA) on the fatty acid status, growth and visual evoked potential (VEP) of term infants. Thirty-six infants were fed formula containing a 10:1 ratio of LA:ALA while 37 infants were fed formula with a 5:1 ratio from birth to 34 weeks of age. The 10:1 formula included 35% oleo, 27% coconut oil, 23% soy oil, and 15% high-oleic safflower oil (percentage of total fatty acids in formula). The 5:1 formula included 35% palm olein, 25% coconut oil, 29% canola oil, and 11% soy oil. An additional reference group of breastfed infants (number not reported) was included in the study. At birth, 6, 16 and 34 weeks of age, all infants were assessed for weight, length, and head circumference, and a blood sample was taken to determine plasma and erythrocyte phospholipid fatty acid concentrations. Visual acuity was measured at 16 and 34 weeks of age. The infants in the 5:1 formula group were heavier, longer and had a greater head circumference than the infants in the 10:1 group from birth, and this pattern continued throughout the study. The rate of growth was not different between the formula groups, but was higher than the breastfed group between 16 and 34 weeks of age. The infants in the 5:1 formula group had higher plasma and erythrocyte phospholipid concentrations of DHA than infants in the 10:1 formula group. However, the DHA concentrations in the 5:1 group were less than those in the breastfed group. The VEP was not significantly different between the formula fed infants and the breastfed infants throughout the study. The authors concluded that the moderate increase in DHA concentrations in the 5:1 formula fed group was not associated with growth rate and VEP acuity.

Von Berg et al. (2003) studied the effect of hydrolyzed cow's milk formula for allergy prevention during the first year of life in infants. Between the years 1995 and 1998, 2285 infants with a hereditary disposition for allergic disease were included in the German Infant Nutrition Intervention Study and randomly assigned to one of 4 formulas: 1) cow's milk formula (CMF), 2) partially hydrolyzed whey formula, 3) extensively hydrolyzed whey formula, and 4) extensively hydrolyzed casein formula (eHF-C). The standard cow's milk formula and extensively hydrolyzed whey formula both contained 25 % fat blend as canola oil.[18] Mothers were advised to attempt exclusive breast-feeding for at least 4 months, and the timing of weaning and formula introduction were left up to the mother. The study formulas were provided until the infants were at least 6 months old. After six months, mothers were advised to feed no more than one solid food type per week and to avoid milk and dairy products. The infants were examined for allergic manifestations at 1, 4, 8 and 12 months of age. Allergic manifestations were defined as atopic dermatitis (AD), gastrointestinal manifestation of allergy, allergic uticaria or a combination of these factors. At the conclusion of 12 months, 945 infants had been exposed to one of the study formulas. The researchers found that using eHF-C formula compared with CMF significantly reduced the incidence of allergic manifestations. The incidence of atopic dermatitis was significantly reduced by the eHF-c or partially hydrolyzed whey formula. The authors concluded that dietary intervention with a hydrolyzed formula may play a role in preventing allergic diseases in the first year of life.

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17 Personal communication, Danone.
18 Personal communication, Danone.

Bakker-Zierikzee et al. (2005) studied how the addition of the prebiotics, galacto- and fructo-oligosaccharides (GOS/FOS,) or the probiotic, *Bifidobacterium animalis* (Bb-12), to infant formula affected the intestinal microflora of infants during the first four months of life. Nineteen infants that received standard, non-supplemented formula were compared with 19 infants that received standard formula supplemented with 6 g/L of GOS/FOS (prebiotic) and 19 infants that received the standard formula containing 6.0 x 10^{10} viable Bb-12 cells/L (probiotic). The standard formula used in this study was Nutrilon I™ (Nutricia, Zoetermeer, The Netherlands), which contains up to 20% canola oil.[19] An additional group of 63 breastfed infants was also used for comparison. Fecal samples were taken at postnatal days 5, 10, 28 and once every 4 weeks thereafter. Compared with infants fed standard formula and Bb-12 formula, the infants that were fed GOS/FOS formula had a higher fecal acetate ratio, lactate concentration, and pH after 16 weeks. The metabolic activity of the intestinal flora in infants that were fed GOS/FOS formula was most similar to that of breastfed infants. The metabolic activity of the intestinal flora in infants that were fed the Bb-12 formula was most similar to that of infants fed standard formula.

Bouwstra et al. (2005) studied the effects of long-chain polyunsaturated fatty acid (LC-PUFA) supplementation in infant formula for two months on the neurological development outcome at 18 months. Full term infants were randomly divided into three feeding groups for two months: 169 infants were fed a control, standard formula; 146 infants were fed a LC-PUFA supplemented formula; and 159 infants were breastfed. The standard formula and the base formula for the LC-PUFA group was Nutrilon Premium™ (Nutricia, Zoetermeer, The Netherlands), which has a canola oil content of up to 20%.[20] This was a continuation of a study where the authors showed that LC-PUFA supplementation results in a beneficial neurological effect at 3 months of age. In the present study, the authors wanted to address the same indices of neurological development at 18 months of age---the Hempel and Bayley scales. The Hempel assessment results in a clinical neurological diagnosis, while the Bayley scale is an analysis of mental and psychomotor development. The groups did not show significant differences in neurological condition or psychomotor and mental development indices. The authors concluded that there were no effects of feeding on neurological condition at 18 months of age.

Bakker-Zierikzee et al. (2006) studied the effects of the prebiotic galacto-oligosaccharides (GOS)/fructo-oligosaccharides (FOS) and the probiotic *Bifidobacterium animalis* (Bb-12) in infant formula on fecal secretory immunoglobulin A (SIgA) secretion. The role of SIgA in the gastrointestinal tract is to increase the neutralization and clearance of viruses, and SIgA is transferred to infants from the maternal breast-milk. The addition of a probiotic or probiotic to infant formula may support the endogenous production of SIgA in formula-fed infants. At birth, 19 infants were assigned to receive standard infant formula, 19 infants were assigned the standard formula containing 0.6 grams of GOS/FOS per 100 milliliters of formula, and 19 infants were assigned standard formula containing 6 x 109 colony forming units of Bb-12 per 100 milliliters of formula for 32 weeks. The standard formula used was Nutrilon I™ (Nutricia, Zoetermeer, The Netherlands), which contains up to 20% canola oil.[21] A group of 63 breastfed infants were used for comparison. Fecal samples were taken on day 5, 10 and week 4 after birth to determine the SIgA levels by enzyme linked immunosorbent assay (ELISA). The SIgA concentrations were highest in the breast fed infants compared to the standard formula fed infants throughout the study. With the infants that were fed the GOS/FOS formula, the fecal SIgA concentrations tended to be higher compared to the infants fed the standard formula. There were no differences between the group that was fed the Bb-12 formula and the standard formula. The authors concluded that infants may benefit from the addition of GOS/FOS to formula because of increased fecal SIgA secretion.

Von Berg et al. (2007) investigated the allergy preventive effect of three hydrolyzed infant formulas compared with cow's milk formula fed for the first 4 months of life until 3 years of age. This study was part of the German Infant Nutritional Intervention study between 1995 and 1998, which followed the development of atopic manifestations in 2252 infants with single or biparental heredity for atopy. These infants were allocated to a group that received cow's milk formula, partially hydrolyzed whey formula, extensively hydrolyzed whey formula or extensively hydrolyzed casein formula for the first 4 months of life. The standard cow's milk formula and extensively hydrolyzed whey formula both contained 25% fat blend as canola oil.[22]The current study included 889 breastfed infants and 904 per protocol (PP) infants who were compliant feeding recommendations. Another group of 1363 intention-to-treat

[19] Personal communication, Danone.
[20] Personal communication, Danone.
[21] Personal communication, Danone.
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ITT) infants (which was made of all randomized infants) was also analyzed, The main outcomes that were studied were allergic manifestations, atopic dermatitis (AD) and asthma. There was a significant reduction in the incidence of AD in the ITT and PP infants that were fed the extensively hydrolyzed casein formula. None of the formulas reduced the incidence of asthma. The authors concluded that the incidence of AD, but not asthma, can be reduced with certain cow's milk hydrolyzates.

3. Other Clinical Studies of Interest in Infants

Henderson et al. (1992) investigated the effect of fish oil on the fatty acid composition of human milk and the erythrocytes of mothers and their infants. Five lactating women, 2-5 weeks postpartum, were given 6 grams of fish oil every day for 21 days. Supplementation increased dietary levels to 3,092 mg of total n-3 fatty acids (FA) and 2,006 mg of very long-chain n-3 FA. All infants were solely breastfed throughout the study. Milk samples were collected immediately prior to ingestion of fish oil capsules each day. On days 1, 7, 14 and 21, samples were also collected at 4, 8, 12 and 16 hours post-supplementation. Maternal and infant blood was collected on days 1 and 21. Milk n-3 FA content increased within 8 hours and reached steady state levels within one week. The n-6 fatty acid content decreased. Erythrocyte eicosapentaenoic (EPA) and DHA content increased in mothers and in infants. There was no significant change in DHA or n-6 fatty acid content. There were no significant changes in maternal or infant tocopherol or platelet aggregation responses. The authors noted that the most significant finding of the study was that maternal fish oil supplementation increased the n-3 fatty acid and decreased the n-6 fatty acid content of milk.

Boehm et al. (1995) investigated the fecal cholesterol excretion in very low birth weight infants (< 1501 grams) that were fed either a low or high cholesterol formula compared with low birth weight infants that were fed fortified breast milk. Forty-four infants were admitted to the study in Leipzig, Germany to determine the fecal cholesterol effects of the formulas, and 29 infants were studied in Milan, Italy to determine the serum cholesterol effects of the formulas. After the second week of life and full enteral feeding was established, 18 infants from Leipzig and 9 from Milan received fortified breast milk with a mean cholesterol content of 15.3 mg/dL. The additional infants (number of infants unclear) were fed either a high cholesterol formula with 30 mg of cholesterol/dL and a modified lipid composition or a standard formula with 5.5 mg cholesterol/dl. The fat blend of the standard formula was a mixture of vegetable oils and cow's milk fat. The fat blend of the high cholesterol formula was vegetable oil blend, cow's milk fat and egg yolk lipids (to give a fatty acid composition similar to breast milk). The canola oil content of these formulas was not reported. In the Leipzig study, a 3-day stool collection was performed after a 10-day feeding period. In the Milan study, blood samples were taken after a 14-day feeding period. The fecal cholesterol excretion was significantly higher in the group that was fed the high cholesterol formula compared to the low cholesterol formula and the human milk-fed groups. The serum cholesterol concentrations were similar between the high cholesterol-fed infants and the human milk-fed infants, but were significantly higher than the group fed the low cholesterol formula. The authors suggested that preterm infants are able to regulate a higher cholesterol intake by increasing fecal cholesterol excretion.

Jacobs et al. (1996) studied the effect of long chain unsaturated fatty acids (LPC) supplementation in infant formula on lipid peroxidation and antioxidants in preterm infants during the first six weeks of life. Thirty-five preterm infants (gestational age 30-35 weeks) were divided in the following feeding groups: 13 infants received formula supplemented with *n*-3 LCP, 15 infants received standard formula and 7 infants were fed breast milk. The standard preterm formula was Nenatal® (Nutricia BV, Zoetermeer, The Netherlands). The canola oil content of Nenatal® was not reported. In the LCP formula, the linoleic acid was lowered and replaced by *n*-3 LCP from fish oil and oleic acid. Blood samples were collected on days 1, 3, 7, 14, 21 and 42 after birth in the formula groups and on days 1, 21 and 42 in the breast milk group. There were no abnormalities in weight gain, length and head circumference in any of the groups. In the group that was supplemented with LCP, erythrocyte DHA levels remained stable, while in the unsupplemented group, these levels declined. The supplemented group also had a reduced susceptibility to peroxide-induced oxidative stress compared to the unsupplemented group. There were no differences between the formula groups in any other parameters of lipid peroxidation. The authors suggested that healthy infants may be able to cope with any peroxidative stress produced by LCP supplementation.

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Harmsen et al. (2000) compared the intestinal flora of breastfed infants with that of formula fed infants by using novel molecular identification methods. It has been previously shown that the intestinal flora of breastfed and formula fed infants differ, and the authors of the present study used techniques such as fluorescent *in situ* hybridization (FISH) with a molecular 16S rRNA based technique to verify those results. Six breastfed and six formula fed infants were included in the study, and fecal samples were

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collected at 4, 5, 7, 8, 12 and 20 days of life. The formula that was used in the study was a whey-predominant formula called Nutrilon Premium (Nutricia, Zoetermeer, The Netherlands). The novel techniques were indeed able to demonstrate the intestinal flora differences between the infants. For example, the breastfed infants the bifidobacteria levels were dominant while in formula fed infants similar amounts of bifidobacteria and *Bacteroides* were found. The techniques were also used to differentiate between the minor components of the intestinal flora in both groups.

Rigo et al. (2001) studied how an infant formula that is free of glycomacropeptide effects hyperthreoninemia in formula fed preterm infants. Hyperthreoninemia is a well-known occurrence in infants that are fed whey-protein predominant formula. Sweet whey formulas are commonly used infant formulas that contain whey proteins and threonine-rich glycomacropeptides (GMP). The authors hypothesize that these GMP may be responsible for increased plasma threonine levels, resulting in hyperthreoninemia, which may affect brain development because of its possible influence on glycinergic neurotransmission. Unlike sweet whey, acid whey does not contain GMP. In the present study, 14 preterm infants (about 34 weeks gestational age) were fed either formula that was sweet whey based or a formula that was acid whey (GMP-free) based for a feeding period of seven days. At the end of the feeding period, the nutrition of each infant was switched to the opposite formula for an additional feeding period of seven days. The canola oil content of the formulas was not reported. At the end of each feeding period, the concentrations of threonine, creatinine, urea and amino acids were measured in the plasma and the concentrations of creatinine and amino acids were measured in the urine. In the plasma, the threonine levels were significantly lower in the group that was fed the GMP-free formula than in the group that was fed the sweet-whey formula. In the GMP-free formula group, the renal excretion of threonine was lower while the excretion of lysine, histidine and glycine was higher. The creatinine levels were not influenced by diet. The authors did not discuss how the cross-over (switching nutrition after a feeding period) design affected the infants. The authors concluded that feeding an acid whey based formula reduces the hyperthreoninemia that is commonly found in formula fed preterm infants.

Boehm et al. (2002) tested whether the addition of oligosaccharide to a standard bovine milk formula increased the fecal *bifidobacteria* counts in preterm infants. The test formula was supplemented with 90% galacto-oligosaccharide and 10% fructo-oligosaccharide at a concentration of 10 grams/liter to resemble the composition of human milk. The supplemented formula was given to 15 preterm infants and the results were compared to 15 infants that were fed formula supplemented with maltodextrin as a placebo. The canola oil content of the formulas was not reported. A reference group of 12 infants that were fed fortified human milk was included in the study. The gestational age of all of the infants in the study was about 31 weeks. Fecal flora, stool characteristics and side effects were observed on days 1, 7, 14 and 28. Day 1 was defined as the first full day of formula feeding. During the study, the number of fecal *bifidobacteria* of the group that was fed oligosaccharide supplemented formula was in the upper range of the number that was observed in the human-milk fed reference group, and the difference between the supplemented and unsupplemented group was highly significant ($p = 0.0008$). The stool frequency in the supplemented group resembled that of the human-milk fed reference group, while the stool frequency of the unsupplemented formula group was significantly lower. The different diets had no effect on the occurrence of side effects such as vomiting and crying, and there was no difference in weight gain or length gain between the groups. The authors concluded that the supplementation of formula with oligosaccharides may help establish balanced intestinal flora in preterm infants.

Carlson et al. (1987) studied the effect of fish oil supplementation on the n-3 fatty acid content of red blood cell membrane in preterm, very low birth weight infants. Preterm infants tend to have significantly reduced DHA in red blood cell membranes following delivery unless they are fed human milk. The purpose of this study was to determine if a dietary source of DHA (MaxEPA, R. P. Scherer Corporation, USA) could prevent this decline in formula fed preterm infants. Nineteen unsupplemented infants and 20 supplemented infants were observed for 4 weeks. The infants ranged in age from 10 to 53 days at the time of enrollment. Red blood cell DHA was measured bi-weekly during the study, and was found to decrease without supplementary DHA. However, the infants that were supplemented with MaxEPA maintained the same DHA levels as at the time of enrollment. The authors concluded that the pattern of red blood cell phospholipid fatty acids in the infants that were supplemented with MaxEPA was the same as preterm infants that were fed human milk.

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Picaud et al. (2002) investigated the effect of dietary cholesterol on vitamin D metabolism in formula fed preterm infants. Thirty infants (10 per group) were randomly assigned to a formula that contained cholesterol levels that were lower, similar to or higher than human milk. The formulas therefore contained <0.03 g/L, 0.15 g/L or 0.30 g/L cholesterol, respectively. The canola oil content of the formulas was not reported. All infants were around 29 weeks gestational age at the start of the study and were fed the

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formulas until 40 weeks gestational age. Growth, serum hydroxy-vitamin D and 1,25-dihydroxy-vitamin D concentrations were measured at the beginning and end of the study. Serum cholesterol and lymphocyte 3-hydroxy-3-methylglutaryl coenzyme A reductase mRNA was also measured in a subgroup of 14 infants. There were no differences in any of these parameters between the three groups. The authors concluded that there were no disadvantageous effects of formula with a cholesterol content similar to or higher than human milk, but the long-term effects require additional study.

Boehm et al. (2003) tested whether the prebiotic effect of human milk oligosaccharides can be duplicated by the addition of galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS) to formula in preterm infants. In the experimental formula, a mixture of 90% GOS and 10% FOS was added to a standard preterm formula. This mixture closely resembles the molecular mass of the neutral human milk oligosaccharide fraction. In a control group, a similar quantity of maltodextrins was added to formula as a placebo. The canola oil content of the formulas was not reported. At a gestational age of approximately 31 weeks, 15 infants received the control formula, 12 infants received the GOS/FOS formula while 15 infants that were fed human milk were used as a reference. Microbiological analysis of the stool was performed at birth and days 7, 14 and 28 after the start of supplementation. After 28 days, the group that was fed GOS/FOS formula had a fecal *bifidobacteria* number that was in the upper range of the reference group, while the group that was fed the control formula had a *bifidobacteria* number that fell in the lower range of the reference group. The characteristics of the stool in the GOS/FOS group, such as frequency and consistency, resembled that of the human milk-fed reference group as well. The authors concluded that the addition of prebiotic mixtures may help in improving tolerance to formula feeding in preterm infants.

Forsyth et al. (2003) studied how infant formula supplemented with long chain polyunsaturated fatty acids (LCPUFAs) influenced blood pressure in later childhood. This study was a follow-up to a 1992 multicentered trial in Europe where full-term infants were fed either a standard formula or a formula that was supplemented with 0.3-0.4 grams of AA and 0.15 to 0.25 grams of DHA (per 100 grams of fat). The present study included 65 children that had been fed LCPUFA supplemented formula, 71 children that had been fed standard formula and a reference group of 83 breastfed children. The LCPUFA source was egg yolk. The canola oil content of the formulas was not reported. At a mean age of 70.1 months, the systolic, diastolic and mean blood pressure was measured in all of the children. The group that had been fed LCPUFA supplemented formula had a significantly lower mean and diastolic blood pressure than the non-supplemented group. Systolic blood pressure was also non-significantly lower. The diastolic pressure of the breastfed children did not differ from that of the children fed LCPUFA formula. The authors concluded that supplementation with LCPUFA during infancy may be associated with lower blood pressure and decreased cardiovascular risk during adulthood.

Fanaro et al. (2005) tested the effects of three oligosaccharide components in formula to mimic the composition of human milk on the intestinal flora of full-term infants. A group of 16 infants received a formula that was supplemented with 0.2 g/dL of acidic oligosaccharides derived from citrus pectin, which mimics the concentration of acidic oligosaccharides in human milk. A second group of 15 infants received formula with 0.2g/dL of acidic oligosaccharides in combination of 0.6g/dL of galacto-oligosaccharide (GOS)/fructo-oligosaccharide (FOS) mixture, which mimics the total concentration of oligosaccharides as well as the ratio between neutral and acidic oligosaccharides in human milk. A control group of 15 infants were fed a formula that was supplemented with 0.8g/dL maltodextrin as a placebo. The canola oil content of the formulas was not reported. The study duration was from day 1 (when formula feeding was established) to six weeks. At these time points, fecal samples were collected. In the group that received acidic oligosaccharides and GOS/FOS, the fecal *bifidobacteria* and *lactobacilli* counts were significantly higher than the other two formula groups. In addition, the stool frequency was increased in this group compared to the control group. There were no differences in growth, vomiting and regurgitation frequency between the groups. The authors concluded that the supplementation with acidic oligosaccharides did not affect intestinal flora because the observed effect is what has been previously seen in a group that received GOS/FOS supplemented formula alone.

Fianese et al. (2008) investigated whether a short period of total parenteral nutrition (TNP) in newborns would increase phytosterol levels in plasma and induce their accumulation in the red blood cell membranes (RBCM). Phytosterols, cholesterol, and other sterol levels were quantified by gas chromatography-mass spectrometry in 15 healthy control infants, 22 patients after TPN, and 11 patients before TPN. Sterols of lipid emulsions were quantified. All of the patients studied received 75% of energy from parenteral nutrition according to standard protocols based on parenteral nutrition according to standard protocols based on age-related nutrient requirements. The lipid component of TPN was provided through a lipid emulsion based on soybean oil. A significant increase in plasma and RBCM phytosterol levels were noted compared to control as well as before and after TNP. The days of TPN

and the total amount of infused lipids correlated significantly with RBCM phytosterol. Correlations for plasma were positive but not significant. No correlation was observed with plasma bilirubin, gamma-glutamyltransferase, or alanine transaminase. Higher phytosterol levels were observed in some patients that could have been due to their individual variability in phytosterol metabolism and/or clearance. The investigator suggested that a greater accumulation of phytosterols in membranes may induce TPN-related cholestasis. It should be noted that in this study the route of administration was intravenous.

4 Toxicology Studies Related to Cardiotoxicity Since 1985

The only toxicology studies found for cardiotoxicity conducted since 1985 were done in the Wistar Kyoto (WKY) rat including various substrains that are spontaneously hypertensive. Many researchers believe that the spontaneously hypertensive rat (SHR) is a good model for genetic hypertension because it develops heart failure due to aging, much like humans (Bing et al., 2002). The SHR develops hypertension and cardiac hypertrophy during the first quarter of life. The SHR develops hypertension at around two months of age, which tends to remain stable until about 18 months of age, when impaired function is observed.

The SHR has been a useful model to study the mechanisms that contribute to heart failure, as well as potential preventions and treatments. Several studies are summarized below that used the SHR as a model to determine how dietary fatty acids affect these animals that already have a tendency to display impaired cardiac function. The spontaneously hypertensive rat (SHR) can also be used to study other cardiovascular diseases such as stroke.

Many studies have been done to investigate the effect of lipids in these models. Studies conducted since 1985 on canola oil and its phytosterols are summarized below. However, many findings in these rats are contrary to experience in humans. For instance, SHR rats fed canola or corn oils have shorter life spans in comparison with animals fed soybean oil. Olive oil shortens survival to a greater extent of any other oil (Ratanayake et al., 2000a, b). Canola phytosterols also shorten survival and some of the studies reviewed in this section further studied the effects of canola phytosterols in these rat models. As discussed above vegetable oils contain basically the same phytosterols only in varying amounts. However, phytosterol content cannot be the entire answer observed for the effects of vegetable oils because olive oil has much lower phytosterol content than other oils tested.

Huang et al. (1996) studied the effects of rapeseed and other dietary oils on the survival time of stroke-prone spontaneously hypertensive (SHR-SP) rats. The animals (age and number not reported) were fed one of 8 diets: a conventional diet that was supplemented with 5 or 10% of either canola oil, soybean oil, microbial oil or perilla oil. Four experiments were performed but were problematic because the authors reported that the diets had varying water contents and speculated that some deaths were due to a failure to properly control the housing temperature. Despite these limiting conditions, the authors report that the rapeseed oil group had a significantly shorter survival time in all four experiments. The relative mean survival times were 50-59% (rapeseed oil group), 78-106% (soybean oil group) and 86% (microbial oil group) as compared with the group fed perilla oil (100%). Histological and physiological observations on rats revealed paralysis of the limbs in 2 out of 16 rats fed rapeseed oil and thrombosis-related vacuole formation in 2 out of 6 rats in the rapeseed oil group. The authors concluded that rapeseed oil may contain one or more factors that are toxic to SHR-SP rats.

Miyazaki et al. (1998) investigated how partially hydrogenated vegetable oils affected mortality in stoke-prone spontaneously hypertensive (SHR-SP) rats. Male rats (seven animals each) were weaned at 4 weeks of age to a conventional diet supplemented with 10% (w/w) rapeseed oil, soybean oil, partially hydrogenated rapeseed oil or partially hydrogenated soybean oil for 11 weeks. The animals also received a 1% sodium chloride in water drinking solution. Both partially hydrogenated rapeseed and soybean oils as well as rapeseed oil shortened the survival time of SHR-SP rats by 40% as compared with soybean oil. No significant differences were observed in the plasma free cholesterol, total cholesterol, total triacylglycerol, total phospholipids, aortic total cholesterol or aortic phospholipids among the three dietary groups. There were also no differences in blood pressure due to any treatment. Only soybean oil and partially hydrogenated soybean oil affected the fatty acid profiles of plasma and aortic lipids in a similar manner. The authors concluded that it will be important to identify the reason certain oils shorten the life span of the SHR-SP rat.

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Maito et al. (2000a) studied the effect on the blood pressure in WKY rats of a 13-week diet that contained either 10% canola oil or soybean oil as the only dietary fat source. Twenty male WKY rats (5 weeks old) were divided into two groups and fed diets that

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contained 10% canola oil or 10% soybean oil. Blood pressure (from 5 animals from each group), water intake and food consumption were measured weekly. At week 13, urine samples were collected to determine electrolyte levels. After the 13-week feeding, blood samples were taken from the inferior vena cava vein. There were no differences between the groups in water intake, food consumption and body weights. The blood pressures in the canola oil group were significantly higher than those in the soybean oil group from week 5 until the conclusion of the experiment. The canola oil group also showed a decreased density of platelets and a higher density of neutrophils compared to the soybean group. Plasma levels of glucose and potassium decreased, and total cholesterol, free cholesterol, triglycerides and phospholipids increased in the canola oil group compared with those in soybean oil group. There were no differences in urine volume or electrolyte excretion between the two groups. After sacrifice, liver examination showed depressed activities of catalase and superoxide dismutase in the hepatic cytosol in the canola oil group. The authors concluded that further studies are needed to understand the meaning of these changes.

Naito et al. (2000b) studied the blood coagulation and osmolar tolerance of erythrocytes in stroke-prone, hypertensive rats given rapeseed (canola oil) or soybean oil as the only dietary fat. Twenty male stroke-prone, spontaneously hypertensive rats (7 weeks old) were divided into two groups and fed diets that contained 10% canola oil or 10% soybean oil as the only fat source for four weeks. After four weeks, blood samples were taken from the inferior vena caval vein for analysis. The authors found that in the canola oil group, the activated partial thromboplastin time was significantly shorter than in the soybean oil group. In addition, the erythrocytes in the canola oil group were less tolerant to low osmotic pressure than the soybean oil group. There were no differences in plasma calcium, platelet density and platelet aggregation between the two groups. The authors concluded that the coagulation time and erythrocyte fragility in the canola oil group may contribute to the cause of strokes in these rats.

Naito et al. (2000c) studied the effect of the dietary intake of canola oil or soybean oil as the only fat source on the blood pressure and pathophysiology of Wistar Kyoto (WKY) rats and spontaneously hypertensive rats (SHR). Animals of each strain were divided into two groups of ten animals at five weeks of age. The animals were either fed a diet of 10% canola oil or 10% soybean oil as the only fat source for 26 weeks. Drinking water containing 1% sodium chloride (NaCl) was given to the animals, which has shown to elevate blood pressure. Food consumption, water intake and blood pressure were measured once a week. At 26 weeks, urine samples were taken for electrolyte analysis. At the end of the 26th week, blood samples were taken for the inferior vena cava vein. The thoracic aorta was isolated from the animals whose blood pressure was measured, and one aortic ring of 3 mm in length was prepared. The preparation was constricted by $3x10^{-6}$ M phenylephrine and dilation responses induced by acetylcholine (10^{-9}~10^{-5}M) and nitroprusside (10^{-5} M) were examined. Isolated, perfused mesenteric arteries were also prepared, and the effect of veratridine (a sodium channel activator) was examined in the mesenteric arteries of other animals than those for which blood pressure had been measured. The gross observation and urinalysis results show that there were no differences in food intake, water intake or electrolyte balance between the groups. In both strains, systolic blood pressures in the canola oil group were higher than the soybean oil group from 10 weeks until the completion of the study. Platelet counts were decreased in the canola oil group in both strains compared to the soybean group, and neutrophil counts were increased in the canola oil group compared to the soybean oil group in SHR only. In the canola oil group of SHR, the plasma glucose and potassium levels decreased. In both strains, the total cholesterol, free cholesterol, triglyceride, high density lipoprotein and phospholipid levels were higher in the plasma of the canola oil group compared to the soybean oil group. Gross necropsy showed that the heart weight was higher in the canola oil group in the SHR. In the WKY rats, the kidney weights were higher in that canola oil group compared to the soybean oil group. Acetylcholine- and nitroprusside-induced dilating responses of isolated aortic rings and norepinephrine- and veratridine-induced increases in vascular tone of isolated perfused mesenteric arteries were not different between the two groups in each strain. These results demonstrate that canola oil elevates blood pressure of the rat provided with drinking water containing 1% NaCl through mechanisms other than blunt dilating response of the blood vessel due to dysfunction of the endothelium or vascular smooth muscle, the augmented response to norepinephrine in the arteries, and the increased amount of norepinephrine in the sympathetic nerve endings. The authors conclude further studies are needed to illustrate the mechanism of these changes.

Ratnayake et al. (2000a) studied how dietary oils affected the life span of the stroke-prone spontaneously hypertensive (SHR-SP) rat. One hundred and eighty four SHR-SP rats, at about 47 days old, were split into 8 dietary groups of 23 rats each. The diets were standard rat chow (control) or had the addition of one of the following: 10% canola oil, high-palmitic canola oil, low sulfur canola oil, soybean oil, high-oleic safflower oil, a fat blend that mimicked canola oil or a fat blend that was high in saturated fatty acids. A 1% sodium chloride solution was used as drinking water to induce hypertension. After a dietary period of 37 days, 5 rats from each group were sacrificed for blood and tissue samples, while the remaining animals were studied for their life span. The rats

that were fed non-canola oil diets lived longer than those on canola oil diets by a mean of 6-13 days. There was no difference in survival time between rats fed canola oil and rats fed low sulfur canola oil, suggesting that the sulfur content in canola oil has no effect on survival time. The high-palmitic canola oil group lived slightly longer than the other two canola oil groups. There were no differences in blood pressure or hematological indices between the groups. The campesterol, sitosterol and phytosterol levels in the liver and brain of the animals fed the canola oil diets were highest. The presence of these sterols in the diet, liver and brain of these animals showed a strong inverse relationship to survival time. The authors concluded that the high content of phytosterols may contribute to the life shortening mechanism in SHR-SP rats. In another study, Ratnayke et al. (2000b) reported that the high concentration of phytosterols in CA and the addition of phytosterols to other fats make the cell membrane more rigid, which might be a factor contributing to the shortened life span of SHRSP rats. In this study, although phytosterols, including brassicasterol were present all oils that were fed to rats, these sterols were barely detectable in erythrocytes of all of the dietary groups.

Naito et al. (2003) studied the effect of ingested rapeseed oil on hypertension related symptoms in stroke-prone spontaneously hypertensive rats (SHR-SP). Forty male SHRSP (5 weeks old) were divided into two equal groups and fed a diet containing either 10% canola oil or 10% soybean oil as the only fat source for their entire life span. The erucic acid content of the canola oil diet was < 4%. The animals were also provided with drinking water that contained 1% sodium chloride (NaCl) to accelerate the progress of hypertension. The parameters measured were life span and onset of stroke symptoms. An additional 4-week study was conducted with 20 SHR-SP (5 weeks old), divided into the two above feeding groups, but without the 1% NaCl in the drinking water. Among the parameters measured in this experiment were blood pressure, sterol measurement, and sodium and potassium ATPase activity. In the lifespan study, the life span of the canola oil group was an average of 6 days shorter than the soybean oil group. Although the onset of stroke symptoms was observed in both groups, the onset was earlier in the canola oil group than the soybean group. There were no differences in the incidence of cerebral hemorrhage or lesion type between the groups. In the second experiment, the systolic blood pressure in the canola oil group was higher than that of the soybean oil group at the conclusion of the 4 weeks. In the canola oil group, the levels of campesterol and β-sitosterol were found to be higher in both plasma and erythrocyte membranes than in the soybean oil group. The activity of the sodium and potassium ATPase was elevated in the brain, heart and kidney of the canola oil group. The authors concluded that the changes in the 4-week study may be the underlying mechanisms for the shortened life span in canola oil group in the life span study.

Ogawa et al. (2003) investigated possible effects of phytosterol additives on blood pressure and stroke onset in spontaneously hypertensive rats. Four groups of 8 male SHR-SP were provided with one of the following diets at 6 weeks of age: 1) 10% canola oil; 2) 10% soybean oil; 3) soybean oil plus 0.6% phytosterol (SO + 0.06P diet); or 4) soybean oil plus 4.5% phytosterol (SO + 0.45P diet). All animals had access to drinking water containing 1% sodium chloride to accelerate the progress of hypertension. The duration of the study was the entire life span of the animals. Blood pressures were measured on the 24th day of the study. All animals were observed for the onset of stroke and were examined following death. In addition, to characterize the intestinal phytosterol absorption, the intestinal mRNA expression of Abcg5 and Abcg8 (ATP binding cassette transporters G5 and G8), which are involved in the selective transport of dietary sterols in the intestine were examined. For this aspect of the study, SHR-SP, Wistar Kyoto and Wistar rats that were fed the soybean oil diet and plain water for two weeks were evaluated at 7 weeks. During the rat life span study, systolic blood pressure in the SO + 0.45P and the SO + 0.06P groups increased compared to the soybean oil group. The survival time after the onset of stroke was shortest in the canola oil group and the onset of stroke time was shortest in the SO + 0.45P group. The SO + 0.45P group and the canola oil group had the shortest life spans overall. In the mRNA experiment, there was a significant decrease in the intestinal expression of Abcg5 and Abcg8 in SHRSP and Wistar Kyoto rats compared to Wistar rats. The authors conclude that this may result in an accumulation of phytosterol in the intestines and may be an explanation for the early onset of stroke in these animals. In the life span study, the authors concluded that the addition of phytosterol to soybean oil in the diet elevated the systolic blood pressure and promoted early onset of stroke. However, a five-fold greater level of phytosterol in soybean oil was required to produce an effect equivalent to that seen with canola oil.

Tatematsu et al. (2004) investigated the effect of dietary canola oil and soybean oil (and particularly their phytosterols) fed to rat dams on the growth and survival of pups. Female SHR-SP at 4 weeks of age were randomly divided into two groups of 9-12 rats each. The two groups either received a basal diet with the addition of soybean oil or canola oil at a ratio of 9:1 (basal diet: fat additive or 10%). At 11 weeks of age, the rats were mated with male SHR-SP. Four weeks after parturition, the resulting pups were divided into 2 groups: half received the same diet as their mothers (Can→Can and Soy→Soy groups) and the other half received the other diet (Can→Soy and Soy→Can groups). The growth rate of the pups that were from the canola oil fed dams

was severely reduced and there were also fewer pups per dam in the canola oil fed group. The survival rate of the male pups only was monitored, and was found to be significantly lower in the Can→Can group than in the Soy→Can group. The survival rate of the pups was also found to be significantly lower in the Can→Soy group than in the Soy→Soy group. The fatty acid and sterol composition of the dams' serum and milk lipids were analyzed. The serum lipids of the canola oil group consisted of significantly more octadecenoic and less linoleic acid than those of the soy group. The proportions of EPA and DHA were greater in the canola oil group than in the soybean oil group. The composition of the diets in these studies was well characterized. Neither the canola oil nor soybean oil used contained measurable erucic acid. As typically occurs, the canola oil was richer in linoleic acid than the soybean oil and the soybean oil was richer in linolenic acid than canola oil. The canola oil contained 1.7 times more total phytosterol than the soybean oil (1.63 µmol/g diet vs 0.95 µmol/g). Canola oil contained low levels of brassicasterol (0.18 µmol/g diet) while none was detected in the soybean oil. The canola oil contained about 3 times more campesterol than the soybean oil (1.28 µmol/g diet vs 0.95 µmol/g). In addition, the proportion of phytosterols in the total sterol fraction and the phytosterol/cholesterol ratio was greater in the canola oil diet than in the soybean oil diet. The authors discussed the fact that SHR and the parent WKY strain are more susceptible to phytosterolemia than other strains of rats. They also noted that the beneficial effects of canola oil in the Lyon Diet Heart Study, where canola oil and olive oil were found to be effective in the prevention of coronary heart diseases. The authors pointed out that there is little epidemiological evidence to suggest that rapeseed oil is detrimental to human health. Therefore, the authors concluded that, although canola oil is detrimental to SHR-SP rats, there is no evidence that these findings are related to humans.

Chi et al. (1997) studied the effect of dietary fat on blood pressure and plasma lipids in SHR. Four-week-old male rats were divided into 5 dietary groups of 11 rats each for 12 weeks. The major fat source in diet 1 was beef tallow (source of saturated fatty acids), in diet 2 was olive oil (source of n-9 monounsaturated fatty acids), in diet 3 was corn oil (source of n-6 polyunsaturated fatty acids), and in diet 4 was fish oil (source of n-3 polyunsaturated fatty acids). Diet 5 contained the same amounts of EPA and DHA and similar amounts of other fatty acids as in diet 4. Systolic blood pressure was taken before the experiment and on weeks 4, 8, 10 and 12. At the end of the 12th week, rats were fasted overnight, and blood samples were taken by heart puncture. The increases in blood pressure of SHR rats fed the diet containing fish oil were significantly decreased compared to the diets containing beef tallow or corn oil. The reduction of blood pressure was due to n-3 fatty acids in fish oil since the rats fed diet 5 exhibited similar reduction in blood pressure. Plasma triglycerides, cholesterol and HDL cholesterol were also significantly reduced in rats fed the diets containing fish oil or n-3 ethyl esters. The authors concluded that dietary n-3 fatty acids from fish oil positively affect hypertension and plasma lipids. Although this study did not examine the effects of canola oil, it is still a relevant analysis on the effect of specific fatty acids on physiological status in rats.

In a 26-week study, Ohara et al. (2008) investigated effects of dietary intake of canola oil and soybean oil on plasma lipid levels, G6PD activity andcyclooxygenase-2 (COX-2) expression in spontaneously hypertensive rats. The rats were fed for 26 weeks on a chow containing either, 10 % of corn oil, or soybean oil (control). Elevated plasma lipids and G6PD activation in the liver and erythrocyte were found in SHR fed canola oil compared to soybean oil, while anti-oxidative enzymes other than G6PD were not activated. The canola oil feeding caused significant vascular lesions in the kidney, in which abundant COX-2 positive foci were immunochemically located in the juxtaglomerular apparatus. The results of this study suggest that dietary canola oil induces a hyperlipidemic condition, in which G6PD may serve as an NADPH provider, and aggravates genetic diseases in SHR (also, probably, in SHRSP). The results obtained in the rats may not be directly applicable to humans, because the dynamics and metabolism of lipids in the liver may differ between rodents and other species, including humans.

In another 13-week study, Ohara et al. (2008) investigated whether fatty acid composition unique to canola oil causes the adverse effect. Canola oil or an interesterified canola oil mimic (ICOM) consisting of safflower oil, flaxseed oil and erucic acid was fed as a dietary fat for 13 weeks to Wistar Kyoto (WKY) rats, and clinical and pathological signs were compared. WKY rats were used to avoid the difficulty in evaluating the results in SHRSP due to irregular deterioration in conditions by stroke. Compared to a standard diet, both diets containing canola oil or ICOM similarly elevated blood pressure, increased plasma lipids, activated hepatic glucose-6-phosphatedehydrogenase, decreased platelets, shortened blood coagulation times and induced abnormalities in the kidney. The results of this study indicate that canola oil specific fatty acid composition affect the pathophysiology of the rat and produce consequent aggravation of pathological status, especially in SHRSP. However, the existence of causative factors other than fatty acids was suggested by increased neutrophil count exclusively induced by CO.

5. Role of Lipids in Blood Cell Membrane Structure & Function

It is evident that there is a significant relationship between dietary fatty acid intake and hemostatic physiology. Hemostasis controls the fluidity of the blood, an imbalance of which contributes to atherosclerosis, thrombosis, and eventually coronary heart disease. Blood fluidity is in part regulated by platelets and the blood coagulation system. Dietary fatty acids can modulate platelet function by modifying the phospholipid fatty acid composition of platelet membranes. Phospholipid fatty acid composition may have effects on the properties of the cell membranes, which can cause changes in receptor activities and intracellular signaling (Freese, 2001). Several studies on the role of dietary lipids in blood cell and platelet membrane structure and function in rats and humans are summarized below.

Mawatari et al. (2003) studied how high dietary cholesterol affected erythrocyte membrane lipids in rats. Sixteen male Wistar rats, 10 weeks old, were fed a basal diet for 10 days. After the ten days, 8 of the rats remained on the basal diet while 8 received a basal diet that was supplemented with 0.5% cholesterol and 0.15% sodium cholate for six weeks. In an additional experiment, 18 rats (10 weeks old) were fed a basal diet for 7 days and were then divided into 3 groups of 6 rats each. One group remained on the basal diet, the second group received 0.5% cholesterol supplemented diet, and the third group received 0.5% cholesterol and 0.15% sodium cholate for two weeks. After the experiment duration, blood samples were taken from all animals to examine erythrocyte membranes. There were not many significant differences between feeding the rats the various diets for two weeks and six weeks. Diets containing 0.5% cholesterol 0.15% sodium cholate induced a decrease in the erythrocyte membrane cholesterol, an increase in α-tocopherol and changes in the fatty acid phospholipids. The two-week diet that was supplemented with cholesterol alone also induced these changes, which indicates that it is the cholesterol addition that is responsible, not the sodium cholate. The authors concluded that a high cholesterol diet does in fact influence erythrocyte membrane composition.

Erman et al. (2006) investigated how the consumption of different types of oil affected the fatty acid composition of leukocyte membranes in humans. All subjects were males between the ages of 20 and 40. They ate three similar meals each day with a total daily calorie intake of 1,800-2,000 KCal (carbohydrate 40%, proteins 20%). The study duration was four months, and the oil intake by each participant totaled 40 g of fat per day. Fifteen participants were categorized as butter (2.5 mg/g cholesterol), 15 were categorized as fluid (Olive oil brand name: Taris), 15 were categorized as margarine (brand names: Sana Yag, Yayla Yag, and Evin Yag) and 15 were categorized as mixed types (fluid+ cow butter + margarines) of oil user. Venous blood samples were taken after duration of the study to compare leukocyte membrane fatty acid composition. Arachidic, dihomo-γ-linolenic and palmitoleic acids were more prominent in the butter and mixed oil-user groups. Eicosadienoic, eicosamonoenoic, dihomo-γ-linolenic and behenic acids were more prominent in the fluid oil group, while heptanoic, valeric, eicosadienoic and linolenic acids were more prominent in the margarine group. The authors concluded that the consumption of different oils does affect the fatty acid membrane composition of leukocytes.

Allman et al. (1995) studied the effects of dietary sunflower seed oil and flaxseed oil on the platelet composition and function in healthy men. Eleven subjects (~ 22 years of age) were recruited to consume either 40 g of flaxseed oil (5 subjects) or sunflower seed oil (6 subjects) for 23 days. When the oils were added to the diet, the subjects' fat consumption did not exceed 40% of total energy. Fasting blood samples were collected at the beginning and end of the study for analysis of platelet aggregation and platelet fatty acid composition. The platelet eicosapentaenoic acid (EPA) more than doubled in the flaxseed oil group but remained unaffected in the sunflower seed group. The EPA:arachadonic acid (AA) ratio increased in this group, considered a marker for thrombaxane production, which increases the potential for platelet aggregation. The aggregation response of the platelets was induced by using 0.75 and 2 μg/ml of the collagen. The aggregation response in the platelets from the flaxseed group was reduced. The authors concluded that flaxseed oil, which is rich in α-linolenic acid, has the tendency to decrease the aggregation potential of platelets.

Ruiz-Gutierrez et al. (1997) studied the effects of dietary olive oil (OO) and high oleic sunflower oil (HOSO) on human plasma and erythrocyte membrane lipids. Twelve females, aged 54±3 years were fed OO or HOSO as the sole source of monounsaturated fatty acids (MUFA) over two 4-week periods, with a 4-week wash out period between the two diets. Venous blood was obtained before and after each phase of the study. Plasma low-density lipoprotein (LDL) cholesterol decreased by 9 and 6% after OO andHOSO diets, respectively. There was a significant increase of 18% after OO diet and 12% after HOSO diet in plasma high-

density lipoprotein (HDL) cholesterol. Free cholesterol decreased by 20% and esterified cholesterol decreased by 41% in the erythrocyte membrane after OO diet, but did not after HOSO diet. Erythrocyte phospholipids were also increased by 10% after OO diet after following the OO diet, which decreased the molar ratio of cholesterol to phospholipids in the membrane. The authors concluded that even though the MUFA content in OO and HOSO is similar, dietary OO may be more beneficial in reducing the atherogenic risk profile in humans. Therefore, these benefits may not be due to the MUFA content alone.

Berra et al. (1998) compared the effects of a diet with normal or high oleic acid sunflower oil (HOSO) on serum lipids and the lipid composition of red blood cell membranes in humans. Nineteen subjects (age 50-70) that showed angiogenic signs of artheriopathy and moderate signs of dyslipidemia were used for the six-month study. Ten subjects received vitamin E enriched HOSO containing 83% oleic acid, and 9 subjects received standard sunflower oil (SSO). Red blood cell membrane lipid composition was determined at the beginning and end of the study and after 3 and 6 months. In the HOSO group, a significant increase in high density lipoprotein (HDL) cholesterol was observed after three and six months. In the red blood cell membranes, the phospholipid and cholesterol content decreased in the HOSO group. In addition, the linoleic and AA decreased significantly in the HOSO group. The authors concluded that red blood cell membranes are influenced by dietary fatty acids.

Vicario et al. (1998) supplemented the diets of healthy adults with olive oil to study the effect on membrane fatty acid content and platelet function. Ten male and female subjects, aged 29-47, were given a daily supplement of 30 g of olive oil for six weeks. Fasting blood and cheek cell samples were taken before the study, after 21 and 42 days of supplementation and 30 days after finishing the supplement. Cheek cell samples were analyzed because they are a good indicator of short-term dietary changes due to their rapid turnover. Oleic acid (C18:1 n-9) was significantly increased in platelet and cheek cell phospholipids. No significant change in C18:1 n-9 was found in erythrocytes. There was a nonsignificant decrease in platelet phospholipid AA (20:4 n-6) after the supplementation. C18:1 n-9 did not remain in platelet membranes 30 days after the subjects stopped consuming the olive oil supplement. There were no significant changes in serum lipids throughout and after the study. Platelet aggregation potential, induced by adenosine diphosphate, remained unaffected by olive oil supplementation throughout the study. The authors concluded that olive oil supplementation induces significant changes in platelet and erythrocyte membrane composition without changing serum lipid levels, and further work needs to be done to assess the importance of these changes.

Vongild et al. (1998) investigated the influence of various dietary marine oils and olive oil on the fatty acid composition of serum and platelets as part of an extensive study of the effects of these oils on symptoms associated with cardiovascular/thrombotic diseases. Healthy subjects (124 females and 149 males), aged 16-69 years of age were randomly assigned to consume 15 mL/day of cod (Gadus morhua) liver oil (CLO),olive (Olea europea) oil, refined and unrefined oil from the blubber of minke whale (Balaenoptera acutorostrata), mixtures of CLO and harp seal (Phoca groenlandica) blubber oil (1:1, vol/vol), CLO and olive oil (1:1, vol/vol), and no oil (control group) for 12 weeks. Blood samples were taken before the start of the study and 8 and 12 weeks after supplementation. In the CLO, seal oil/CLO, and whale oil groups, serum levels of eicosapentaenoic acid (EPA) were increased. In platelets, EPA was increased in the CLO, seal/CLO, and olive oil/CLO groups. No significant differences in triacylglycerols (TG), total cholesterol, or high-density lipoprotein cholesterol were observed. Mean platelet volume increased significantly in both whale oil groups and the CLO/olive oil group. Platelet count was significantly reduced in the refined whale oil group only. Lipopolysaccharide-stimulated blood tended to generate less thromboxane B_2in CLO, CLO/seal, and CLO/olive groups. Thromboxane B_2 is regarded as a parameter of platelet functionality and there is a nonlinear relationship between thromboxane production and platelet aggregation. The authors concluded that the intake of various marine oils affect platelet membranes in a favorable, antithrombotic way.

6. Polyunsaturated Fatty Acids and Platelet Function

Many studies have been conducted on the effects of marine oils, which are rich in n-3 polyunsaturated fatty acids, on platelet function. In 1944, Dr. Hugh Sinclair observed that cardiovascular disease was uncommon in Greenland Eskimos compared to mainland Danes and this difference appeared to be due to diet. He noted that the Eskimos consumed a large amount of marine oils high in eicosapentaenoic acid (EPA), while the mainland Danes consumed foods containing linoleic acid. Sinclair also observed that the Eskimos bled excessively from wounds, and proposed that the diet high in polyunsaturated fatty acids (PUFA) was responsible for decreased platelet aggregability, which was a protective effect against thromboembolic problems causing cardiovascular disease (*Marine Oils and Platelet Function in Man*, 1984).

Knapp et al. (1986) further studied this epidemiological comparison by simulating an Eskimo diet by EPA supplementation (10 grams per day) for one month in six patients with peripheral vascular disease and seven healthy control patients. Results showed that this dose of EPA only mildly altered the synthesis of thromboxanes and prostacyclins. Thromboxanes are potent vasoconstrictors and platelet agonists, while prostacyclins are potent vasodilators and platelet inhibitors. From this study, the effect of EPA as an antithrombotic agent is undetermined.

More recent studies in humans demonstrate similarly inconclusive results. Barradas et al. (1990) studied seven healthy adults that ingested 21 grams of olive oil daily for 8 weeks. The results showed that platelet aggregation (in platelet rich plasma and in whole blood) decreased. In addition, thromboxane A2 release, a substance released by platelets that cause blood clotting and vessel constriction was reduced after supplementation. In another study, the relationship between dietary fat and fish oil on platelet function in men at risk of cardiovascular disease was performed by Mori et al. (1997). The study involved 120 men between the age of 30 and 60 years, with mildly elevated blood pressure and cholesterol who were randomly allocated to one of 5 high fat or two low fat diets for 12 weeks. The authors found that dietary fatty acids from fish or fish oil had beneficial affects on cardiovascular risk factors, including a reduction in platelet aggregation.

In contrast, a four–week study conducted by Butcher et al. (1990) involved 18 healthy volunteers who consumed very low fat diets (<7% of daily energy) enriched with different sources of long chain fatty acids (PUFA). Three diets provided 500 g/day of fish caught in the tropical waters of Australia (rich in AA and DHA), fish caught in the southern waters of Australia (rich in DHA), or kangaroo meat (rich in linoleic acid and AA). The fourth diet was vegetarian, and contained no PUFA. Results showed that there was no effect on platelet function or bleeding time, and the authors suggest that this may be a result of the short duration of the diet, or relatively low amounts of AA, DHA and EPA in the diets. These findings also support a study where eight healthy male volunteers (aged 22-39 years) supplemented their normal daily diet with 15 grams of encapsulated fish oil (MaxEPA) for a 6 week period (Gibney and Bolton-Smith, 1998). There were no significant effects on platelet function after the study period.

Several animal studies have also been conducted. Ping et al. (1993) studied the effects of teaseed oil, which contains 76% oleic acid, on blood lipids and platelet function in adult rabbits. A standard diet was given with either 5 mL of coconut, teaseed or safflower oil via stomach tube for 8 weeks. Platelet aggregation, thromboxane levels and serum cholesterol were significantly reduced in the teaseed oil group compared to the coconut oil group, but not the safflower oil group. An additional study by the same investigators used Wistar rats that were fed for 9 weeks with semisynthetic diets containing 15% coconut oil, teaseed oil or safflower oil and 1% cholesterol, respectively. Teaseed oil was found to cause a lower level in platelet aggregation and a significant reduction in plasma thromboxane levels. The conclusion from the two studies is that oleic acid could be important in the prevention of cardiovascular disease caused by thromboembolic events. In contrast, Winocour et al. (1987) found that diets containing different types of dietary fat did not affect platelet function in rats. Animals were fed 1.5 or 16% safflower or corn oil or 16% milk fat for 15 weeks played no part in modifying *in vitro* platelet function, platelet survival or the influence of thrombosis in rats. The author reported that safflower oil, corn oil, and milk fat contain approximately 70%, 50% and 3% of linoleic acid, respectively.

Fish oils contain PUFAs that may differ in concentration and composition depending on the species of fish. The PUFAs are very susceptible to peroxidation, and lipid peroxides accumulate in tissues of animals, especially with inadequate vitamin E intake (Bruckner, 1986). Currently, the cardiovascular effects of fatty acids from other types of oils are under extensive study to demonstrate their dietary importance. It is clear that while the n-3 family of fatty acids may be beneficial in decreasing the incidence of thromboembolitic events, further investigation into the specificity of this mechanism is necessary.

7 Polyunsaturated Fatty Acids & Erythrocyte Fragility

Investigating how dietary fats modify the lipid composition of the erythrocyte membrane constitutes another area of research that has attracted much attention. This research has shown that a modified lipid composition in the diet influences membrane characteristics such as osmotic fragility, membrane receptors, and enzyme kinetics (Vajreswari et al., 1983). These membrane abnormalities likely play an important role in the development of cardiovascular disease.

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Membrane phospholipids and cholesterol changes due to dietary fats have been the focus of several studies. Muriana et al. (1997a) studied how a four-week diet rich in olive oil or high-oleic sunflower seed oil (as a good source of monounsaturated fatty acids, MUFA) affected the transbilayer movement of erythrocyte membranes in eight patients with normal cholesterol levels and eight hypercholesterolemic hypertensive patients. The authors concluded that the dietary olive oil was helpful in normalizing the transbilayer movement of erythrocyte membrane cholesterol in both groups. This effect was not seen in the high-oleic sunflower oil diet, and therefore cannot be attributed to MUFA content alone.

Muriana et al. (1997b) conducted an additional study on the effects of olive oil and high-oleic sunflower oil enriched diets on erythrocyte membrane cholesterol distribution and sodium-lithium (Na^+-Li^+) countertransport activity in twelve control subjects and sixteen patients with untreated essential hypertension; the study lasted for four weeks. There is evidence that transbilayer distribution of membrane cholesterol, which may be a marker of hypertension, is strongly correlated with Na^+-Li^+ countertransport. The authors found that the dietary olive oil, but not the high-oleic sunflower seed oil, was helpful in normalizing the impaired distribution of membrane cholesterol and reducing the elevated Na^+-Li^+ countertransport in the erythrocytes of hypertensive patients.

A study by Vajreswari et al. (1983) in rats examined the effect of the prolonged feeding of erucic acid-containing oils on erythrocyte fragility and membrane bound enzyme activity, such as sodium and potassium-ATPase and calcium and magnesium-ATPase. Male and female rats were fed low erucic acid rapeseed (LEAR) and high erucic acid mustard oils (HEAM) for 16 months, while rats fed groundnut oil were used as controls. The erythrocytes from HEAM-fed rats were resistant to hypotonic hemolysis, which was associated with an increase in the cholesterol/phospholipid molar ratio in male rats only. In addition, HEAM fed male rats had increased activity in sodium and potassium-ATPase activity. The authors concluded that high erucic acid oils generate abnormalities in erythrocyte membranes, and these abnormalities may be sex-specific.

An additional study in weanling rats on the effects of dietary olive oil, corn oil and medium-chain triglycerides (MCT) on erythrocyte membrane lipid fluidity was conducted by Periago et al. (1990). Weanling rats were fed for three or five weeks either high fat diets containing olive oil, corn oil or a mixture of MCT with olive oil or corn oil. The results showed that only olive oil feeding induced important changes in the fatty acid composition of the erythrocyte membrane compared to corn oil. More specifically, they demonstrated that olive oil feeding favors the deposition of 20:4 n-6 and long-chain (n-3 PUFA. The cholesterol and phospholipid content, two important determinants of membrane fluidity, were unaffected by this type of diet.

There are many studies on the effects of PUFA on erythrocyte membrane composition. However, more attention is being paid to the influence of other fats, such as olive oil, and other sources of MUFA and MCT. This is due to the evidence that a lower intake of PUFA and a higher intake of monounsaturates result in a reduced incidence of cardiovascular disease (Periago et al., 1990).

8. Vitamin E & Dietary Unsaturated Fatty Acids

Unsaturated fatty acids are prone to oxidization, and supplementation with these fatty acids in infant formulas may influence the antioxidant status of the infant. A high dietary intake of fatty acids may overwhelm the antioxidant defenses, which increases the need for dietary antioxidant supplementation. Vitamin E is an essential, potent antioxidant that is influenced by dietary fatty acid intake. Many countries suggest fixed daily requirements for dietary vitamin E, but also indicate that the vitamin E requirement increases with increasing polyunsaturated fatty acid (PUFA) intake (Farwer et al., 1994). The four studies in rats from 1989-1994 that are summarized below suggest that there is an increased requirement of vitamin E in diets that are high in PUFA. On the other hand, the Desci et al. (1995) study summarized below in full-term infants suggests that when fatty acid supplementation in formula resembles the fatty acid content of human milk, there are no adverse effects on antioxidant levels.

Garrido et al. (1989) studied how the ingestion of fish oil increases the susceptibility of cell membranes to oxidative stress. Twenty male Wistar rats, fed a standard diet received 4 g of sardine oil per kg body weight daily during 7 or 14 days. Five control animals received an equivalent amount of mineral oil. At the end of 14 days, blood samples and livers were taken and erythrocyte and hepatic microsome membranes were examined for lipid peroxidation, assessed as thiobarbituric acid reactive products (TBAR). Feeding of rats with sardine oil during either 7 or 14 days produced an increase in some polyunsaturated fatty acids in erythrocyte membranes and hepatic microsomes. Feeding rats with sardine oil during 7 or 14 days increases the content of EPA and DHA in

the erythrocyte and hepatic microsomal membranes by 2 to 6%. These membranes show increased susceptibility to the induction of oxidative stress, expressed as lipid peroxidation, when they are exposed to Fe^{2+}-ascorbate and to NADPH-Fe^{3+}-ADP, which are inducers of lipid peroxidation in erythrocytes and hepatic microsomes, respectively. The authors concluded that in order to prevent increased susceptibilityto lipid peroxidation, supplementation with larger amounts of antioxidants may be required in diets that contain fish oil.

Leibovitz et al. (1990) studied the effects of dietary vitamin E, menhaden (fish) oil, and corn-oil lard on lipid peroxidation in rats. Male Sprague-Dawley rats were divided into six groups and six rats each and were fed the following diets for five weeks: 10% (w/w) menhaden oil (MO) or a 10% corn oil-lard (COL) mixture (1:1) in diets with 65 IU or 62 IU/kg vitamin E, respectively, or the same diets supplemented with d-α-tocopberyl succinate to a total of 35 and 180 IU vitamin E/kg, respectively. The MO and COL diets contained 3.3% and 3.7% polyunsaturated fatty acids (PUFA), respectively. After the five weeks, slices of liver and heart were used to study lipid peroxidation *in vitro*. As an indication of lipid peroxidation, thiobarbituric acid reactive substances (TBARS) were measured in the medium after incubation at 37°C for 1 hour in the absence (uninduced) and presence of 0.5 mM tert-butyl hydroperoxide (induced). The release of TBARS from slices of heart and liver from rats fed either lipid decreased with increasing levels of dietary vitamin E. At the same level of vitamin E, TBARS release was greater for slices of liver and heart from the MO-fed rats. Of the 16:0-22:6 fatty acids measured in liver from MO-fed rats, 15.4% was n-6 fatty acids and 29.9% was n-3 fatty acids; in liver from COL-fed rats, 37.4% was n-6 fatty acids and 3.7% was n-3 fatty acids. Liver and kidney vitamin E levels were unaffected by dietary lipid. The dietary fish oil increased *in vitro* lipid peroxidation in the heart and liver, and vitamin E supplementation decreased TBARS. The authors concluded that there might be an increased requirement for vitamin E when dietary fish oil supplements are used.

Garrido et al. (1993) studied the effects of high doses of dietary fish oil on the susceptibility of cellular membranes to the induction of oxidative stress in young and aged rats. Male Wistar rats that were either 60 days old (i.e., young) or 18 months old (i.e., aged) were fed a standard diet. One third of the young and aged rats received 10 grams of sardine oil/kg of body weight by intragastric cannula daily. Another third received the fish oil but with a supplementation of dl-α tocopherol (vitamin E, 1 g/kg oil). A third of the animals, fed only the standard diet, were used as a control. After the 14-day study period, blood samples were taken and erythrocyte membranes were examined for lipid peroxidation, protein content and fatty acid content. In both young and aged rats receiving both fish oil and oil with dl-α tocopherol supplementation, there was a decrease in the content of oleic acid (18:1, n-9) and AA (20:4 n-6), and a significant increase in the contents of EPA and DHA. On the other hand, in the aged rats only, the content of linoleic acid (18:2 n-6) decreased. In both the young and aged rats, feeding fish oil drastically reduced membrane tocopherol levels. Supplementation with dl-α tocopherol restored these levels to an extent in both the young and aged rats, but to different degrees. The authors concluded that these results show that ingestion of high doses of fish oil are accompanied by a susceptibility to the induction of oxidative stress in erythrocyte membranes in both young and aged rats. Supplementation of the oil with dl-α tocopherol may prevent this oxidation but only in membranes obtained from young rats.

Farwer et al. (1994) investigated the vitamin E nutritional status of rats fed diets high in fish oil, linseed oil or sunflower seed oil. Twelve groups of eight rats and two control groups of sixteen rats were given semisynthetic diets with 40% energy as fat for a period of 76 days. All diets contained a minimum of 3% energy as linoleic acid and basal levels of D-α- and D-γ-tocopherol. The diets high in polyunsaturated fatty acids (PUFA) were either rich in fish oil (FO; groups 1-4; 10% energy as fish oil PUFA), linseed oil (LN; groups 1-4; 10% energy as α-linolenic acid) or sunflower seed oil (SF; groups 1-4; 10 + 3% energy as linoleic acid). The control groups were given a diet high in monounsaturated fatty acids (MUFA; CO 1; 10 + 13% energy as oleic acid) or a diet with an 'average' linoleic acid content (CO 2; 8.5%energy as linoleic acid). Of each high PUFA diet, three groups were supplemented with graded levels of DL-α-tocopheryl acetate. The animals on the high-PUFA diets were checked for steatitis in the adipose tissue, which is an early symptom of vitamin E deficiency that occurs when diets are rich in fatty acids with three or more double bonds. Steatitis was observed only in the adipose tissue of the FO groups, even in the group with the highest DL-α-tocopheryl acetate supplementation. The α- and γ-tocopherol levels were also examined in the liver and serum. The α-tocopherol levels were found to be positively correlated and liver and serum γ-tocopherol levels negatively correlated with dietary DL-α-tocopheryl acetate. The groups on the FO diets had significantly lower liver and serum tocopherol levels in comparison with the groups on the other high-PUFA diets. With the supplementation scheme used for the FO groups the liver a-tocopherol levels comparable to both control groups were reached, but not in the serum. The authors concluded that dietary PUFA influences the vitamin E status in rats and more work needs to be done to determine the optimal vitamin E status in these diets.

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Decsi et al. (1995) studied how formula enriched with ω-6 and ω-3 long-chain polyunsaturated (LCP) fatty acids affected growth, plasma retinol and α-tocopherol concentrations in full-term infants. Healthy, full-term, infants were enrolled at 5 days of age. Ten infants were assigned to receive either a conventional infant formula (F) based on cow's milk protein and vegetable fats devoid of LCP (Pre-Aptamil, Puch/Salzburg, Austria), and 12 infants received the same formula enriched with egg lipids and evening primrose oil (Pre-Aptamil with Milupan, LCP-F) to achieve both ω-3 and ω-6 LCP levels similar to the contents in human milk. Anthropometric data were obtained and fatty acid (FA) compositions of plasma phospholipids, triglycerides and sterol esters, as well as plasma retinol and α-tocopherol concentrations, were determined at 5 days and 1, 2, 3 and 4 months of age. Gains in weight and increases in length, and head circumference did not differ between the two groups throughout the study period. Plasma FA values did not differ at 5 days of age. Between 1 and 4 months of age, plasma phospholipids of infants fed LCP-F consistently had significantly higher percentages of AA. Plasma retinol and a-tocopherol concentrations did not differ between the two groups throughout the study. The authors concluded that supplementing formula for full-term infants with ω-6 and ω-3 LCP fatty acids in amounts similar to average human milk contents effectively enhances LCP status without adverse effects antioxidant levels.

9. Deleterious Effects of Various Vegetable Oils

Duthie et al. (1988) studied the chronic oral toxicity and carcinogenicity due to the feeding of partially hydrogenated fish oil compared to partially hydrogenated soybean oil and refined rapeseed oil in rats. This study investigated partially hydrogenated fish oil (PHFO) of differing 22:1 levels in comparison with partially hydrogenated soybean oil (PHSBO) and refined rapeseed oil (LEAR) in a rat lifespan study, preceded by a breeding period in which the experimental lipids were fed to male and female parents. Wistar weanling rats, 200 of each sex, were the subjects for the breeding period. A total of 555 weanlings from each parental treatment group were allocated to the five dietary treatments of the lifespan period of the study. Commercially produced PHFO were selected to represent the lower (PHFO-L) and upper (PHFO-U) range of 22 1 contents, 4.3 and 13.8%, respectively. A third test oil was prepared from a 50:50 blend of these (PHFO-M) to provide and intermediary 22:1 level. For the lifespan period of the study, which was terminated after 107 to 110 weeks of treatment, 50 subjects were allocated to each of the PHSBO, PHFO-L and PHFO-U dietary groups, and 50 males to each of the LEAR and PHFO-M groups. The remaining subjects were allocated to subgroups for sacrifice 4 days or 26 weeks after introduction of the lifespan period diets. All decedent and terminated lifespan group subjects were subjected to *post mortem* examination, and 16 organs were weighed. Samples of most organs and tissues were processed, including multiple heart sections. Heart and skeletal muscle samples from subjects terminated after 4 days and 26 weeks were examined histopathologically. Hearts were macroscopically normal in appearance at all three stages of examination. Examination of hearts taken from animals after four days feeding showed the presence of very fine lipid droplets in the PHFO-L, PHFO-M and PHFO-U treatments. Low grade lipidosis was present in hearts from control subjects fed PHSBO and LEAR. After 26 weeks, heart lipid levels in male rats on the PHFO-L and PHFO-M treatments were reduced compared to the four-day findings. Few rats from the fish oil or control treatments had traces of lipid in cardiac cells on termination at the end of the life span period. After the life span period, few of these lesions were found, which were not related to treatment or sex. Blood enzyme studies were not indicative of degenerative heart processes. The authors concluded that this study demonstrates that there are no adverse pathological effects due to the consumption of the partially hydrogenated vegetable and animal oils as a group or in comparison with the control refined vegetable oil LEAR.

Rand et al. (1988) studied the effects of dietary palm oil on arterial thrombosis and platelets in rats. Five-week-old male Wistar rats were fed one of the following three diets for 8 weeks: sunflower seed oil (control); a diet containing 50% sunflower seed oil (which is high in polyunsaturated fatty acids); or a diet containing 50% palm oil (which is high in saturated and monounsaturated fatty acids). After the feeding period, arterial thrombosis tendency, which was measured by the aorta loop technique, tended to be lowered by the palm oil diet and was lowered significantly by the sunflower seed oil diet, compared with the control. Aggregation of platelets in whole blood that was activated with collagen was not altered by palm oil feeding, but was enhanced in the sunflower seed oil group, compared with the control. Thromboxane A_2 (TXA_2), a prostanoid that stimulates platelet aggregation, was decreased by palm oil feeding. Platelet membrane fluidity, which was measured by fluorescence polarization, was not altered in the palm oil group and was significantly increased only by sunflower seed oil feeding compared to the control. The authors concluded that palm oil did not increase arterial thrombosis tendencies and decreased platelet aggregation compared to the sunflower seed oil, which is an interesting result considering that palm oil is high in saturated fatty acids.

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Badawy et al. (1994) compared the effects of dietary high and low erucic acid rapeseed oil on biological and pathological parameters in rats. Five groups of 8 Sprague-Dawley rats (90-95 grams) were fed one of the following diets for 6 weeks: 1) control (10% Corn oil), 2) hydrogenated rapeseed oil (10%), 3) partially hydrogenated rapeseed oil (10%), 4) high erucic acid rapeseed oil (10%), or 5) low erucic acid rapeseed oil (10%). Blood samples, kidney and liver were harvested from each animal at the end of the experiment. The maximum body weight gain over six weeks was from the control group, followed by the low erucic acid rapeseed oil. Animals fed the high erucic rapeseed oil showed a decrease in weight gain compared to the control. The animals that were fed the low erucic rapeseed oil had the maximum feed efficiency ratio followed by the control group, while the animals that were fed hydrogenated rapeseed oil had the lowest ratio. In the blood serum analysis, the partially hydrogenated and hydrogenated rapeseed oil groups had a significant decrease in cholesterol. The triglyceride levels were significantly increased in the group that was fed the low erucic rapeseed oil. Plasma phospholipids were significantly increased in rats fed the partially hydrogenated diet. In the histopatholgical examination of the liver, the rats fed hydrogenated rapeseed oil showed albuminous degeneration with fat globules in the hepatocyte with mononuclear inflammatory cells. The kidney tubules also showed degenerative changes. In the partially hydrogenated rapeseed oil group, the hepatocytes were swollen with increased number of fat globules, and the kidney showed mild degeneration. In the rats fed high erucic acid rapeseed oil, hepatocytes and mononuclear aggregation were observed around blood vessels in the liver. The kidney showed degenerative changes with mononuclear inflammatory cells. In the low erucic acid group, hepatocytes and mononuclear aggregation were seen and the kidney showed mild degenerative changes. From the data, it is clear that the type of dietary oil influences biological and pathological parameters in rats.

Clark et al. (2001) describe the effects of a large volume of canola oil that was accidentally ingested by 9 dairy cattle. On June 15, 2000, 3 Holstein dairy cattle were examined. All cows showed symptoms including anorexia, failure to drink, profuse diarrhea, and a cessation of milk production. The animals were also recumbent for long periods of time. The farmer reported that, in the cattle barn, there was a tank of feed-grade canola oil that was used as an additive in the cows' daily feed. During the night of June 12, the 80 cows in the barn may have released the tank valve, spilling the entire contents (estimated to be 800 liters) onto the barn floor. The farmer did not know how many cows had drunk the oil during the night. Nine cows total were affected. Seven to 8 days after exposure, four cows died and three were necropsied. No single cause of death could be identified. In one cow, acute renal tubular necrosis was seen in the kidney. In another cow, there were several irregular coalescing pale foci within the myocardium of the wall of the left ventricle and the interventricular septum. Of the 5 remaining cattle with clinical signs, 4 ceased lactation and only 1 started lactating again. However, 2 months after the exposure, all were able to support and retain a pregnancy. The authors concluded that canola oil remains a safe component in daily feed in the recommended quantities.

References

Allman, M.A., Pena, M.M., Pang, D., 1995. Supplementation with flaxseed oil versus sunflowerseed oil in healthy young men consuming a low fat diet: effects on platelet composition and function. Eur J Clin Nutr 49,169-178.

Badawy, I.H., Atta, B., Ahmed, W.M., 1994. Biochemical and toxicological studies on the effect of high and low erucic acid rapeseed oil in rats. Die Nahrung 38, 402-411.

Bakker-Zierikzee, A.M., Alles, M.S., Knol, J., Kok, F.J., Tolboom, J.J.M., Bindels, J.G., 2005. Effects of infant formula containing a mixture of galacto- and fructo-oligosaccharides or viable *Bifidobacteriumanimalis* on the intestinal microflora during the first four months of life.Br J Nutr 94, 783-790.

Bakker-Zierikzee, A.M., Tol, E.A., Kroes, H., Alles, M.S., Kok. F.J., Bindels, J.G., 2006. Faecal SIgA secretion in infants fed on pre- or probiotic infant formula. Pediatr Allergy Immunol 17,134-140.Barradas, M.A., Christofides, J.A., Jeremy, J.Y., Mikhailidis, M.D.,

Fry, D.E., Dandona, P., 1990. The effect of olive oil supplementation on human platelet function, serum cholesterol-related variables and plasma fibrinogen concentrations: a pilot study. Nutr Res 10, 403-411.

Barradas, M.A., Christofides, J.A., Jeremy, J.Y., Mikhailidis, M.D., Fry, D.E., Dandona, P., 1990. The effect of olive oil supplementation on human platelet function, serum cholesterol-related variables and plasma fibrinogen concentrations: a pilot study. Nutr Res 10:403-411.

Berra, B., Montorfano, G., Adorni, L., Zoppi, S., Mazzali, P., Rapelli, S., 1998. Serum lipids and lipidic composition of red blood cell membranes after diet with sunflower oil at normal or high content of oleic acid. Rivista Italiana delle Sostanze Grasse 75, 127-132.

Billeaud, C., Bougle, D., Sarda, P., Combe, N., Mazette, S., Babin, F., Entressangles, B., Descomps, B., Nouvelot, A, Mendy, F., 1997. Effects of preterm infant formula supplementation with alpha-linolenic acid with a linoleate/alpha linolenate ratio of 6:1: A multicentric study. Eur J Clin Nutr 51, 520-526.

Bing, O.H.L., Contrad, C.H., Boluyt, M.O., Robinson, M.T., Brooks, W.W., 2002. Studies of prevention, treatment and mechanisms of heart failure in the aging spontaneously hypertensive rat. Heart Fail Rev 7, 71-88.

Boehm, G., Moro, G., Müller, D.M., Müller, H., Minoli, I., 1995. Fecal cholesterol excretion in preterm infants fed breast milk or formula with different cholesterol contents. Acta Paediatr 84(3):240-244.

Boehm, G., Lidestri, M., Casetta, P., Jelinek, J., Negretti, F., Stahl, B., Marini, A., 2002. Supplementation of a bovine milk formula with an oligosaccharide mixture increases counts of fecal bifidobacteria in preterm infants. Arch Dis Childh 86, F178-181.

Boehm, G., Fanaro, S., Jelinek, J, Stahl, B., Marini, A., 2003. Prebiotic concept for infant nutrition. Acta Paediatr Suppl 441, 64-67.

Borg, K., 1975. Physiopathological effects of rapeseed oil: a review. Acta Medica Scandinavica Supplementum 587, 5-13.

Bouwstra, H., Dijck-Brouwer, D.A.J., Boehm, G., Boersma, E.R., Muskiet, F.A.J., Hadders-Algra, M., 2005. Long-chain polyunsaturated fatty acids and neurological developmental outcome at 18 months in healthy term infants. Acta Paediatr 94, 26-32.

Bruckner, G., 1986. Fats, their potential isomers, and platelet function. J Med Technol 3:24-28.

Butcher, L.A., O'Dea, K., Sinclair, A.J., Parkin, J.D., Smith, I.L., Blombery, P., 1990. The effects of very low fat diets enriched with fish or kangaroo meat on cold-induced vasoconstriction and platelet function. Prostag Leukotr Ess 39, 221-226.

Carlson, S.E., Rhodes, P.G., Rao, V.S., Goldgar, D.E., 1987. Effect of fish oil supplementation on the n-3 fatty acid content of red blood cell membranes in preterm infants. Pediatr Res 21 507-510.

Chi, M.S., Ray, R.L., Williams, D.C., Tuig, M.V., Galbreath, K., 1997. Effects of dietary fat on blood pressure and plasma lipids in spontaneously hypertensive rats. Nutr Res 19, 917-925.

Clark, C., Radostits, O., Petrie, L., Allen, A., 2001. Toxic effects in dairy cattle following the ingestion of a large volume of canola oil. Can Vet J 42, 721-723.

Desci, T. and Koletzko, B., 1995. Growth, fatty acid composition of plasma lipid classes, and plasma retinol and a-tocopherol concentrations in full-term infants fed formula enriched with w-6 and w-3 long-chain polyunsaturated fatty acids. Acta Paediatr 84: 725-32.

Duthie, I.F., Barlow, S.M., Ashby, R., Tesh, J.M., Whitney, J.C., Sauders, A., Chapman, E., Norum, K., R., Svaar, H., Opstvedt, J., 1988. Feeding of partially hydrogenated fish oils to rats in comparison with partially hydrogenated soybean oil and refined rapeseed oil: a combined chronic oral toxicity and carcinogenicity study combined with in utero phase. Acta Med Scand 726, 1-89.

Erman, F., Suleyman, A., Demir, Y., Akcay, F., Bakan, E., 2006. Determination of saturated and unsaturated fatty acids amount in leukocyte membranes from subjects fed with solid and fluid oils. J Biochem Mol Biol 39, 516-521.

Fanaro, S., Jelinek, J., Stahl, B., Boehm, G., Kock, R., Vigi, V., 2005. Acidic oligosaccharides from pectin hydrolysate as new component for infant formulae: effect on intestinal flora, stool characteristics, and pH. J Pediatr Gastroenterol Nutr 41, 186-90.

Farwer, S.R., Der Boer, B.C.J., Haddeman, E., Kivits, G.A.A., Wiersma, A., Danse, B.H.J.C., 1994. The vitamin E nutritional status of rats fed on diets high in fish oil, linseed oil or sunflower seed oil.Brit J Nutr 72, 127-145

Forsyth, J.S., Willatts, P., Agostoni, C., Bissenden, J., Casaer, P., Boehm, G., 2003. Long chain polyunsaturated fatty acid supplementation in infant formula and blood pressure in later childhood: follow up of a randomised controlled trial. BMJ 326, 953.

Freese, R., 2001. Low erucic rapeseed oil and platelet function. Eur. J. Lipid Sci. Technol 103, 483–489.

Garrido, A., Garrido, F., Guerra, R., Valenzuela, A., 1989. Ingestion of high doses of fish oil increases the susceptibility of cellular membranes to the induction of oxidative stress. Lipids 24, 833-835.

Garrido, A., Garate, M., Campos, R., Villa, A., Nieto, S., Valenzuela, A., 1993. Increased susceptibility of cellular membranes to the induction of oxidative stress after ingestion of high doses of fish oil: effect of aging and protective action of dl-a tocopherol supplementation. J Nutr Biochem 4, 118-122.

Gibney, M.J., Bolton-Smith, C., 1988. The effect of a dietary supplement of n-3 polyunsaturated fat on platelet lipid composition, platelet function and platelet plasma membrane fluidity in healthy volunteers. Brit J Nutr, 60, 5-12.

Gibson, R.A., Makrides, M., Neumafi, M.A., Jeifrey, B., Lien, E., 1998. Effect of lowering the dietary linoleic acid (LA) to alpha-linolenic acid (ALA) ratio through the use of canola oil on visual and growth outcomes of term infants. Proceedings of the Nutrition Society of Australia 22, 289.

Green, T.J., Innis, S.M., 2000. Low erucic acid canola oil does not induce heart triglyceride accumulation in Neonatal pigs fed formula. Lipids 35, 607-612.

Harmsen, H.J., Wildeboer-Veloo, A.C., Raangs, G.C., Wagendorp, A.A., Klijn, N., Bindels, J.G., Welling, G.W., 2000. Analysis of intestinal flora development in breastfed and formula-fed infants by using molecular identification and detection methods. J Pediatr Gastroenterol Nutr 30, 61-67.

Henderson, R.A., Jensen, R.G., Lammi-Keefe, C.J., Ferris, A.M., Dardick, K.R., 1992. Effect of fish oil on the fatty acid composition of human milk and maternal and infant erythrocytes. Lipids 27, 863-869.

Huang, M., Naito, Y., Watanre, S., Kobayashi, T., Kanai, H., Nagai, H., Okuyama, H., 1996. Effect of rapeseed and dietary oils on the mean survival time of stroke-prone spontaneously hypertensive rats. Biol Pharm Bull 19, 554-557.

Innis, S.M., 1991. Essential fatty acids in growth and development. ProgLipid Res 30, 39-103.

Innis, S.M., 1993. The colostrum-deprived piglet as a model for study of infant lipid nutrition. J Nutr 123, 386-390.

Innis, S.M., Dyer, R.A., 1999. Dietary canola oil alters haematological indices and blood lipids in neonatal piglets fed formula. J Nutr 129,1261-1268.

Jacobs, N.J., Van Zoeren-Grobben, D., Drejer, G.F., Bindels, J.G., Berger, H.M., 1996. Influence of long chain unsaturated fatty acids in formula feeds on lipidperoxidation and antioxidants in preterm infants. Pediatr Res 40, 680-686.

Jensen, C.L., Chen, H., Fraley, J.K., Anderson, R.E., Heird, W.C., 1996. Biochemical effects of dietary linoleic/alpha linolenic acid ratio in term infants. Lipids 31, 107-113.

Jensen, C.L., Prager, T.C., Fraley, J.K., Chen, H., Anderson, R.E., Heird, W.C., 1997. Effect of dietary linoleic/alpha linolenic acid ratio on growth and visual function of term infants. J Pediatr 131, 200-209.

Kennedy, K., Fewtrell, M.S., Morley, R., Abbott, R., Quinlan, P.T., Wells, J.C.K., Bindels, J.G., Lucas, A., 1999. Double-blind, randomized trial of a synthetic triacylglycerol in formula-fed term infants: effect on stool biochemistry, stool characteristics, and bone mineralization. Am J Clin Nutr 70, 920-927.

Knapp, H.R., Reilly, I.A.G., Alessandrini, P., Fitzgerald, G.A., 1986. In vivo indexes of platelet and vascular function during fish oil administration in patients with atherosclerosis. N Engl J Med 314, 937-942.

Kramer, J.K., Farnworth, E.R., Johnston, K.M., Wolynetz, M.S., Modler, H.W., Sauer, F.D., 1990. Myocardial changes in newborn piglets fed sow milk or milk-replacer diets containing different levels of erucic acid. Lipids 25, 729-737.

Kramer, J.K., Sauer, F.D., Farnworth, E.R., Wolynetz, M.S., Jones, G., Rock, G.A., 1994. Hematological and lipid changes in newborn piglets fed milk-replacer diets containing vegetable oils with different levels of n3 fatty acids. Lipids 29, 859-868.

Kramer, J.K., Sauer, F.D., Farnworth, E.R., Stevenson, D., Rock, G.A., 1998. Hematological and lipid changes in newborn piglets fed milk-replacer diets. Lipids 33, 1-10.

Leibovitz, B.E., Hu, M., Tappel, A.L., 1990. Lipid Peroxidation in Rat Tissue Slices: Effect of Dietary Vitamin E, Corn Oil-Lard and Menhaden Oil. Lipids 25, 125-129.

Lu, F.C., 1988. Acceptable daily intake: inception, evolution and application. Regul Toxicol Pharmacol.8:45-60.

Makrides, M., Neumann, M.A., Jeffrey, B., Lien, E.L., Gibson, R.A., 2000. A randomized trial of different ratios of linoleic to α-linolenic acid in the diet of term infants: effect on visual function and growth. Am J Clin Nutr 71,120-129.

Marine oils and platelet function in man, (no authors listed), 1984. Nutr Rev 42, 189-191.

Mawatari, S., Ohnishi, Y., Kaji, Y., Maruyama, T., Murakami, K., Tsutsui, K., Fujino, T., 2003. High-cholesterol diets induce changes in lipid composition of rat erythrocyte membrane including decrease in cholesterol, increase in α-tocopherol and changes in fatty acids of phospholipids. Biosci Biotechnol Biochem 42(5) 189-191.

Miyazaki, M., Huang, M., Watanabe, S., Kobayashi, T., Okuyama, H., 1998. Early mortality\ effect of partially hydrogenated vegetable oils in stroke-prone spontaneously hypertensive rats (SHRSP). Nutr Res 18, 1049-1056.

Mori, T.A., Beilin, L.J., Burke, V., Morris, J., Ritchie, J., 1997. Interactions between dietary fat, fish, and fish oils and their effects on platelet function in man at risk of cardiovascular disease. Arterioscl Throm Vas 17, 279-286.

Muriana, F.J.G., Villar, J., Ruiz-Gutierrez, V., 1997a. Intake of olive oil can modulate the transbilayer of human erythrocyte membrane cholesterol. Cell Mol Life Sci 53, 496-500.

Muriana, F.J.G., Ruiz-Gutierrez, V., Guerrero, A., Mantilla, C., Leon-Camacho, M., Villar, J., 1997b. Olive oil normalizes the altered distribution of membrane cholesterol and Na+-Li+ countertransport activity in erythrocyte of hypertensive patients. Nutr Biochem 8, 205-210.

Naito, Y., Kasama, K., Yoshida, H., Ohara, N., 2000a. Thirteen-week dietary intake of rapeseed oil or soybean oil as the only dietary fat in Wistar Kyoto rats - Change in blood pressure. Food Chem Toxicol 38, 811-816.

Naito, Y., Konishi, C., Ohara, N., 2000b. Blood coagulation and osmolar tolerance of erythrocytes in stroke-prone spontaneously hypertensive rats given rapeseed oil or soybean oil as the only dietary fat. Toxicol Lett 116, 209-215.

Naito, Y., Yoshida, H., Nagata, T., Tanaka, A., Ono, H., Ohara, N., 2000c. Dietary intake of rapeseed oil or soybean oil as the only fat nutrient in spontaneously hypertensive rats and Wistar Kyoto rats - Blood pressure and pathophysiology. Toxicology 146, 197-208.

Naito, Y., Nagata, T., Takano, Y., Nagatsu, T., Ohara, N., 2003. Rapeseed oil ingestion and exacerbation of hypertension-related conditions in stroke prone spontaneously hypertensive rats. Toxicology 187, 205-216.

Ogawa, H., Yamamoto, K., Kamisako, T., Meguro, T., 2003. Phytosterol additives increase blood pressure and promote stroke onset in salt-loaded stroke-prone spontaneously hypertensive rats. Clin Exp Pharmacol P 30, 919-924.

Ohara, N., Naito, Y., Nagata, T., Tachibana, S., Okimoto, M., Okuyama, H., 2008. Dietary intake of rapeseed oil as the sole fat nutrient in Wistar rats--lack of increase in plasma lipids and renal lesions. J Toxicol Sci 33(5):641-5

Periago, J.L., Suarez, M.D., Pita, M.L., 1990. Effect of dietary olive oil, corn oil and medium-chain triglycerides on the lipid composition of rat red blood cell membranes. J Nutr 120, 986-994.

Pianese, P., Savia, G., Campanozzi, A., D'Apoloto, O., Dello Russo, A., Pettoello-Mantovani, M., Corso, G., 2008. Sterol profiling in red blood cell membranes and plasma of newborns receiving total pareteral nutrition. J Pediatr Gastroenterol Nutr 47 (5)645-651.

Picaud, J.P., Boucher, .P, Lapillone, A., Berthouze, M., Delvin, E., Boehm, G., Claris, O., Laborie, S., Reygroellet, B., Papillonne, H., Clorieux, F.H., Salle, B.L., 2002. Infleunce of dietary cholesterol on vitamin D metabolism in formula fed preterm infants. J Pediatr Gastroenterol Nutr 35,180-184.

Ping, W., Chunrong, W., Jian, Z., Wenxun, F., 1993. Effect of teaseed oil on blood lipids and platelet function in animals. Acta Nutrimenta Sinica 15, 377-384.

Raiten, D.J., Talbot, J.M., Waters, J.H. (Eds.), 1998. Assessment of nutrient requirements for infant formulas. J Nutr 128, 2089S-2107S.

Rand, M.L., Hennissen, A.A.H.M., Hornstra, G., 1988. Effects of dietary palm oil on arterial thrombosis, platelet responses and platelet membrane fluidity in rats. Lipids 23, 1019-1023.

Ratnayake, W.M.N., Plouffe, L., Hollywood, R., L'Abbe, M.R., Hidiroglou, N., Sarwar, G., Mueller, R., 2000a. Influence of sources of dietary oils on the life-span of stroke-prone spontaneously hypertensive rats. Lipids 35, 409-420.

Rigo, J., Boehm, G., Georgi, G., Jelinek, J., Nyambugabo, K., Sawatzki, G., Studzinski, F., 2001. An infant formula free of glycomacropeptide prevents hyperthreoninemia in formula-fed preterm infants. J Pediatr Gastroenterol Nutr. 32(2), 127-130. Erratum in: J Pediatr Gastroenterol Nutr 32, 620.

Ruiz-Gutierrez, V., Muriana, F.J.G., Guerrero, A., Villar, J., 1997. Olive oil and high-oleic sunflower oil on human plasma and erythrocyte membrane lipids. Nutr Res 17, 1391-1399.

Rzehak, P., Koletzko, S., Koletzko, B., Sausenthaler, S., Reinhardt, D., Grübl, A., Bauer, C.P., Krämer, U., Bollrath, C., von Berg, A., Berdel, D., Wichmann, H.E., Heinrich, J., 2011. The GINI Study Group: Growth of infants fed formula rich in canola oil (low erucic acid rapeseed oil). Clinical Nutrition 30:339-345.

Sauer, F.D., Farnworth, E.R., Belanger, J.M.R., Kramer, J.K.G., Miller, R.B., Yamashiro, S., 1997. Additional vitamin E required in milk-replacer diets that contain canola oil. Nutr Res 17, 259-269.

Silva, A.P., Guimarães, D.E., Mizurini, D.M., Maia, I.C., Ortiz-Costa, S., Sardinha, F.L., do Carmo, M.G., 2006. Dietary fatty acids early in life affect lipid metabolism and adiposity in young rats. Lipids41, 535-541.

Tatematsu, K., Fuma, S.Y., Nagase, T., Ichikawa, Y., Fujii, Y., Okuyama, H., 2004. Factors other than phytosterols in some vegetable oils affect the survival of SHRSP rats. Food Chem Toxicol 42, 1443-1451.

Vicario, I.M., Malkova, D., Luna, E.K., Johnson, I.T., 1998. Olive oil supplementation in healthy adults: effects in cell membrane fatty acid composition and platelet function. Ann Nutr Metab 42, 60-169.

Vajreswari, A., Srinivasa Rao, P, Kaplay, S.S., Tulpule, P.G., 1983. Erythrocyte membrane in rats fed high erucic acid-containing mustard oil: osmotic fragility, lipid composition, and (Na^+, K^+)-and (Ca^{2+}, Mg^{2+})-ATPases. Biochem Med 29:74-84.

Vongild, E., Elvevoll, E.O., Brox, J., Olsen, R.L., Barstad, H., Aursand, M., 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.

Von Berg, A., Koletzko, S., Grub,.A., Fillpiak-Pittroff, B., Wichmann, H-.E., Bauer, C.P., Reinhardt, D., Berdel, D., 2003. The effect of hydrolysed cow's milk formula for allergy prevention in the first year of life: The German Infant Nutritional Intervention Study, a randomised double-blind trial. J Allergy Clin Immunol 111, 533-540.

Von Berg, A., Koletzko, S., Filipak-Pittroff, B., Laubereau, B., Grubl A, Wichmann, H-.E., Bauer, C-.P., Reinhardt, D., Berdel, D., 2007. Certain hydrolysed formulas reduce the incidence of Atopic dermatitis but not that of asthma: Three year results of the German Infant Nutritional Intervention Study. J Allergy Clin Immunol 119, 718-725.

Winocour, P.D., Kinlough-Rathbone, R.L., Morazain, R., Mustard, J.F., 1987. Effect of the amount and type of dietary fat on platelet function, platelet survival and response to continuous aortic injury in rats. Atherosclerosis 68, 221-230.

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APPENDIX C

Summary of Studies in Human Infants Conducted or Sponsored By Danone Using Infant Formulas Containing Canola Oil

Summary of Studies in Human Infants Conducted or Sponsored By Danone Using Infant Formulas Containing Canola Oil

Many clinical studies have been conducted in term and pre-term infants using formulas of varying compositions which incorporated canola oil as one of the constituent oils. In the term infant studies, over 900 infants were fed formulas containing canola oil. The levels of canola oil in the formula ranged from 3 to 40%, and the feeding of formula lasted for up to 12 months. In the pre-term studies over 125 infants received the formulas containing canola oil for up to 36 weeks. These studies make an important contribution to the knowledge on the safety and efficacy of formulas incorporating canola oil. The numbers of infants exposed to canola oil-containing formulas in these studies, the canola oil content of the formulas and the main outcomes are summarized in the tables which follow. The studies are described more fully in Appendix B, section 2. Publications based on these studies will not always provide details of the canola content of the fat blend in the infant formula, as the canola content was not the focus of the study. However, a number of these trials used infant formulas manufactured by Danone. As a result, data on the composition of the fat blend used in these infant formulas is available and has been provided by Danone. The following Table summarizes information on the canola oil content and vitamin E and K levels of formulas used in these Danone sponsored or supported studies as well as details of ethical considerations and subject follow-up.

The maximum erucic acid content of the canola oil used for manufacture of the Danone infant formula used in these clinical trials was 1% total fatty acids or less. The maximum unsaponifiable matter content of the canola oil used for manufacture of the Danone infant formula used in these clinical trials was 1.5% or less.

Clinical Studies Sponsored or Supported by Danone Baby and Medical Nutrition B.V.: Summary of Canola Oil Content, Vitamin E & K Supplementation, Ethical Considerations & Subject Follow-Up

Publication	Formula consumed	% Canola oil Erucic acid levels	Vit E & K concentration/ 100kcal	Number of patients taking canola oil formula	Ethical approval/ Numico sponsorship	Time taking formula/length of follow up
Bakker-Zierikzee et al, 2005; 2006	Nutrilon 1	% Fat = 26.8% Canola oil = 19% of total fatty acids (5.01% of final formulation)	Vit E: 1.7mgTE/100kcal Vit K: 7.5mcg/100kcal	N=57	Approved by ethical committee of University Medical Centre St Radboud, Nijmegen, NL. Written informed consent from parents prior to enrolment. Numico were involved directly in study.	Recruitment: Jan 2000-May 2003 Formula consumed for 16 wks from birth.
Boehm et al, 2003	Preterm formula + GOS/FOS Prematil	% Fat = 28.3% Canola oil = 21.2% of total fatty acids (5.58% of final formulation)	Vit E:3.7mg TE/100kcal Vit K: 10mcg/100kcal	N=30	Ethical approval not described Numico were involved directly in study.	Formula consumed for 28 days from birth.
Boehm et al, 2002					Study protocol approved by ethical committee of Hospital Mangiagalli, Milan. Study was supported by a grant from Numico Research Germany	Formula consumed for 28 days from birth.
Boehm et al, 1995	Prematil + milupan fat blend	% Fat = 28.3% Canola oil = 21.2% of total fatty acids (5.58% of final formulation)	Vit E:3.7mg TE/100kcal Vit K: 10mcg/100kcal	N=44	Study protocol approved by Ethics Committees of Leipzig&Milan.	Formula consumed for 10 days from birth.
Bouwstra et al, 2005; 2003	Nutrilon premium +/- LCP	% Fat = 26.8% Canola oil = 19% of total fatty acids (5.01% of final formulation)	Vit E: 1.7mgTE/100kcal Vit K: 7.5mcg/100kcal	Nutrilon premium control formula n=167 + LCP n=145	Study approved by Ethics Committee of the GroningenUniversityHospital (MEC 95/08/207) Supported by Numico Research.	Recruitment: Feb 1997-Oct 1999 Formulas were consumed for 2 months. Patients were followed up at 18 months (2005 study)
Fanaro et al, 2005	Std formula (Nutrilon premium) + GOS/FOS/ AOS	% Fat = 26.8% Canola oil = 19% of total fatty acids (5.01% of final formulation)	Vit E: 1.7mgTE/100kcal Vit K: 7.5mcg/100kcal	N=46 (on 3 formulas based on std formula)	Approved by Ethics Committee of the University of Ferrara, Written informed consent obtained for each infant Supported by Numico Research	Formulas were consumed for 6 weeks.

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PUBLICATION	FORMULA CONSUMED	% CANOLA OIL ERUCIC ACID LEVELS	VIT E & K CONCENTRATION/ 100KCAL	NUMBER OF PATIENTS TAKING CANOLA OIL FORMULA	ETHICAL APPROVAL/ NUMICO SPONSORSHIP	TIME TAKING FORMULA/LENGTH OF FOLLOW UP
Forsyth et al, 2003 Forsyth et al, 1999 Arch Dis Child; 81: 253-256. Willatts et al, Lancet 1998; 352: 688-91(additional papers not included in original GRAS dossier)	Std formula (Aptamil) or Aptamil + fat supplement Milupan.	Aptamil: %Fat = 27.4% Canola oil = 19% (5.13% of final formulation) Aptamil + Milupan %Fat = 27.4% Canola oil = 21% (5.57% of final formulation)	Vit E: 1.1mgTE/100kcal Vit K: 5.8mcg/100kcal Vit E: 1.8mgTE/100kcal Vit K: 5.8mcg/100kcal	N= 147	Approved by Tayside Committee on Medical Research Ethics Research grant from Milupa (Numico)	Formula was consumed for first 4 months after birth. Follow up of patients at 10 months & 6 years to investigate blood pressure in later childhood.
Harmsen et al, 2000	Nutrilon Premium	% Fat = 26.8% Canola oil = 19% of total fatty acids (5.01% of final formulation)	Vit E: 1.7mgTE/100kcal Vit K: 7.5mcg/100kcal	N= 6	Part of U-Gene study (Utrecht, NL) Supported by Numico Research.	Formula was consumed for 20 days after birth.
Jacobs et al, 1996	Nenatal & Nutrilon Premium	% Fat = 26.8% Canola oil = 19% of total fatty acids (5.01% of final formulation)	Vit E: 1.7mgTE/100kcal Vit K: 7.5mcg/100kcal	N=28	Approved by the Scientific Committee of the Dept of pediatrics of the UniversityHospital of Leiden & The Ethical Committee of the JulianaChildrenHospital, the Hague. Supported by a grant from Nutricia N.V., The Netherlands.	Formula was consumed for 6 weeks from birth.
Kennedy et al, 1999	Std Formula (Nutrilon premium) + betapol	% Fat = 26.8% Canola oil = 19% of total fatty acids (5.01% of final formulation)	Vit E: 1.7mgTE/100kcal Vit K: 7.5mcg/100kcal	N=203	Ethical approval obtained from the Cambridge Health Authority & local MRC ethics committees. Supported by Nutricia UK, and a MRC training fellowship.	Formula was consumed for 12 weeks from birth.
Lidestri et al, 2003 (see also Boehm et al 2002, 2003 above)	Prematil	% Fat = 28.3% Canola oil = 21.2% of total fatty acids (5.58% of final formulation)	Vit E:3.7mg TE/100kcal Vit K: 10mcg/100kcal	N=30	Approved by ethical committee of Hospital Mangiagalli, Milan. Informed parental consent obtained before enrolment.	Formula was consumed for 4 weeks.

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PUBLICATION	FORMULA CONSUMED	% CANOLA OIL ERUCIC ACID LEVELS	VIT E & K CONCENTRATION/ 100KCAL	NUMBER OF PATIENTS TAKING CANOLA OIL FORMULA	ETHICAL APPROVAL/ NUMICO SPONSORSHIP	TIME TAKING FORMULA/LENGTH OF FOLLOW UP
Moro et al, 2006	EHF-W formula + GOS/FOS Nutrilon pepti	% Fat = 28.5% Canola oil = 19% of total fatty acids (5.27% of final formulation)	Vit E: 1.5mgTE/100kcal Vit K: 7.9mcg/100kcal	N=259 (both formulas had canola oil component)	Approved by the Ethical committee of the MacedonioMelloniMaternityHospital Study was partially supported by a grant from Numico Research, Germany.	Formula was consumed for 6 months after birth.
Moro et al, 2005	Nutilon premium + 0.8g/100ml GOS/FOS	% Fat = 24.8% Canola oil = 30% (7.36% of final formulation)	Vit E: 1.6mgTE/100kcal Vit K: 6.5 mcg/100kcal	N=32	Approved by Ethics Committees of Macedonio Melloni Maternity Hospital & Mangiagalli Hospital, University of Milan, Italy. Parents gave informed consent before enrolment.	Formula was consumed for 28 days.
Moro et al, 2002	Nutrilon premium + GOS/FOS	0.4 Nutrilon Premium % Fat = 25.9% Canola oil = 22% of total fatty acids (5.6% of final formulation) 0.8 Nutrilon Premium % Fat = 24.8% Canola oil = 30% (7.36% of final formulation)	Vit E: 1.7mgTE/100kcal Vit K: 7.3mcg/100kcal Vit E: 1.6mgTE/100kcal Vit K: 6.5 mcg/100kcal	N=90 (3 formulas – placebo; 0.4g or 0.8g/100ml GOS/FOS)	Approved by Ethics Committees of Macedonio Melloni Maternity Hospital & Mangiagalli Hospital, University of Milan, Italy. Parents gave informed consent before enrolment. Supported by grant from Numico Research.	Formula was consumed for 28 days.
Moro et al, 1999	Unnamed preterm formula			N=6	Not described	
Picaud et al, 2002	Preterm formula + differing amounts of cholesterol. Which formula? Contains canola (MvE)			N=30	Approved by Ethics committee of ClaudeBernardUniversity (Lyon) & by the French Health Ministry in accord with the Helsinki declaration of 1975 revised in 1983. Milupa Research Centre (Numico, Germany) provided study formulas & financial support for lab analysis.	Preterm infants received formula until theoretical term age (40 week post conception), maximum length of supplementation 10-12 weeks.

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Publication	Formula consumed	% Canola oil Erucic acid levels	Vit E & K concentration/ 100kcal	Number of patients taking canola oil formula	Ethical approval/ Numico sponsorship	Time taking formula/length of follow up
Rigo et al, 2001	Preterm formula free of Glycomacr opeptide			N=14	Approved by Ethics Committee of Hospital de la Citadelle, Dept of Neonatology, University of Liege, Belgium. Informed parental consent before study enrolment. Numico Research Germany were directly involved in study.	Formula was consumed for 2 weeks.
Von Berg et al, 2003	Nutrilon premium & Nutrilon pepti	Nutrilon premium: % Fat = 25% canola Canola oil = (7 % of final formulation) Nutrilon pepti: % Fat = 25% canola oil Canola oil = ((6.9% of final formulation)	Vit E: 1.7mgTE/100kcal Vit K: 7.5mcg/100kcal Vit E: 1.5mgTE/100kcal Vit K: 7.9mcg/100kcal	Nutrilon premium (CMF) n= 556 Nutrilon pepti (EHF-W) n=559	Study approved by the ethics committees of each of the study centres. Supported by research grants from a number of nutrition companies including Milupa, Numico.	Formula was consumed until the infant was 6 months of age. Patients were followed up to 12 month.

References

Bakker-Zierikzee, A.M., Alles, M.S., Knol, J., Kok, F.J., Tolboom, J.J.M., Bindels, J.G., 2005. Effects of infant formula containing a mixture of galacto- and fructo-oligosaccharides or viable Bifidobacteriumanimalis on the intestinal microflora during the first four months of life. Br J Nutr 94, 783-790.

Bakker-Zierikzee, A.M., Tol, E.A., Kroes, H., Alles, M.S., Kok. F.J., Bindels, J.G., 2006. Faecal SIgA secretion in infants fed on pre- or probiotic infant formula. Pediatr Allergy Immunol 17,134-140.

Boehm, G., Moro, G., Müller, D.M., Müller, H., Minolil., 1995. Fecal cholesterol excretion in preterm infants fed breast milk or formula with different cholesterol contents. Acta Paediatr 84, 240-244.

Boehm, G., Lidestri, M., Casetta, P., Jelinek, J., Negretti, F., Stahl, B., Marini, A., 2002. Supplementation of a bovine milk formula with an oligosaccharide mixture increases counts of fecal bifidobacteria in preterm infants. Arch Dis Childh 86, F178-181.

Boehm, G., Fanaro, S., Jelinek, J, Stahl, B., Marini, A., 2003. Prebiotic concept for infant nutrition. Acta Paediatr Suppl 441, 64-67.

Bouwstra, H., Dijck-Brouwer, D.A., Wildeman, J.A., Tjoonk, H.M., van der Heide, J.C., Boersma, E.R., Muskiet, F.A., Hadders-Algra, M., 2003. Long-chain polyunsaturated fatty acids have a positive effect on the quality of general movements of healthy term infants. Am J Clin Nutr 78, 313-318.

Bouwstra, H., Dijck-Brouwer, D.A.J., Boehm, G., Boersma, E.R., Muskiet, F.A.J., Hadders-Algra, M., 2005. Long-chain polyunsaturated fatty acids and neurological developmental outcome at 18 months in healthy term infants. Acta Paediatr 94, 26-32.

Fanaro, S., Jelinek, J., Stahl, B., Boehm, G., Kock, R., Vigi, V., 2005. Acidic oligosaccharides from pectin hydrolysate as new component for infant formulae: effect on intestinal flora, stool characteristics, and pH. J Pediatr Gastroenterol Nutr 41, 186-90.

Forsyth, J.S., Varma, S., Colvin, M., 1999. A randomised controlled study of the effect of long chain polyunsaturated fatty acid supplementation on stool hardness during formula feeding. Arch Dis Child 81, 253-256.

Forsyth, J.S., Willatts, P., Agostoni, C., Bissenden, J., Casaer, P., Boehm, G., 2003. Long chain polyunsaturated fatty acid supplementation in infant formula and blood pressure in later childhood: follow up of a randomised controlled trial. BMJ 326, 953.

Harmsen, H.J., Wildeboer-Veloo, A.C., Raangs, G.C., Wagendorp, A.A., Klijn, N., Bindels, J.G., Welling, G.W., 2000. Analysis of intestinal flora development in breastfed and formula-fed infants by using molecular identification and detection methods. J Pediatr Gastroenterol Nutr 30, 61-67.

Jacobs, N.J., Van Zoeren-Grobben, D., Drejer, G.F., Bindels, J.G., Berger, H.M., 1996. Influence of long chain unsaturated fatty acids in formula feeds on lipidperoxidation and antioxidants in preterm infants. Pediatr Res 40, 680-686.

Kennedy, K., Fewtrell, M.S., Morley, R., Abbott, R., Quinlan, P.T., Wells, J.C.K., Bindels, J.G., Lucas, A., 1999. Double-blind, randomized trial of a synthetic triacylglycerol in formula-fed term infants: effect on stool biochemistry, stool characteristics, and bone mineralization. Am J Clin Nutr 70, 920-927.

Lidestri, M., Agosti, M., Marini, A., Boehm, G., 2003. Oligosaccharides might stimulate calcium absorption in formula-fed preterm infants. Acta Pediatr Suppl 91, 91-92.

Moro, G.E., Minoli, I., Boehm, G., Georgi, G., Jelinek, J., Sawatzki, G., 1999. Postprandial plasma amino acids in preterm infants: influence of the protein source. Acta Pediatr 88, 885-889.

Moro, G.E., Minoli, I., Mosca, M., Fanaro, S., Jelinek, J., Stahl, B., Boehm, G., 2002. Dosage-related bifidogenic effects of galacto- and fructooligosaccharides in formula-fed term infants. J Pediatr Gastroenterol Nutr 34, 291-295.

Moro, G.E., and Arslanoglu S., 2005. Reproducing the bifidogenic effect of human milk in formula-fed infants: why and how? Acta Pediatr Suppl 94, 14-17.

Moro, G.E., Arslanoglu, S., Stahl, B., Jelinek, J., Wahn, U., Boehm, G., 2006. A mixture of prebiotic oligosaccharides reduces the incidence of atopic dermatitis during the first six months of age. Arch Dis Child 91, 814-819.

Picaud, J.P., Boucher, .P, Lapillone, A., Berthouze, M., Delvin, E., Boehm, G., Claris, O., Laborie, S., Reygroellet, B., Papillonne, H., Clorieux, F H., Salle, B.L., 2002. Infleunce of dietary cholesterol on vitamin D metabolism in formula fed preterm infants. J Pediatr Gastroenterol Nutr 35,180-184.

Rigo, J., Boehm, G., Georgi, G., Jelinek, J., Nyambugabo, K., Sawatzki, G., Studzinski, F., 2001. An infant formula free of glycomacropeptide prevents hyperthreoninemia in formula-fed preterm infants. J Pediatr Gastroenterol Nutr. 32, 127-130. Erratum in: J Pediatr Gastroenterol Nutr 32, 620.

Von Berg, A., Koletzko, S., Grub,.A., Fillpiak-Pittroff, B., Wichmann, H-.E., Bauer, C.P., Reinhardt, D., Berdel, D., 2003. The effect of hydrolysed cow's milk formula for allergy prevention in the first year of life: The German Infant Nutritional Intervention Study, a randomised double-blind trial. J Allergy Clin Immunol 111, 533-540.

Willatts, P., Forsyth, J.S., DiModugno, M.K., Varma, S., Colvin, M., 1998. Effect of long-chain polyunsaturated fatty acids in infant formula on problem solving at 10 months of age. Lancet 352, 688-691.

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APPENDIX D

Specification for Low Erucic Acid Rapeseed Oil (Canola Oil) for Term Infant Formula

Product Specification for Canola Oil (Low Erucic Acid Rapeseed (LEAR oil))

Low Erucic Acid Rapeseed Oil (Canola Oil) is a refined, bleached and deodorised vegetable oil from rapeseed (*Brassica Napus/ Brassica Rapa*).

Physical Appearance A clear pale yellow liquid, with bland odour.

Product Specification Parameter	Unit	Min	Max	Method of Analysis
Lovibond Colour Red Yellow			 1.5 15	**Lovibond 5 ¼"cell**
Moisture	%		0.05	**Karl Fischer**
Unsaponifiable Matter	%		1.5	
Free Fatty Acids	g/100 g fat		0.1	**M=282, As Oleic Acid**
Peroxide Index	meq O2/kg Fat		0.5	**Titrimetric**
Iodine Index		105	125	**AOCS CD 1-85 (Wijs)**
Parameter	**Unit**	**Min**	**Max**	**Method of Analysis**
Fatty Acids				**Capillary GC/FID**
Palmitic Acid C16:0	g/100 g fatty acids	3.8	5.6	
Stearic Acid C18:0	g/100 g fatty acids	1.0	2.5	
Oleic Acid C18:1	g/100 g fatty acids	56	66	
Linoleic Acid C18:2	g/100 g fatty acids	17	23	
Linolenic Acid C18:3	g/100g fatty acids	7.0	11.0	
Erucic Acid C22:1	g/100 g fatty acids		1	
Parameter	**Unit**	**Min**	**Max**	**Method of Analysis**
Lead	ppb		100	**AAS**
Mercury	ppb		20	**AAS**
Cadmium	ppb		20	**AAS**
Arsenic	ppb		100	**AAS**
Parameter	**Unit**	**Min**	**Max**	**Method of Analysis**
Total Polychlorinated Biphenyl Dioxin-Like	pg WHO-TEQ/g fat		1.5	**EN 1528 prt 1-4**
All Pesticide Residues	ppb		10	
PAH b[a]p: Benzo(a)pyrene	ppb		2	**DFG C III 17A(1997)**
Dioxins (PCDD + PCDF)	pg WHO-TEQ/g fat		1.5	
Aflatoxin B1	ppb		0.2	**IAS/HPLC**

[redacted]

	Temp.	Unit	n	c	Threshold (or m)	Reject value (or M)
Aerobic Plate Count	30^0C	/g				100
Enterobacteriaceae		/g				10
Moulds		/g			50	100
Yeast		/g			50	100
Salmonella spp.		/25g				Neg

APPENDIX E

Manufacturing Process for Low Erucic Acid Rapeseed Oil (Canola Oil)

(with Hexane Specification)

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Manufacturing Process for Low Erucic Acid Rapeseed oil (Canola oil)

Canola oil is manufactured from crushing oilseeds of canola or low erucic rapeseed (Brassica rapa/ B. napus). Following extraction from the oilseeds, the crude oil is further refined in three steps described below. The aim of the refining process is to produce a safe, edible oil with bland taste and bright color and to remove any unwanted components from the oil.

The crushing and refining uses conventional processes used in the vegetable oil refining industry. For example, the same type of processes are used in the manufacture of soyabean oil.

Crushing

Crushing is the term for the processes used to obtain the oil from the rapeseed. Crushing of the oilseeds is done either by pressing the oilseeds or combining pressing with solvent extraction. To separate fat from the canola oilseeds the seeds are subject to pressing, which is the physical separation of fat and solid matter by means of pressure. The solid matter may then be fully de-oiled by solvent extraction. Hexane is used as solvent, the maximum residue limit for hexane in the canola oil is 1 mg/kg as specified in European Directive 2009/32/EC*. The solvent is removed from the oil by distillation. A complete removal of the solvent is guaranteed by the final deodorisation step.

The resulting canola oil is washed and filtered and pumped to a crude oil tank.

Refining

The purpose of refining the crude oil is to remove the impurities derived from the oilseed such as free fatty acids, phosphatides and oxidation products. It is also important to remove any contaminants such as pesticide residues and environmental contaminants. At the same time the natural antioxidants in the crude oil shall be preserved to as high extent as possible. This is ensured by selecting the correct processing conditions and subjecting them to strict control.

The refining process is an alkaline refining and comprises the following steps: degumming, neutralization, bleaching and deodorization.

Degumming

The purpose of degumming is to remove phospholipids and free fatty acids from the oil. The oil is mixed with acidified water using phosphoric acid, which makes the phospholipids water-soluble and thus possible to separate together with the water phase.

Neutralization

In the neutralization the oil is mixed with diluted lye, transforming the free fatty acids into soaps with can be separated together with the washing water.

Bleaching

The neutralized oil is then subject to bleaching in order to remove pigments and reduce the color of the oil. Metals and some oxidation products are also removed in this process. The oil is treated with citric acid and bentonite clay, which has the capacity of adsorbing the impurities. Bentonite is a naturally occurring clay, washed and sifted before use. In some cases activated carbon is used, in order to remove heavy polyaromatic hydrocarbons. Bentonite and activated carbon are removed through filtering.

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Deodorization

Deodorization is the final refining step in which all volatile substances are removed from the oil, including taste and odour compounds and contaminants such as pesticide residues and poly aromatic hydrocarbons. Any residues of the hexane solvent used for the extraction of the oil is efficiently removed in the deodorization. The deodorization is a steam distillation which is attained by leading steam through the oil at high temperature and in a vacuum. The balance between temperature and time guarantees that substances with intermediate volatility, like vitamins and sterols, remain in the oil. The applied process leaves even the sensitive polyunsaturated fatty acids unaffected. Citric acid is added to the deodorised oil as a metal ion chelator.

Refined oil

The fully refined canola oil passes through a final filter before it is being packed and distributed. The result of the refining process is an oil with substantially improved keeping properties and bland taste, whereas the chemical composition of the oil has been left unchanged.

Reference

1. Directive 2009/32/EC of the European Parliament and of the Council of 23rd April 2009 on the Approximation of the laws of the Member States on Extraction Solvents Used in the production of Foodstuffs and Food Ingredients

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ExxonMobil Chemical

PERFORMANCE FLUIDS

Effective : June 8, 2009

ART. NR	PROD. KOD
823 010	

GODKÄND
DAT: 2009-07-4
(b) (6)
SIGN:

GODKÄND
DAT:
SIGN:

Exxsol™ Hexane
Hydrocarbon Fluid

Specifications

Property	Units	Min values	Max values	Typical values^A	Test method* (latest method, unless specified otherwise)
Distillation range	°C				ASTM D 1078
- Initial boiling point		65		66 / 66	
- Dry point			70	69 / 69	
Total aromatic content *^B	mg/kg		10	< 10 / < 10	UV
Benzene content *^B	mg/kg		5	1 / 1	UV
Bromine Index *	mg/100g		30	< 5 / < 5	ASTM D 2710 Mod / D 2710
Colour Saybolt	-	+ 30		+30 / +30	ASTM D 6045
n-Hexane content *	wt%	44		47 / 47	GC
Cyclohexane content *	wt%		4	2 / 1	GC
Total paraffins *	wt%	75		82 / 80	GC
Non-volatile matter	g/m³		10	< 10 / < 10	ASTM D 1353
Sulphur content	mg/kg		1	< 1 / < 1	ASTM D 4045

^A : When two typical values are presented on one line, the first corresponds to the quality manufactured in the Antwerp plant, the second to the Trecate plant quality.
* : When two test methods are presented on one line, the first one is the one used in the Antwerp plant, the second one is used in the Trecate plant.
^B : For sea transportation, other than in BLC, we only guarantee a maximum of 50 mg/kg aromatics and 10 mg/kg benzene.

Other properties

Property	Units	Typical values^A	Test method (latest method, unless specified otherwise)
Aniline point	°C	63	ASTM D 611
Composition *	wt%		GC
- 2-Methylpentane		18	
- 3-Methylpentane		16	
- Methylcyclopentane		17	
Evaporation rate (n-BuAc = 100) *	-	1200	EC-M-F01
KB value	-	29	ASTM D 1133
Refractive index at 20°C	-	1.360	ASTM D 1218
Solubility parameter *	(cal/cm³)^1/2	7.3	EC-A-S03

^A : Typical values refer to Antwerp production.

* Value may be determined by ExxonMobil procedures equivalent to industry standard test methods. Applicable sampling and testing methods are subject to change without notice and are available for review on request.

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MAR 16 2012
Division of
Biotechnology and
GRAS Notice Review

Ramos-Valle, Moraima

From: Bob McQuate [mcquate@gras-associates.com]
Sent: Friday, March 16, 2012 3:06 PM
To: Ramos-Valle, Moraima
Cc: Mary CREAN; 'Cathy BUSHELL'; Rich Kraska
Subject: Completed Expert Panel Signature Page for Danone Canola Oil GRAS Notification
Attachments: CANOLA OIL FINAL 03 06 12 P. 46 Innis Sig.pdf

Dear Ms. Ramos-Valle,

Attached you will find the replacement page 46 associated with the GRAS notification that was submitted on behalf of Danone regarding the intended canola oil use in infant formulas.

You may recall that we were not able to obtain the final review and authorization for signature inclusion for Professor Innis at the time we issued our GRAS notification to you. Please accept the attached as a replacement page.

We are relaying three additional copies to you via US Priority mail, and they should arrive in College Park, MD early next week.

We look forward to the review and feedback that you will provide in the coming months.

Sincerely,

Bob
Robert S. McQuate, Ph.D.
CEO & Co-Founder
GRAS Associates, LLC
20482 Jacklight Lane
Bend, OR 97702-3074
541-678-5522
mcquate@gras-associates.com
www.gras-associates.com

[redacted]

VIII. CONCLUSIONS

The members of the Expert Panel consisted of: Richard C. Kraska, Ph.D. (Chair), DABT, Madhusudan G., Soni, Ph.D., Robert S. McQuate, Ph.D., Stanley T. Omaye, Ph.D., Walter H. Glinsmann, MD, and Sheila M. Innis, Ph.D., RDN. Each has an extensive technical background in the evaluation of the safety of food ingredients.[16]

> The Panel concludes that the use of canola oil produced in compliance with current Good Manufacturing Practices requirements and which meets Food Chemicals Codex specifications is generally recognized as safe (GRAS) when used as a component of the fat blend in infant formulas for term infants, with a typical intended use level of up to 31% of the fat blend, such that the fat blend provides a nutritionally balanced blend of essential fatty acids, saturated, monounsaturated and polyunsaturated fatty acids in line with dietary recommendations for fatty acid intakes for infants.

(b) (6)

Richard C. Kraska, Ph.D., DABT - Chair

(b) (6)

Madhusudan G. Soni, Ph.D., FACN, FATS

(b) (6)

Robert S. McQuate, Ph.D.

(b) (6)

Stanley T. Omaye, Ph.D., DATS

(b) (6)

Walter H. Glinsmann, M.D.

(b) (6)

Sheila M. Innis, Ph.D., RDN

March 6, 2012

[16] The detailed educational and professional credentials for some of the individuals serving on the Expert Panel can ble found on the GRAS Associates website at www.gras-associates.com. Dr. Kraska served as Chair of the Panel. Drs. Kraska and McQuate worked on GRAS and food additive safety issues within FDA's GRAS Review Branch earlier in their careers and subsequently continued working within this area in the private sector. Dr. Omaye is a professor of nutrition at the University of Nevada, Reno and has published extensively on the nutritional and toxicological aspects of food ingredients. Dr. Soni's credentials can be accessed at http://www.soniassociates.net. Dr. Glinsmann has extensive experience in clinical nutrition and food safety that includes service with FDA's Division of Nutrition where he addressed a broad range of food and human nutrition topics. Dr. Innis is currently Director, Nutrition & Metabolism Research Program, CFRI; and Professor, Department of Pediatrics, University of British Columbia. Dr. Innis' credentials can be accessed at http://www.cfri.ca/our_research/researches/search_researchers/researcher_detail.asp?ID=61.

[redacted]

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mcquate@gras-associates.com

March 16, 2012

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Division of
Biotechnology and
GRAS Notice Review

Ms. Moraima J. Ramos Valle, MS
Division of Biotechnology & GRAS Notice Review (HFS-255)
Office of Food Additive Safety
Center for Food Additive Safety & Applied Nutrition
Food & Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: GRAS Notification – Canola Oil Use in Infant Formulas

Dear Ms. Ramos Valle:

As described in our March 6, 2012 transmittal letter to you on behalf of Danone Trading BV, we are providing the replacement Expert Panel signature page for the GRAS notification for Canola Oil Use in Infant Formulas. The attached letter now includes the signature as authorized by Professor Sheila Innis.

We hope that the composite information provided will enable your review to proceed efficiently. If additional clarification is needed as this process advances, please feel free to contact me *via* telephone or email.

We look forward to your feedback.

Sincerely,

(b) (6)

Robert S. McQuate, Ph.D.
CEO & Co-Founder
GRAS Associates, LLC
www.gras-associates.com

Enclosure: Expert Panel Signature Page for Danone GRAS Notification for Canola Oil Use in Infant Formulas (in triplicate)

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[redacted]

VIII CONCLUSIONS

The members of the Expert Panel consisted of: Richard C. Kraska, Ph.D. (Chair), DABT, Madhusudan G., Soni, Ph.D., Robert S. McQuate, Ph.D., Stanley T. Omaye, Ph.D., Walter H. Glinsmann, MD, and Sheila M. Innis, Ph.D., RDN. Each has an extensive technical background in the evaluation of the safety of food ingredients.[16]

> The Panel concludes that the use of canola oil produced in compliance with current Good Manufacturing Practices requirements and which meets Food Chemicals Codex specifications is generally recognized as safe (GRAS) when used as a component of the fat blend in infant formulas for term infants, with a typical intended use level of up to 31% of the fat blend, such that the fat blend provides a nutritionally balanced blend of essential fatty acids, saturated, monounsaturated and polyunsaturated fatty acids in line with dietary recommendations for fatty acid intakes for infants.

(b) (6)

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March 6, 2012

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SUBMISSION END