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Trans Fatty Acids Labeling

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TRANS FAT LABELING

Effective January 1, 2006, *trans* Fat Labeling on Food Products is required per the Federal Register of July, 2003. The US Food and Drug Administration (FDA) amended the food regulations on nutrition labeling (21 CFR §101.9 and 21 CFR §101.36) to require that the amount of *trans* fatty acids in a food, including dietary supplements, be included in the Nutrition Facts. FDA has used "*trans* fatty acids" and "*trans* fat" interchangeably in the same manner it has used "saturated fatty acids" and "saturated fat". Specifically, 21 CFR §101.9(c)(2)(ii) (ii) ''*Trans* fat'' or ''*Trans*'': A statement of the number of grams of *trans* fat in a serving, defined as the sum of all unsaturated fatty acids that contain one or more isolated (i.e., nonconjugated) double bonds in a *trans* configuration, except that label declaration of *trans* fat content information is not required for products that contain less than 0.5 gram of total fat in a serving if no claims are made about fat, fatty acid or cholesterol content. The word ''*trans*'' may be italicized to indicate its Latin origin. *Trans* fat content shall be indented and expressed as grams per serving to the nearest 0.5 (1/2)-gram increment below 5 grams and to the nearest gram increment above 5 grams. If the serving contains less than 0.5 gram, the content, when declared, shall be expressed as zero. Except as provided for in paragraph (f) of this section, if a statement of the *trans* fat content is not required and, as a result, not declared, the statement ''Not a significant source of *trans* fat'' shall be placed at the bottom of the table of nutrient values. For more information, see the following website: (http://www.fda.gov/OHRMS/DOCKETS/98fr/94p-0036-nfr001.pdf)

Basis for *Trans* Fat Regulation

On July 10, 2002 the Panel on Macronutrient Dietary Reference Intakes (DRI) of the Food Nutrition Board, Institute of Medicine of the National Academies of Science, at the request of the USFDA released a letter report on *trans* fatty acids. The report concludes that dietary *trans* fatty acids are a risk factor for coronary heart disease, showing a linear trend between intake and total and LDL cholesterol. The panel concluded there was no safe upper intake level. But, appreciating the fact that *trans* fatty acids are unavoidable in ordinary diets because of natural occurrence in foods, and that extreme or drastic dietary changes can result in inadequate intake of other essential nutrients, the panel did not propose an upper intake limit, but recommends that "*trans* fatty acid consumption be as low as possible while consuming a nutritionally adequate diet".

WHAT IS *TRANS* FAT

Fats are a regular part of our everyday diet. The Federal Register in 1993 (1) published regulations relevant to the Nutrition Labeling and Education Act (NLEA) of 1990 requiring the nutrition labeling on foods that are regulated by United States Food and Drug Administration (FDA) and Department of Agriculture (USDA). Under NLEA, fat consists of fatty acids, which are present as either free fatty acid, or are esterified in a variety of forms, such as mono-, di-, or triglycerides, phospholipids, or glycolipids. These fatty acids have carboxylic acids with chain lengths of four to 30 carbons. The chains may be aliphatic (saturated), or olefinic (unsaturated, having one or more double bonds between carbon atoms). The olefinic chains may have one (monounsaturates), or more (polyunsaturates) double bonds.

Unsaturated fatty acids can have the same chemical formula but different chemical and physiological properties due to different geometrical configuration. The double bond of the unsaturat-

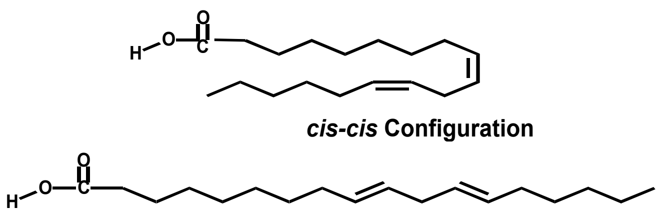

FIGURE 1: *cis* and *trans* Isomerization

ed fatty acid can be in either *cis* or *trans* configuration (See Figure 1). Most of the dietary unsaturated fatty acids are in a *cis* configuration i.e. the two hydrogen atoms are on the same side of the two carbons of the double bond. This gives the fatty acid chain of the *cis* isomer a bent configuration, effectively prohibiting it from packing tightly with neighboring molecules. This result in the fat being a low melting solid or an oil.
In the *trans* configuration, the hydrogen atoms are on the opposite side of the two double bond carbons. *Trans* isomers have a lower energy state than *cis* isomers. Thus *trans* isomers are formed during the hydrogenation process of the unsaturated fatty acids when reversible partial hydrogenation results in formation of the lower energy *trans* bond. In addition, partial hydrogenation of polyunsaturated fatty acids may result in a shift of the double bonds positions in the fatty acid chain. Similarly, fat oxidation results in reversible reactions that may shift the double bond position and convert *cis* double bonds to *trans* double bonds in the fatty acid chain. During the hydrogenation process, the bent *cis* isomer opens so to speak to the *trans* isomer to stabilize the molecule into a straight configuration which enables the molecules to pack easily together resulting in higher melting points, providing shelf life and flavor stability. These more stable fats are used in margarines and shortenings. In addition to the occurrence of *trans* bonds in fats as a result of hydrogenation of oils, trans fats occur naturally in dairy fats and in animal body fats such as lard and tallow as well.

Stearic acid is an eighteen carbon saturated fatty acid and is represented symbolically as C18:0. The zero denotes the fatty acid is saturated, that is, it contains zero double bonds. Similarly, eighteen carbon fatty acids like oleic acid with one double bond and linoleic acid with two double bonds are represented as C18:1, and C18:2 respectively. Linolenic acid is a polyunsaturated fatty acid with three double bonds and is represented as C18:3. It is considered linolenic acid only if all the double bonds are in the nonconjugated *cis* configuration. To designate fatty acid as *cis* or *trans,* a *c* or *t* is sometimes placed behind the bond number, i.e. C18:1*c*, C18:2*cc*, C18:2*ct* etc.

TYPICAL FATTY ACID PROFILE FOR A FOOD CONTAINING HYDROGENATED FAT

Reading and understanding a fatty acids report:
COLUMN 1: Names the individual fatty acids that are found in edible fats, by the number of carbons in the chain of the molecule, followed by a colon, then followed by a number that represents the number of double bonds along the carbon chain. The common name of the fatty acid follows. Fatty acids with a nonconjugated double bond in the *trans* configuration are noted with "*trans*" in the name, while *cis* configured bonds are only noted in special cases.
COLUMN 2: Shows the fatty acid profile of the fat, normalized by weight. Fats from various sources have characteristic profiles, so this pattern can be useful in identification or quality assurance of fat source.
COLUMN 3: Shows the amount of each fat as its triglyceride, along with the sum of the fats added together. Nutrition labeling requires total fat content be expressed as triglycerides.
COLUMN 4, 5, 6, 7 Show the amount of each type of fat, i.e. saturated, monounsaturated, polyun-

Fatty Acid	Normalized by Weight %	Triglyceride in Product %	Saturated Fatty Acid in Product %	Mono Unsaturated Fatty Acid in Product %	***Cis-Cis* Poly** Usaturated Fatty acid in Product %	***Trans* Fatty Acid in Product %**
4:0 Butyric	0.049	0.010	0.009			
6:0 Caproic	0.020	0.004	0.004			
8:0 Caprylic	0.034	0.007	0.006			
10:0 Capric	0.044	0.009	0.008			
11:0 Undecanoic						
12:0 Lauric	0.226	0.046	0.043			
13:0 Tridecanoic						
14:0 Myristic	0.236	0.048	0.045			
14:1 Myristoleic						
14:1 *trans*-Myristelaidic						
15:0 Pentadecanoic	0.034	0.007	0.007			
15:1 Pentadecenoic						
16:0 Palmitic	5.486	1.117	1.064			
16:1 *trans*-Palmitelaidic	0.113	0.023				0.022
16:1 Palmitoleic	0.221	0.045		0.043		
17:0 Margaric	0.069	0.014	0.013			
17:1 Margaroleic	0.064	0.013		0.012		
18:0 Stearic[a]	4.533	0.923	0.884			
18:1 *trans* 6-Petroselenic						
18:1 *trans*-Elaidic	16.518	3.363				3.218
18:1 *trans* 11-Vaccenic						
18:1 Petroselenic						
18:1 Oleic[a]	56.724	11.549		11.053		
18:1 Vaccenic						
18:1 Octadecenoic						
18:2 *trans*-Linolelaidic	4.892	0.996				0.953
18:2 *trans* 9-Linolelaidic						
18:2 *trans* 12-Linolelaidic						
18:2 Linoleic	5.314	1.082			1.035	
20:0 Arachidic	0.737	0.150	0.144			
18:3 g-Linolenic	0.500	0.102			0.098	
20:1 Eicosenic *cis* 5						
20:1 Eicosenic *trans* 11						
20:1 Eicosenic *cis* 8						
20:1 Eicosenic *cis* 11						
20:1 Eicosenic *cis* 13	1.572	0.320		0.307		
18:3 Linolenic	0.447	0.091			0.087	
18:2 Linoleic conjugated **						

18:2 Linoleic conjugated **						
21:0 Heneicosanoic	0.359	0.073	0.070			
18:2 Linoleic conjugated **	0.039	0.008				0.008
18:4 Octadectetraenoic	0.015	0.003			0.003	
20:2 Eisocadienoic	0.034	0.007			0.007	
22:0 Behenic	0.383	0.078	0.075			
20:3 g-Eicosatrienoic						
22:1 Cetoleic						
22:1 Erucic	0.118	0.024		0.023		
20:3 Eicosatrienoic						
20:4 Arachidonic	0.025	0.005			0.004	
23:0 Tricosanoic	0.029	0.006	0.006			
22:2 Docosadienoic						
24:0 Lignoceric	0.177	0.036	0.035			
20:5 Eicosapentaenoic	0.333	0.068			0.065	
24:1 Nervonic	0.187	0.038		0.037		
22:3 Docosatrienoic						
22:4 Docosatetraenoic						
22:5 Docosapentaenoic						
22:6 Docosahexaenoic	0.476	0.097			0.093	
Total	100.00	20.36	2.41	11.48	1.39	4.19
			Saturated Fatty Acid	Mono Unsaturated Fatty Acid	***Cis-Cis*** **Poly** Usaturated Fatty Acid	***Trans*** **Fatty Acid**
Percentage of Fatty Acid Components Based on Total Fat			12.40	58.93	7.14	21.52
**These may be *trans* fatty acids, but because they have conjugated sets of double bonds rather than being isolated *trans* double bonds, they would not be included in the required *trans* fatty acid labeling						

saturated, and containing a nonconjugated *trans* double bond respectively. The sums at the bottom of each respective column are used on nutrition labels to indicate the respective quantity of that category of fat.

Fatty Acids Highlighted in Red are *Trans* fatty acids
Fatty Acids Highlighted in Green are Conjugated Linoleic Acids

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PROPERTIES OF TRANS FAT

The properties of *trans* fatty acids typically fall between those of the corresponding saturated fat, and the *cis* isomer. The *cis* configuration makes it difficult for the fat molecules to pack tightly next to each other, hence a respective *cis* isomer fat is either a lower melting solid or a liquid oil. For instance, the C18 saturated fat, stearic acid, has a melting point of 69°-70° C. The monounsaturated *cis* isomer, C18:1*c*, oleic acid has a melting point of 4°C, while elaidic acid, the *trans* isomer, C18:1*t* has a melting point of 44°-45°C. The configuration of the double bond affects the digestibility of the fat, i.e. the *cis* isomer being more readily processed by human enzymes than the *trans* form. Naturally occurring polyunsaturated fatty acids are primarily in the *cis-cis* configuration with a methylene group between the double bonds. This is a form that is metabolized easily in the human digestive system. Common polyunsaturated fatty acids in food are the all *cis* linoleic acid (C18:2), linolenic (C18:3) and arachidonic acid (C20:4).

About two-thirds of dietary fats consumed are of vegetable origin in the form of margarine and shortening (2) produced by the hydrogenation of vegetable oils. This results in a high intake of saturated fat, unsaturated fat and *trans* fatty acids. A publication in 1993 from the National Cholesterol Education Program (NCEP) confirms that *trans* fatty acids raise LDL-cholesterol levels (3). It also states that *trans* fatty acids account for about 3 % of total calories in the American diet; this amount causes a definite increase in LDL-cholesterol levels, but of course less of an increase than results from the more abundant cholesterol-raising saturated fatty acids. National food consumption survey data also estimate the average consumption of dietary *trans* fatty acids in the U.S., range from about 3 g/day to about 13 g/day (4). The 1995 Dietary Guidelines for Americans, a joint publication from the U.S. Department of Agriculture (USDA) and U.S. Department of Health and Human Services (DHHS) also states that partially hydrogenated vegetable oils, such as those used in many margarines and shortenings, contain a particular form of unsaturated fat known as *trans*-fatty acids that may raise blood cholesterol levels, although not as much as saturated fat (5).

Controlled intervention studies in the USA and other countries provide evidence that *trans*-fatty acids increase the level of LDL-cholesterol (6). Significant association of *trans*-fatty acid with increased levels of LDL-cholesterol was provided from observational studies of general populations (7).

Several countries and the World Health Organization have taken regulatory initiatives on the intake of *trans* fatty acid levels and proposed revisions to the criteria for nutrient content claims. Canada has proposed to revise the definition of "saturated fat free" to less than 0.2 g saturated fatty acids and less than 0.2 g *trans* fatty acids per labeled serving. Canada also proposed to define "low in saturated fat" as not more than 2 grams saturated and *trans* fatty acids combined per reference amount.

In the Federal Register of July, 2003, the US FDA amended its regulations on nutrition labeling to require that the amount of *trans* fatty acids in a food, including dietary supplements, be included in the Nutrition Facts [see box, page 1].

ANALYSIS OF TRANS FAT

Analyses to quantitate the *trans* fatty acid content of fats, oils, margarines and shortenings can be carried out using either gas chromatography (GC), AOAC Official Methods of Analysis 985.21 or 996.06 or infrared spectrophotometry (IR), AOAC 965.34 or 994.14. For the *trans* fatty acid content of foods, the modified AOAC International Official Method of Analysis 996.06 (modified in 1991), for fats in foods by gas chromatography works well. Infrared spectrometry is the classical method for determination of *trans* fatty acids with isolated *trans* double bonds. This method can be used for oils, margarines, shortenings and partially hydrogenated fats and oils where the *trans* fat is more than 1% of total fat (8).

Analytical methods to measure total fat in foods consistent with the NLEA definitions, have been developed, validated, and adopted as Official Methods of Analysis by AOAC International (9). The AOAC International method, 996.06 originally developed at Medallion Laboratories was recently modified at Medallion to include better separation and identification of *cis* and *trans* isomers of fatty acids and this method is applicable to all foods (10). The method was modified to improve the resolution of these unsaturated fatty acids by using an SP2560 100 m x 0.25 mm with 0.20 µm film column. Typical retention times of the fatty acids found in foods on the SP2560 column are presented in table 1. Thus this GC method provides an accurate means of determining *trans* fat in foods. Method 996.06 involves a complete hydrolytic extraction of fatty acids in all forms from the food matrix, followed by methylation using boron trifluoride in methanol and gas chromatographic quantitation of the derivatized individual fatty acids. Hydrolysis conditions are adjusted as necessary depending on the sample matrix (based upon historically proven AOAC methods). Acid hydrolysis is used for most foods, although base hydrolysis is used for certain matrices such as dairy-based foods and food products containing dairy. An internal standard is added at the beginning of the analytical sequence to assure accurate quantitation. An antioxidant is used to protect unsaturated fatty acids against oxidation and against any *cis*-*trans* isomerization during analysis. The quantity of each fatty acid measured is mathematically converted to its respective triglyceride equivalent and the triglyceride equivalents are summed to give the total fat content of the sample. Saturated and unsaturated (and conjugated) fatty acids (not as their triglyceride equivalents) are summed to provide saturated and unsaturated fat levels that comply with the NLEA definition. All fatty acids with an isolated *trans* double bond are summed for total *trans* fat as defined by the regulations. Table 1 also shows a typical analytical report of the fatty acid makeup of a food containing 20% hydrogenated shortening.

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