The Grocery Manufacturers Association (GMA) submits this petition to the Food and Drug Administration (FDA) which demonstrates that partially hydrogenated vegetable oils (PHOs) meet the "reasonable certainty of no harm" safety standard required for food additives and generally recognized as safe (GRAS) ingredients. GMA continues to believe PHOs are GRAS on the basis of common use in foods; nonetheless, GMA is committed to working cooperatively with FDA and submits this food additive petition in the interest of furthering the science and understanding regarding PHOs. This petition details the safety of partially hydrogenated vegetable oils (PHOs) (CASRN, 68334-28-1) manufactured from the following vegetable oils: soy (CASRN 8001-22-7), cottonseed (CASRN 8001-29-4), coconut (CASRN 8001-31-8), canola (CASRN 120962-03-0), palm (CASRN 8002-75-3), palm kernel (CASRN 8023-79-8) and sunflower (CASRN 8001-21-6) oils, or blends of these oils, under the identified conditions of use.

As described in more detail in the sections below, the uses of PHOs satisfy the reasonable certainty of no harm standard for the following reasons:

- Recent intervention studies, meta-analyses and a mode of action (MOA) study support a 1.5 %en/day threshold level below which TFA does not have a significant effect on changes in LDL-C.
- The 90th percentile exposure of TFAs from the petitioned PHO uses combined with the intrinsic ruminant *trans* fatty acids (rTFAs) is 1.33 %en/day, a level of TFA exposure below the 1.5 %en/day threshold.
- The non-threshold linear model relied upon in the previous assessment by FDA did not consider recent intervention studies, meta-analyses and a mode of action study that support a 1.5 %en/day threshold level below which TFAs do not have a significant effect on change in LDL-C.
- Nonetheless, application of a linear dose model similar to that used by FDA in the past, and which FDA asked GMA specifically to consider, demonstrates that the petitioned PHO uses do not increase hypothetical CHD risk.

Triglycerides are the main components of vegetable oils, comprised predominantly of fatty acids present in the form of esters of glycerol. Mixtures of triglycerides composed of both saturated and unsaturated fatty acids are present in vegetable oils. Chemical hydrogenation is the process by which hydrogen atoms are added to unsaturated sites on the carbon chains of fatty acids, in the presence of catalysts, thereby reducing the number of double bonds. "Partial hydrogenation" describes an incomplete saturation of the double bonds, in which some double bonds remain but

may shift to a different position along the carbon chain and alter their configuration from *cis* to *trans*. The *trans* arrangement of hydrogen atoms results in a relatively straight configuration of the fatty acids and increases the melting point, shelf life, and flavor stability of the partially hydrogenated oil.

The *trans* fat components of partially hydrogenated vegetable oils mainly contain the *trans* isomers of oleic acid, the major one being C18:1 *trans*-9 or elaidic acid and C18:1 *trans*-10. Partially hydrogenated vegetable oils also contain smaller amounts of C18:1 *trans*-8, and C18:1 *trans*-11 or vaccenic acid, lesser amounts of C18:2 *trans* and C18:3 *trans*, and very minor levels, generally reported at less than one percent, of *trans* isomers of alpha-linolenic acid may arise during deep-fat frying.

The *trans* fatty acid (TFA) content of PHOs can vary from approximately 5 to 60 percent of the oil, depending on how the oil is manufactured. The average TFA content will depend upon the product type and vary within this range. The general specifications for partially hydrogenated vegetable oils are provided herein. The manufacturers of the PHOs have authorized GMA to incorporate by reference the information in their food additive master files (FMF Nos. 896, 900, 902 and 903) relating to specifications and analytical results for several non-consecutive production batches demonstrating compliance with the PHO specification and certificates of analysis.

Partially hydrogenated vegetable oils have been used for decades in numerous food applications. PHOs are used in the production of foods, food ingredients, processing aids and incidental additives to improve the oxidative stability and/or melting and crystallization properties of food grade oils and fats, and combinations thereof. The data and information compiled by FDA demonstrate that since 2003, *trans* fat intake exposure from PHOs has been reduced by approximately 80 percent. This food additive petition includes uses of PHOs described in 21 CFR 170.3 (o): (i) as an anti-caking, anti-dusting and free flow agent, (ii) as a dough strengthener, (iii) as an emulsifier, (iv) as a formulation aid, e.g. serving as a carrier, binder, film former, tableting aid, (v) as a humectant, (vi) as a lubricant and release agent, either alone or in combination with other components, (vii) as a processing aid or component thereof, (viii) as a solvent and vehicle for fat soluble ingredients including coloring agents, flavors, flavor enhancers and vitamins, (ix) as a stabilizer or thickener, (x) as a surface-active agent, (xi) as a surface-finishing agent or (xii) as a texturizer (tenderness and moisture retention in glutencontaining foods).

PHOs are also used as (xiii) a heat transfer medium (i.e., in deep frying, heat energy is transferred from the heat source to the food in the PHO).

The food additive petition would cover the following uses of PHOs:

(i) PHO, or a blend of PHOs, as a carrier or component thereof for flavors and flavorings, and as a diluent or component thereof for color additives intended for food use, provided the PHOs in the carrier or diluent contribute no more than 300 parts per million (300 mg/kg) TFA to the finished food as consumed.

(ii) PHO, or a blend of PHOs, as an incidental additive, including as a processing aid, or component thereof, provided the PHOs in the incidental additive contribute no more than 50 parts per million (50 mg/kg) TFA to the finished food as consumed.

(iii) PHO, or a blend of PHOs, in the below-listed foods when the standards of identity established under section 401of the Act do not preclude such use:

Limitation - PHO(s)	Food Categories
0.01g TFA/100g	 Protein drinks, such as instant breakfast drinks, meal supplement drinks, meal replacement drinks, etc.
0.02g TFA/100g	 Tea bags and tea powder mixes
0.05g TFA/100g	 Breakfast Cereals, ready-to-eat Chewing gums Processed meat products, such as emulsified or formed products like bologna, hot dogs (frankfurter), meat loafs, sausage, or meatballs, etc.
0.06g TFA/100g	Artichoke hearts, marinatedPancakes waffles, frozen
0.07g TFA/100g	• Meat alternatives, such as meatless bacon, breakfast links, chicken, fish sticks, frankfurters/hot dogs, luncheon meat and meatballs, vegetarian meat loaf or patties, etc.
0.08g TFA/100g	• Frozen entrees or side dishes, such as vegetables for steaming, Salisbury steak, sirloin with gravy, Swedish meatballs, fried chicken, chicken and noodles, chicken with rice and vegetables, turkey dinner, fish dinner, spaghetti and meatballs, lasagna, scrambled eggs with sausage and hash brown, French fries, etc.
0.10g TFA/100g	Pie fillings
0.12g TFA/100g	Candies, caramel
0.13g TFA/100g	Pudding, dry mix
0.15g TFA/100g	Pizza, frozen
0.16g TFA/100g	• Potatoes and potato meal and side dishes, shelf-stable, including mashed potatoes, scalloped potatoes, au gratin potatoes, potatoes with cheese, julienne potatoes, hashbrown potatoes, potato casseroles, etc.
0.17g TFA/100g	• Soups, canned, including meal starter sauces

Limitation - PHO(s)	Food Categories
contribute no more than	
0.19g TFA/100g	 Cream-based or cheese-based frozen entrees or side dishes, such as vegetables in cheese or cream sauces, chicken divan, chicken or turkey a la king, chicken in cream sauce with noodles and vegetables, fish in lemon-butter sauce, enchilada with chicken, macaroni and cheese, pasta with vegetable and cheese sauce, etc. Tortillas and taco shells, flour, soft
0.20g TFA/100g	 Hot cocoa mix, liquid Ice cream sauces, ready to eat Pinto beans, canned Pudding, ready to eat
0.21g TFA/100g	 Frozen dairy-containing desserts (non-ice cream) and novelties, including frozen custards, gelatos, frozen yogurts and sherbets Ice cream products, including tubs, cups, bars, sticks, sandwiches or cones. Pizza rolls, frozen
0.24g TFA/100g	 Cakes and cupcakes, ready-to-eat, with or without filling or icing
0.25g TFA/100g	• Breakfast or dessert pastry-type foods, such as fritters, strudels, Danishes, doughnuts, tarts, turnovers, etc.
0.28g TFA/100g	Seasoning, dry mixes
0.30g TFA/100g	Cheese cake dry mix
0.35g TFA/100g	• Bread or dough products, refrigerated or frozen, such as hand-held sandwiches (i.e., meat or breakfast turnover), cinnamon rolls, dinner rolls, biscuits, pizza crust, bread sticks, etc.
0.40g TFA/100g	Candies, soft chocolate with nut inclusions
0.42g TFA/100g	• Rice cake type products, including rice cakes, rice crackers, puffed rice cakes, popcorn cakes, etc.
0.43g TFA/100g	Pies and cobblers, frozenSalad dressings
0.47g TFA/100g	Cookies, including ready-to-eat cookies, bars and brownies, ready-to-bake cookies, ice cream cones (cone only), etc.
0.50g TFA/100g	 Pasta or rice dish dry mixes, such as macaroni or noodles with cheese mixes, flavored pasta mixes, rice pilaf mixes, flavored rice and/or pasta mixtures, Spanish rice mixes, etc. Savory snacks, such as crackers, crispbread, corn or cornmeal based salty snacks (i.e., corn/tortilla chips and cheese puffs), multigrain chips, pretzels, potato chips,

Limitation - PHO(s)	Food Cotogonies
contribute no more than	Food Categories
	vegetable chips, etc.
0.55g TFA/100g	• Cake, muffin and quick bread mixes, including frozen or refrigerated muffin batters, etc.
0.60g TFA/100g	• Hot cereal mixes, such as oatmeal, etc.
0.70g TFA/100g	• Nutrition or granola bars, such as breakfast bars, snack bars, protein bars, fiber bars, etc.
0.73g TFA/100g	• Soup and bouillon, dry mixes and pastes
0.74g TFA/100g	• Stuffing, dry mix
0.84g TFA/100g	Candies, hard
0.86g TFA/100g	• Sauces, cheese, ready-to-eat
0.87g TFA/100g	Pancake, waffle and biscuit mixes
0.88g TFA/100g	Dough mix for pizza crust
0.90g TFA/100g	• Frostings and fillings, including whipped toppings and icings, confectionery toppings, etc.
1.0g TFA/100g	 Sugar used as doughnuts coating Margarine sticks, including light margarine, unsalted margarine and lactose-free margarine sticks Popcorn, sweet or savory, including ready-to-eat, microwave, etc. Wafer-containing, chocolate-covered confections
1.04g TFA/100g	Cookie, bar and brownie mixes
1.06g TFA/100g	Sauces and gravies, dry mixes
1.07g TFA/100g	• Cream substitutes, such as frozen, liquid or powdered non- dairy creamers, etc.
1.13g TFA/100g	Cultured dips, ready-to-eat
1.28g TFA/100g	• Pie crust mixes
1.70g TFA/100g	• Breading in meat and poultry products, including breading mixes and breadcrumbs
1.75g TFA/100g	• Pie crusts, frozen
1.91g TFA/100g	Candies, soft fruit snack
3.0g TFA/100g	Shortening

TFA in PHOs is determined by the method entitled "Fatty Acid Composition by Capillary GC for Nutritional Labeling", AOCS Ce 1h-05 (09). Compliance with the proposed TFA limits in foods may be calculated based on the TFA content of the PHO and the amount of PHO called for in the food product recipe. Validated analytical methods may be another option for confirming compliance with the TFA limits (e.g., AOCS Ce 1j-07).

HISTORY OF USE AND REGULATORY STATUS

Partially hydrogenated oils have a long history of use in the United States (U.S.) food supply as ingredients in food. PHOs are formed from the partial hydrogenation of vegetable oils and are semi-solid fats at room temperature. This process was developed in the 1930s and has been used commercially for over seventy years. PHOs were originally used to replace butter and lard which are high in saturated fat; today, they are used to improve flavors, increase shelf-life and provide flavor stability of foods. The presence of PHOs in foods increased during the 1980s to replace tropical oils because of their low saturated fat levels. PHOs have no formal regulatory definition. The two most common PHOs marketed today are partially hydrogenated soybean oil and partially hydrogenated cottonseed oil. PHOs have been determined to be Generally Recognized as Safe (GRAS) at levels consistent with Good Manufacturing Practices (GMP) based on historical use in food prior to the 1958 Food Additives Amendment to the FD&C Act. The Select Committee on GRAS Substances (SCOGS) confirmed the GRAS status of soybean PHO in 1976 and two additional PHOs were affirmed as GRAS by FDA in the mid- and late 1980s, i.e., low erucic acid rapeseed (LEAR) oil per 21 CFR 184.1555(c)(2) and menhaden oil per 21 CFR 184.1472(b). FDA based its GRAS affirmations of the LEAR and menhaden PHOs on the fact that those PHOs are comparable, chemically and biologically, to commonly used PHOs such as corn and soybean PHOs.

FDA issued a final rule on July 11, 2003 (68 FR 41434) requiring declaration of the TFA content in the nutrition label of conventional foods and dietary supplements effective January 1, 2006. As a result, many food manufacturers voluntarily reformulated products to reduce the levels of *trans* fat in food products. At the time of the 2003 labeling proposed rule, the daily mean intake of TFAs from PHOs among adults 20 years of age and older was 4.6 g/day (2 %en/day) and total TFA from both animal and PHO sources was 5.8 g/day (2.6 %en/day) (68 FR 41434). In a later assessment by the FDA using updated food composition data from 2009 and 2010 and food consumption data from 2003-2006, intakes of TFAs from PHOs among the US population was found to have dropped by almost 80%, down to 1.3 g/day (0.59 %en/day) (Doell et al., 2012) when TFA data weighted by market share and representative of the TFA levels in foods available in the markets were used.

In 2004 and 2009, two citizen petitions were submitted to the FDA requesting the revocation of the GRAS status of PHOs. On November 8, 2013, the FDA issued a tentative determination that PHOs used in processed food are not GRAS and requested comments, scientific data, and information. (78 FR 67169, Docket No. FDA-2013-N-1317). In response to FDA's request,

GMA submitted comments addressing scientific, legal, and policy considerations relevant to FDA's Tentative Determination, including a review of scientific and other factors demonstrating continued GRAS status of PHOs under current conditions of use (GMA, 2014a,b).¹

ESTIMATED DAILY INTAKE

Estimated daily intakes (EDI) of TFAs from the following sources were computed: a) Intrinsic (background), b) Petitioned PHO Uses, c) Cumulative (Intrinsic + Petitioned PHO Uses).

Food consumption data were obtained from the 2007-10 What We Eat in America (WWEIA) dietary component of the National Health and Nutrition Examination Surveys (NHANES). Consumption data in the WWEIA NHANES are reported on an "as consumed basis." Several databases were used to map the foods reported consumed in WWEIA, NHANES to their respective ingredients, or to identify nutrients and weights of typical portions, etc. including the Food Intakes Converted to Retail Commodities Database (FICRCD), the Food Patterns Equivalent Database (FPED), the Food Commodity Intake Database (FCID), and the Food and Nutrient Database for Dietary Studies (FNDDS).

The USDA National Nutrient Database for Standard Reference (SR) (Standard Reference, Release 27) (SR27), (USDA 2014b) was the source of the intrinsic TFA concentrations used in the assessment of TFA intakes from intrinsic sources. The distributions of TFA levels from intrinsic sources derived from SR27 for those food groups with at least 25 observations were reviewed and Crystal Ball[®] (Release 11.1.2.0.00) was used to determine the best fitting parametric distribution. Stochastic modeling was used to estimate TFA intakes from intrinsic sources. **The mean TFA intake from intrinsic sources for the US 2+ y is 1.04 g/day (95% CI: 0.94 -1.15) or 0.46 %en/day (95% CI: 0.41 – 0.50).** TFA intakes from beef and dairy products constitute almost 75% of the total TFA intake from intrinsic sources.

For the assessment of TFA intakes from the petitioned uses of PHOs in the above-listed food categories (see above and Table 4 within this petition), food codes reported consumed in NHANES 2007-10 were reviewed and mapped to the listed food categories. Several of the listed food categories are for dry mixes with proposed maximum TFA limits for the foods "as sold" and not as consumed. In these cases percent recipe adjustments were applied to derive the TFA

¹ GMA continues to view all past and current uses of PHOs as GRAS for the reasons set forth in our comments. The submission of this Petition does not constitute evidence that GMA has changed its position that past and current uses of PHOs are GRAS.

level in the foods as consumed. Deterministic modeling was used to estimate TFA intakes from petitioned PHO uses. Each food category was assigned a maximum TFA level contributed by the petitioned use of PHOs in that food category and a deterministic assessment was used to estimate the population mean and 90th percentile using the Food Analysis and Residue Evaluation Program (FARE)TM. The mean TFA intake from petitioned uses of PHOs in the select food categories for the U.S. population 2 years (y) and older (U.S. 2+ y) is 0.77 g/day (95% CI: 0.75 -0.79) or 0.34 %en/day (95% CI: 0.33 – 0.35).

TFA intakes from petitioned PHO uses as flavor carriers, color additive diluents and incidental additives (including processing aids) were derived using a *per capita* intake approach. Specifically, the maximum TFA level of 300 ppm for flavor carriers and color additive diluents, and maximum level of 50 ppm for incidental additives, including processing aids, were applied to a 3 kg diet, and assuming 38% of the diet is from processed foods. **The TFA intake from PHO uses as flavor carriers and color additive diluents is estimated to be 0.34 g/day or 0.15** %en/day. The TFA intake from incidental additive (including processing aid) uses of PHOs is estimated to be 0.057 g/day or 0.026 %en/day.

Stochastic modeling was used to estimate combined TFA intakes from intrinsic sources and petitioned PHO uses in the select food categories. The mean *per capita* intake estimates from flavor carrier and color additive diluent uses and from incidental (including processing aid) uses are added to the total deterministically. The mean cumulative EDI of TFAs from all sources (intrinsic + petitioned uses of PHOs) for the US 2+ y is 2.21 g/day (95% CI: 2.10 - 2.33) or 0.98 %en/day (95% CI: 0.93-1.02). The 90th percentile cumulative EDI from all sources (intrinsic + petitioned uses of PHOs) for the US 2+ y is 3.49 g/day (95% CI: 3.26 - 3.73) or 1.33 %en/day (95% CI: 1.24-1.43).

SAFETY INFORMATION

TFA, Blood Lipid Metabolism, and Coronary Heart Disease (CHD) Risks

Similar to all other triglycerides consumed in the diet (IOM, 2005), PHOs have effects on various lipoproteins and other physiological biomarkers related to blood lipid metabolism. Lipid metabolism is not an adverse effect in and of itself. As discussed in detail below, while high levels of TFA intake contributed by PHOs in the diet are associated with changes of low density lipoprotein cholesterol (LDL-C), there are numerous other dietary constituents (e.g., saturated fatty acids) that also have the potential to impact biomarkers of lipid metabolism, including LDL-C. High density lipoprotein – cholesterol (HDL-C) has in some studies, but not others, been shown to be decreased by high levels of TFA intake (IOM, 2005; IOM, 2010).

In addition to LDL-C and HDL-C, several biological markers of CHD risk have been examined to determine whether associations with TFA intake exist, including Lp(a), apo-B, apo-A1, C-reactive protein (CRP), serum triglycerides (TG), and serum cholesterol ratios (i.e., LDL-C:HDL-C and TC:HDL-C). However, the use of lipoprotein ratios (i.e., LDL-C:HDL-C, apoB: apoA1) can be difficult to interpret for magnitude of effect. In addition, the effect of TFA intake on these alternative measures is not consistently reported in the scientific literature and therefore, many recent reviews have not been able to conduct meta-analyses to quantify overall summary effect sizes for these biomarkers for use in a safety assessment.

No Effects of Low Levels of TFA (< 1.5 %en/day) on LDL-C

There is no evidence from randomized, controlled intervention studies on adverse effects attributable to increased risk of CHD at the petitioned low levels of consumption of PHO (i.e., 90% percentile cumulative intake from naturally-occurring TFA and industrially-produced TFA at 1.33 %en/day.) In fact, a recent randomized, controlled intervention study showed no statistically significant difference in impact on LDL-C levels between the test group which consumed 1.47 %en/day TFA and the control group which consumed 0.399 %en/day TFA (Takeuchi et al., 2013).

The Association between Levels of TFA and LDL-C

An association between TFA intake and increased CHD risk has been observed in US prospective cohorts groups consuming high levels of TFA. Intake of TFA in the individual cohorts ranged from 0.44 %en/day to ~5 %en/day at the upper quintile. Two initial studies examining intakes from the Nurses Health Study (NHS) cohort found significant increase in risk for CHD but this relationship was only statistically significant at levels of TFA intake of 3.2 %en/day compared to 1.3 %en/day (Willett et al., 1993) and 2.9 %en/day (Hu et al., 1997). In a 20 year follow-up study of this population (NHS) (Oh et al., 2005) increased risk for CHD was significantly associated with the third and fifth quintile of TFA consumption (i.e., 1.9 %en/day and 2.8 %en/day) but not with the second and fourth quintile (i.e., 1.6 %en/day and 2.2 %en/day) when compared to the lowest quintile of TFA intake of 1.3 %en/day in models adjusted for dietary fat intake, fiber, and fruits and vegetables. While CHD risk was not significantly associated with each increasing quintile of intake, there was a significant trend (p for trend = 0.01). In the Health Professional Follow-up Study (HPFS) the lowest TFA intake level that was associated with an increased risk of myocardial infarction was 4.3g/day (1.6 %en/day) when compared to 1.5 g/day, but this relationship was no longer significant after adjustment for fiber intake (Ascherio et al., 1996). In the Zutphen Elderly Study (Oomen et al., 2001) there was no significant effect of TFA intake on CHD risk in the middle tertile of TFA intake (median TFA intake = 3.87 %en/day) compared to the lowest tertile (median TFA intake = 2.36 %en/day).

The Alpha-Tocopheral Beta-Carotene Cancer Prevention Study (Pietinen et al., 1997) found no significant relationship between risk for major coronary event and total TFA (including all TFA isomers) but this relationship did reach significance with intake of 5.6 g/d (2.0 %en/day) and risk of coronary death. The results from these studies, while not able to demonstrate causality, provide supporting evidence that although a relationship between increased CHD risk and high levels of TFA intake exists, this observed relationship has not been established at low levels of intake below 1.3 %en/day (i.e., the reference group).

Non-Threshold Model

Quantitative estimates of the effect of TFAs in the diet on LDL-C and HDL-C included several meta-analyses of randomized controlled feeding trials in humans. These analyses include a comprehensive review of the dietary intervention trials conducted between the years 1982-2011. Four analyses included LDL-C (Brouwer et al., 2010; Mensink et al., 2003; Mozaffarian and Clarke, 2009; Trumbo and Shimakawa, 2011) as a measured endpoint while the Ascherio et al (1999) analysis examined the relationship between change in TFA intake and change in calculated ratio of LDL-C to HDL-C.

All of these analyses relied upon application of a dose-dependent linear regression method. With the exception of Trumbo 2011, all analyses made an *a priori* decision to arbitrarily set the intercept at zero, regardless of whether this is biologically plausible for lipoprotein endpoints.

It is important to note that the Ascherio et al (1999) study was the first regression analysis to assume the linear dose response between TFA intakes and blood lipids. Moreover, Ascherio et al (1999) included only one study (i.e., Lichtenstein et al. (1999)) that evaluated TFAs at a dose below 3% (0.36 %en/day change in TFA intakes) and the remainder of the test diets ranged from >3 %en/day to 11 %en/day.

Therefore, this linear dose model draws a straight line to zero relying on a single non-significant finding in the low dose region of the dose response. This approach assumes that there is no threshold for TFA intake below which there is no meaningful effect on LDL-C levels.²

² Issues and concerns presented by the use of regression analyses to support a "zero tolerance" standard for food ingredients have been comprehensively reviewed by GMA and others (GMA 2014a,b) and are not further examined here. For completeness, this Petition evaluates PHO safety from several perspectives, including the non-threshold linear approach relied upon in the Tentative Determination and a threshold approach supported by biological plausibility.

Recent Intervention Trials, Meta-Analyses and a Mode of Action Study Support a 1.5 %en/day Threshold

These early analyses included very few intervention diets with TFA intakes in the low dose range (i.e., with the majority at 4 %en/day to 7 %en/day and as high as 11 %en/day) (Brouwer et al., 2010; Mozaffarian and Clarke, 2009; Mensink et al., 2003). Subsequent to the Brouwer et al (2010) regression-analysis, several dietary intervention trials have investigated the effect of TFA on LDL-C in the lower dose region of the response curve with null findings (Takeuchi et al., 2013; Takeuchi et al., 2011, Labonte et al., 2011) indicating a linear dose response model through zero may not be the most appropriate fit for the data.

Researchers on behalf of the Food Standards Australia New Zealand (FSANZ) recently published an updated meta-analysis (Hafekost et al., 2014) of randomized controlled trials with TC, LDL-C or HDL-C as an outcome and feeding duration >3 weeks. The FSANZ metaanalysis was based on 10 data points from 8 trials that included both sources of TFAs (i.e., ruminant and industrial) and reported no significant effect on LDL-C from a 1 %en/day TFA intake in exchange for mono-unsaturated fatty acids (MUFAs) (0.0145 mmol/L; 95%CI, -0.0375, 0.0664 mmol/L). The TFA intakes in these later trials provides LDL-C responses in the lower TFA dose range (<2 %en/day), allowing a focus on the lower end of the dose response curve where previous trials and meta-analyses were lacking data. These new findings support the existence for a threshold TFA intake below which there is no meaningful effect on LDL-C levels. However, the determination of whether a threshold exists would be based on an examination of the proposed mode of action for TFA, which consists of two receptor-mediated events (i.e., increased very low density lipoprotein (VLDL) synthesis and decreased LDL clearance) that are non-linear biological processes (Haber et al., 2015). Therefore, the relationship between exposure and response would be expected to have a threshold as indicated by the underlying biology.

To address this critical research gap, a group of researchers recently modeled the effect of change in TFA intake on change in LDL using a meta-regression approach to improve the accuracy of estimating the relationship between exposure and response by considering the variance in individual studies and weighting the results appropriately. This work was focused at the low end of the dose response with TFA intake. They concluded that TFA intakes within the range of 1.5 %en/day results in "a negligible increase in the TFA-associated change in LDL-C" (Haber et al., 2015). The slight increases in LDL-C that occur within this range of TFA intake are consistent with accepted measurement variability of LDL-C (i.e., 5%) (Schectman et al., 1996).

Haber et al (2015) also presented a description of the mode of action (MOA) to aid the interpolation and/or extrapolation of the effect of TFAs on blood lipids in the low dose region of the response curve. The MOA for the raising of LDL-C levels from TFA intake results from two key events that are both functions of non-linear biological processes: 1) increased LDL production and 2) decreased LDL clearance. The shape of the dose response curve between TFA intake and LDL-C is hypothesized to be non-linear due to feedback loops and homeostatic controls that regulate blood lipid levels and underlie the two key events (Haber et al., 2015).

Other Health Outcomes

A review of published guidance documents on the level of evidence regarding the association between TFA intake and other health outcomes by national and international scientific expert panels within food safety and public health authorities was conducted. Based on the published guidance documents from the scientific expert panels described above, the conclusions consistently reported limited, inconsistent, and/or weak evidence for any effects of TFA on other health outcomes including diabetes, cancer, and obesity.

Ruminant v. Industrial Sources of TFA

The differentiation of the effect between industrially produced PHOs and natural, or ruminant, sources of TFA in the diet has only been evaluated in a small number of studies with inconsistent findings. Several researchers conclude that null results in the meta-analyses measuring the association between ruminant TFAs (rTFAs) and LDL-C are most likely in part due to a lack of statistical power to detect a significant effect due to the small number of studies. Further, the naturally low intakes of TFA from ruminant sources in the diet make it difficult to estimate effects that are typically measured of industrial TFAs (iTFAs) in clinical trials. The majority of rTFA trials reported intakes <2 %en/day while the iTFA trials are typically in the 5-7 %en/day range and as high as 11-12 %en/day. A cross-over study by Motard-Belanger et al. (2008) showed that when a high intake of iTFA (10.2 g/2500 kcal or 3.7 %en) was compared to a moderate intake of rTFA (4.2 g/ 2500 kcal or 1.5 %en/day), there was a significantly higher LDL-C level among the iTFA diet. However, when a high rTFA was achieved (10.2 g/2500 kcal diet or 3.7 %en/day), LDL-C levels were significantly higher compared to a low TFA diet from any source (0.8 %en/day). This study suggests that when comparable levels of rTFA and iTFA are achieved, the effects on LDL-C are also comparable. Several national and international organizations have evaluated the importance of the source of the TFA on risk of CHD for purposes of dietary recommendations. EFSA summarized results of two human intervention studies (Motard-Belanger et al., 2008; Chardigny et al., 2008) and concluded that the available evidence indicates that rTFA and iTFA have similar adverse effects on blood lipids (EFSA, 2010). In the WHO FAO's third Expert Consultation on Fats and Fatty Acids in Human Nutrition, it was concluded that there is evidence to indicate that TFA from natural sources have

similar effects on the TC:HDL-C ratio to those from industrial sources (FAO, 2010). In the WHO Scientific Update on TFA, it was stated that "despite the inherent differences in chemical structure, limited evidence indicates that industrial and ruminant TFAs may have similar effects on serum lipoproteins when ruminant TFA are consumed in sufficient quantities (much higher than seen with usual dietary intakes) in experimental studies" (Uauy et al., 2009).

SAFETY EVALUATION

While several blood lipid and lipoprotein measurements have been evaluated as surrogate biomarkers for the relationship between dietary fat intakes and risk of CHD, LDL-C has an established biological plausibility (IOM, 2005) and has been the focus of dietary guidelines and recommendations (USDA/DHHS, 2010; NCEP, 2002). In particular, an association between TFA intake, LDL-Cs and an individual's risk of CHD was recognized by the IOM/ NAS in the 2005 Dietary Reference Intakes (DRIs) for macronutrients (IOM, 2005) and relied upon in the FDA's Final Rule on "Food Labeling: Trans Fatty Acids in Nutrition Labeling, Nutrient Content Claims, and Health Claims" (68 Federal Register (FR) 133:41434-41456) Although HDL-C has been found to be independently associated with CHD risk, its causal association is not as well established. The IOM's review of the evidence on biomarkers for disease risk states that LDL-C is a known, independent risk factor for CHD with decades of consistent data supporting this conclusion, but noted that the value of HDL-C as a biomarker for CHD is less clear with controlled intervention studies showing inconsistent results and providing little evidence that an increase in HDL-C confers any predicted benefit (IOM, 2010). While recognizing the complex pathway between TFA intake and CHD risk and that any one biomarker will not be the only contributor to the pathway, given the IOM's review on the CHD predictive value of HDL-C and the robust and measured relationship between LDL-C levels and CHD risks from intervention studies (summarized herein), the associated changes of LDL-C with TFA intake and increased CHD risk is the focus of the dose response assessment and safety evaluation in this petition.

Non-Threshold Linear Dose Response Models

As discussed above, non-threshold linear model relied upon by FDA in its Tentative Determination did not consider the more recent studies, analyses and an MOA study supporting a threshold TFA intake level on LDL-C. Nonetheless, to accommodate consideration of the linear regressions reported in previous nutritional and epidemiological assessments of clinical association, and at the direct request of FDA, a linear dose response model was developed to quantify the change in risk of CHD associated with an incremental increase in dietary intake of TFAs. This model assumes that any TFA intake greater than 0 %en/day is associated with an increase in LDL-C levels that are in turn associated with CHD risk. However, as previously reviewed in the above section, the fundamental limitation of this linear approach is that it is not consistent with the mechanistic data describing the key events in the pathway between diet intake and LDL-C concentrations, which are non-linear. The implementation of the dose-response model to quantify the change in risk of CHD that could result from a given increase in TFA intake (e.g. 1 %en/day) requires estimates for two model parameters: 1) the change in LDL-C associated with changes in TFA intake and 2) the associated risk of CHD events with a change in LDL-C.

To ensure that the totality of the evidence is captured, both intervention and observational studies were considered in the dose response model. Two linear dose response models were implemented based on the available data to capture the range of the magnitude of change in LDL-C associated with a unit change in TFA intake (e.g. 1 %en) and the change in CHD risk associated with a unit change in LDL-C. Replacement of TFA with MUFA in the diet was assumed in the modeling. Although this is not reflective of functional replacement scenarios, it was chosen to represent the most conservative case.

The two dose response models were combined with the estimated daily intake of TFA from background (intrinsic) sources, PHO uses, and total (intrinsic sources + PHO uses), which are below the TFA intake level of 1.5 %en/day that Haber et al (2015) identified as the threshold level below which TFAs do not have a significant effect on changes in LDL-C (see below), to estimate hypothetical CHD risks and 95% CI. Hypothetical risk estimates were derived for mean and 90th percentile TFA intakes from intrinsic sources, PHO uses, and from total combined. To characterize the uncertainty surrounding the magnitude and variability in the estimates of changes in LDL-C and hypothetical CHD risks depending on the source of the safety data (i.e. intervention or observational studies), as well as variability and uncertainty associated with the EDI, hypothetical CHD risk estimates were stochastically derived using Crystal Ball[®] (Release 11.1.2.0.00), a spreadsheet application for predictive modeling and simulation. The mean, 2.5th, and 97.5th percentiles of the 1000 simulated risk values represent the mean risk and associated 95% CI corresponding to the mean TFA intake. A similar approach was used for estimating the hypothetical risk and 95% CI corresponding to the 90th percentile TFA intake.

The predicted percent increase in hypothetical CHD Risks and 95% CI associated with the estimated daily intake of TFA from intrinsic sources, PHO uses and total combined (intrinsic sources + PHO) uses overlap. In all cases, whether at the mean or 90th percentile TFA intake, or linear dose response models 1 or 2, the lower bound difference in hypothetical CHD risk estimates between background TFA intake from intrinsic source and TFA intake from PHO uses or TFA intake from both sources (intrinsic + PHO uses) is 0% (non-significant differences). Therefore, the hypothetical CHD risks associated with background TFA intake from intrinsic sources are not statistically significantly different from hypothetical CHD risks associated with TFA intake from petitioned PHO uses or risks associated with total TFA intake (intrinsic + PHO

uses), irrespective of which linear dose response models are applied. This is true at both the mean and 90^{th} percentile TFA intake estimates.

1.5 %en/day Threshold Level

Recently, there have been several dietary intervention trials that investigated the effect of TFA intakes on LDL-C in the lower dose region of the response curve with null findings (Takeuchi et al., 2013; Takeuchi et al., 2011; Labonte et al., 2011). The recent FSANZ meta-analysis that was based on 10 data points from 8 trials that included both sources of TFAs (i.e., ruminant and industrial) reported no significant effect on LDL-C from a 1 %en/day TFA intake in exchange for MUFAs (0.0145 mmol/L; 95%CI, -0.0375, 0.0664 mmol/L) (Hafekost et al., 2014). The TFA intakes in these later trials provide LDL-C information in the lower TFA dose range (<2 %en), allowing a focus on the lower end of the dose response curve where previous trials and meta-analyses were lacking data. These new findings support a threshold level that must be exceeded for TFA intake to have a significant effect on changes in LDL-C.

In order to assess the available clinical data and define the relationship of TFA and LDL-C, Haber et al. (2015) completed a comprehensive MOA evaluation for the effects of TFA on LDL-C, as well as a meta-regression analysis of this effect. These critical assessments clearly show that the prevailing reliance on the linear non-threshold relationship to describe the relationship of TFA and LDL-C is not biologically correct. The meta-regression analysis of 16 published controlled dietary intervention studies with 34 data points that measured the association between TFA intake and LDL-C levels (Haber et al., 2015) concluded that the best fitting model was nonlinear. Haber et al. identified a threshold level of 1.5 %en/day TFA below which TFA intake does not have a significant effect on change in LDL-C.

The cumulative EDI for TFA from all sources (intrinsic + PHO uses) is 0.98 %en/day and 1.3 %en/day at mean and the 90th percentile, respectively for the U.S. 2+ y. The highest cumulative TFA intake is among boys 13-19y, which is 1.4 %en/day at the 90th percentile. These cumulative EDIs are below the threshold level of 1.5 %en/day.

The MOA for the raising of LDL-C levels from TFA intake further supports a threshold level for TFA. Potential effects from TFA can result from two key events that are both functions of nonlinear biological processes: 1) increased LDL production and 2) decreased LDL clearance. The shape of the dose response curve between TFA intake and LDL-C is hypothesized to be nonlinear due to feedback loops and homeostatic controls that regulate blood lipid levels and underlie the two key events (Haber et al., 2015).

Thus, based on a comprehensive review of all available data, a non-linear model provides the best fit model and demonstrates a threshold of effect. Regardless of the approach used, all

available data reviewed support the safety of PHO added to the diet under the conditions of intended use described in this petition.

SAFETY CONCLUSION

Safety assessments of dietary macro constituents such as PHOs are complex and require consideration of the totality of the relevant evidence, including chemistry, metabolism, nutrition, and toxicological factors. The body's biological response to diet, genetic, and lifestyle factors is dynamic, fluid, and constantly changing through regeneration and repair as part of normal processes of metabolism, utilization, and elimination. Numerous dietary constituents, including saturated and unsaturated fatty acids, fiber, and other food components, have a demonstrated effect on surrogate biomarkers of CHD, and are recognized confounders.

The progression to CHD is a complex pathway and research on this pathway has resulted in hundreds of variables that are statistically associated with CHD outcomes. Of these numerous risk factors, the evidence consistently supports that the majority of CHD events can be explained by a smaller group of factors including dyslipidemia, blood pressure, smoking, and diabetes which are often clustered within individuals. However, even within this smaller group of factors, the pathway is complex and mediated or effected by an individual's characteristics, including genetics and the environment, with some individuals with clearly identified risk factors such as high blood pressure or dyslipidemia never experiencing CHD events. Alternatively, to further add to the complexity of the causal pathway to CHD, relatively normal levels of established risk factors within an individual can interact beyond a simple additive relationship and predict events at a multiplicative rate. As such, removal of a single dietary factor, such as PHOs (and associated TFAs), may not result in meaningful risk reduction.

As recognized by the IOM (2005), "... *trans* fatty acids are unavoidable in ordinary, nonvegan diets, [and] consuming 0 percent of energy [from *trans fats*] would require significant changes in patterns of dietary intake...Such adjustments may introduce undesirable effects (e.g., elimination of commercially prepared foods, dairy products, and meats that contain *trans* fatty acids may result in inadequate intakes of protein and certain micronutrients) and unknown and unquantifiable health risks."

In defining the safety standard for a food additive, FDA has long recognized that "[i]t is impossible ...to establish with complete certainty the absolute harmlessness of the use of any substance." A food additive is therefore deemed safe if "there is a reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use." See 21 CFR § 170.3(i). As described further below, the petitioned uses of PHOs under the intended condition of use as described herein satisfy the reasonable certainty of no harm standard.

The PHOs that are the subject of this petition have a long history of safe use in the food supply and meet appropriate food grade specifications and heavy metals limits.

The estimated mean daily intake of TFAs from the PHOs, under the conditions of use in or on foods as described in this petition, among the US 2+ y and adults 20+ y is 1.17 g/day (0.52 %en/day) and 1.18 g/day (0.52 %en/day), respectively. These estimated intakes assume that all foods in a given category will contain TFAs at the maximum proposed limits, and do not account the fact that some products could contain lower TFA levels, thus the true TFA intake estimates from PHOs could be even lower.

Relative to the mean daily intake of TFAs from intrinsic sources (i.e. meat, milk, dairy and other products), which is 1.042 g/day (0.46 %en/day) among the US 2+ y, the estimated mean daily intake of TFAs from the petitioned uses of PHOs in or on foods as an incidental additive, including as a processing aid, is 0.06 g/day (0.03 %en/day), which is approximately 15 times lower than and within the decimal range of the background TFA intake from intrinsic sources.

Relative to the mean daily intake of TFAs from intrinsic sources, the estimated mean daily intake of TFAs from the petitioned use of PHOs in or on foods as flavor carrier and color additive diluent (0.34 g/day or 0.15 %en/day) is approximately 1/3 of the background TFA intake from intrinsic sources. The estimated mean daily intake of TFAs from the petitioned use of PHOs in or on specified foods in the select categories (0.77 g/day or 0.34 %en/day) is lower than the background TFA intake from intrinsic sources.

For expediency, and to respond most directly to questions FDA has raised about TFAs and LDL-C, this Petition examines the safety of the TFA intake resulting from the petitioned PHO uses from several different perspectives. First, the Petition summarizes systematic/evidence-based reviews and meta-analyses in which possible quantitative relationships between TFAs, blood lipids, and CHD were estimated. Then, to address numerous limitations with analyses that assume a linear dose response relationship, and to consider more recent trials and predictive models investigating the effects of lower intakes of TFA, the Petition reviews support for a threshold TFA intake level below which TFA has no significant effect on change in LDL-C levels. Finally, in response to a direct request from FDA, the Petition applies non-threshold linear dose response models to model hypothetical CHD risk from TFA intake under the conditions of intended use described in the petition.

Until recently, most of the published analyses on the relationship between TFA intake, LDL-C and CHD risks based on feeding trials of TFA from PHOs at high doses assumed a non-threshold linear dose-response relationship, arbitrarily forced through zero in the absence of data at the low levels. Based on this assumption and to capture the evidence and associated uncertainty in the magnitude of effect, both intervention and observational studies are included in the

parameterization of two dose response modes. Since there is no evidence of differential effects between ruminant TFA and TFA from PHOs, these dose response models were applied to TFA intake from both intrinsic and PHO uses. Applying these dose response models to the TFA intake from intrinsic sources, TFA intake from PHO uses, as well as total TFA from combined intrinsic and PHO uses, the predicted percent increase in hypothetical CHD Risks and 95% CI associated with TFA intake from intrinsic sources, TFA intake from PHO uses and total TFAs from combined intrinsic and PHO uses overlap, irrespective of the linear dose response models. In all cases, whether at the mean or 90th percentile TFA intake, or linear dose response models 1 or 2, the lower bound difference in hypothetical CHD risk estimates between background TFA intake from intrinsic source and TFA intake from PHO uses or TFA intake from both sources (intrinsic + PHO uses) is 0% (non-significant differences). Therefore, the hypothetical CHD risks associated with background TFA intake from intrinsic sources are not statistically significantly different from hypothetical CHD risks associated with TFA intake from petitioned PHO uses or risks associated with cumulative TFA intake (intrinsic + PHO uses). This is true at both the mean and 90th percentile intake estimates. In other words, the additional TFA intake from the petitioned uses of PHOs does not alter existing hypothetical CHD risk that is assumed through linear modeling.

Early analyses included very few intervention diets with TFA intakes in the low dose range; several dietary intervention trials have investigated the effect of TFA on LDL-C in the lower dose region of the response curve with null findings (Takeuchi et al., 2013; Takeuchi et al., 2011, Labonte et al., 2011) indicating a linear dose response model through zero may not be the most appropriate fit for the data. Indeed, the recent analysis by Haber et al (2015) identified a TFA intake threshold of 1.5 %en/day below which TFA does not significantly affect change in LDL-C. The cumulative EDI for TFA from all sources (intrinsic + PHO uses) is 0.98 %en/day and 1.3 %en/day at the mean and 90th percentile, respectively for the US 2+y. The highest cumulative TFA intake is found among boys 13-19y, which is 1.4 %en/day at the 90th percentile. These cumulative EDIs are below the TFA threshold intake level of 1.5 %en/day, below which TFA has no significant effect on change in LDL-C. Therefore, there can be no inference of any increase in hypothetical CHD risk via the LDL-C mediated pathway as the result of the proposed uses of PHOs.

There is a reasonable certainty of no harm because the 90th percentile exposure of TFAs from the uses of PHOs covered by this petition combined with the rTFAs is 1.33 %en/day; a level of use lower than the 1.5 %en/day threshold supported by the Haber et al analysis (2015) below which TFAs do not have a significant effect on change in LDL-C. Further, even using the non-threshold linear model, PHO uses covered by this petition would not increase hypothetical CHD risk beyond that which is inherent in the human diet due to consumption of meat, milk, dairy and other products. To the extent that humans continue to consume meat for protein, milk/dairy for

calcium, etc., there will always be TFA in the diet. Accordingly, there is a reasonable certainty of no harm from the additional exposure of the petitioned uses of PHOs because the incremental intake of TFA from the petitioned PHO uses does not alter existing hypothetical CHD risk.