USDA Dietary Supplement Ingredient Database Release 4.0 (DSID-4)

Omega-3 Fatty Acid Dietary Supplement Study

Research Summary

Prepared by

Dietary Supplement Ingredient Database Team

Karen W Andrews, Joel Palachuvattil, Pavel A Gusev, PhuongTan Dang, Sushma Savarala, and Fei Han

March 2015
August, 2017, Minor Revisions

US Department of Agriculture
Agricultural Research Service
Beltsville Human Nutrition Research Center
Methods and Application of Food Composition Laboratory
10300 Baltimore Avenue
Building 005, Room 107, BARC-West
Beltsville, Maryland 20705
Tel.: 301-504-0630, Email: mafclinfo@usda.gov

Web address: https://dsid.usda.nih.gov

1. Introduction

Nearly half of US adults report taking dietary supplements (DS) (1). A single serving of a DS may contain amounts of nutrients or other bioactive compounds that exceed their concentration in foods. During the manufacturing of DS, ingredients may be added in amounts exceeding the label claims in order to compensate for losses during shelf life. However, these amounts are not standardized for specific ingredients or among the different manufacturers. DSID pilot studies have also identified a number of ingredients in a variety of product categories with mean content below label claims. Thus, actual ingredient amounts are unknown to consumers and researchers. Epidemiological studies of nutrient intake and health currently use the manufacturer's label as the source of information on ingredient content in dietary supplements.

In order to provide a tool to more accurately estimate intakes from dietary supplements, an analytically validated database for high priority ingredients in dietary supplement products has been developed. The Dietary Supplement Ingredient Database (DSID; https://dsid.usda.nih.gov) is a collaboration of the Agricultural Research Service (ARS)/ Methods and Application of Food Composition Laboratory (MAFCL), and the National Institutes of Health (NIH)/Office of Dietary Supplements (ODS) with other federal partners (National Center for Health Statistics of the Centers for Disease Control and Prevention, Food and Drug Administration, National Cancer Institute of the National Institutes of Health and National Institute of Standards and Technology [NIST] of the Department of Commerce). ODS is the primary funder of the DSID, which builds on the well-recognized strengths of the MAFCL in developing databases that support assessments of intakes of nutrients from foods. For national DSID studies, representative supplement products are purchased and tested by experienced laboratories for their ingredient content.

2. Overview of the Omega-3 Fatty Acid Study

A study of DS containing omega-3 fatty acids (also known as n-3 fatty acids and n-3 long-chain polyunsaturated fatty acids [n-3 LCPUFA]) estimated the relationship between the content reported on dietary supplement labels and analytical values for omega-3 fatty acids. The study focused on the three most abundant (and most commonly labeled) omega-3 fatty acids: alpha-linolenic acid (ALA; C18:3n-3), eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3).

Omega-3 fatty acid dietary supplements were defined for this study as products containing fish oil, plant oil or fish/plant oil blends sold for the primary purpose of increasing omega-3 fatty acids intake.

Products identified as representative of the US market were purchased for this study. Samples of multiple lots of these products were sent to qualified laboratories for the analysis of fatty acids using validated methods and appropriate quality assurance measures. Most, but not all of the products analyzed had a label claim for the amount of the major individual (ALA, EPA, DHA) omega-3 fatty acids. For the final analytical dataset, relationships between label values and analytical values for ALA, EPA and DHA were evaluated by regression analysis. In addition, the variability in the predicted ingredient content was estimated. These statistical results and their National Health and Nutrition Examination Survey (NHANES) application tables were originally released in DSID-3 in 2015. In DSID-4, an online interactive Omega-3 Fatty Acid DS Calculator was released.

3. Sampling Plan

MAFCL develops sampling plans for food and beverages to select sample units representing the US market from multiple geographic areas of the United States (2). MAFCL has also consulted with statisticians to set up sampling plans for purchasing samples of dietary supplement products for the DSID studies. A sampling frame was developed using US Census data to select purchase locations in six counties representative of the US population (Alabama, California, Colorado, Michigan, Missouri and New York).

MAFCL identified omega-3 fatty acid products for purchase based on information from the NHANES DS files, *Nutrition Business Journal* (3-5) reports, national and local store surveys and internet searches. Products were purchased through three channels:

- Mass market (MM) channel: grocery stores, drug stores, club stores (e.g., Costco) and other retail outlets
- Natural/specialty (NS) channel: vitamin specialty shops and natural food stores
- Direct sales (D) channel: multilevel (network) marketers (e.g., Herbalife or Melaleuca) and internet vendors

The sampling plan included both commonly reported omega-3 fatty acid DS (top market share [TMS] products) and products with a lower market share (LMS).

The primary factor in determining which omega-3 fatty acid supplements to include in the TMS category was the frequency with which they were reported in NHANES. Twenty (15 fish and 5 flax oil) supplements were identified as TMS based on NHANES 2003-06 data (the most recent data available at that time), market share information across sales channels, and the observed prevalence of fish vs. plant oil products. Multiple (3-7) lots of these products were purchased by contracted shoppers in the six states listed above.

After the TMS samples were sent for laboratory analysis, a sampling plan for LMS products was developed. Representative LMS retail products were selected based on results from local and regional store surveys conducted in seven US locations in 2008-09. A larger number of omega-3 fatty acid supplement brands and products were observed in stores than were reported in NHANES 2003-06. The NS stores had higher brand/product/form diversity than MM stores, where the same brands were found in many of the stores.

To identify representative LMS brands for purchase, a score was calculated for each brand based on the frequency with which it was reported in store surveys, industry reports, NHANES and other national surveys. Brands were randomly assigned to a type (i.e., fish oil, flaxseed oil, fish oil/plant oil blend) if the brand sold more than 1 type. Brands with the highest scores comprised 75% of planned retail LMS products. Among the brands with lower scores, brand/type products were randomly selected to be included in the remaining 25% of planned retail LMS products. Products were purchased in the same six US counties as the TMS products. MAFCL directed each shopper to buy 37 different LMS products from 20 different MM and NS stores in a specific sequence.

To identify LMS omega-3 fatty acid supplements sold via direct channels, a similar scoring approach was used. A list of more than 50 brand names was compiled using non-retail data from the sources previously mentioned. All of the top-scoring brands were designated for purchase and a random selection of the lower-scoring brands was also purchased.

Multiple lots of 84 different omega-3 fatty acid products (TMS and LMS) were purchased and analyzed in 2008-2010. Supplements were purchased without consideration of the amount of label information about ingredient content. Of the 84 products, 59 contained fish oil, 17 had flaxseed oil and 8 consisted of fish oil/plant oil blends.

4. Laboratory Analysis and Quality Control

Products were sent to MAFCL for processing. Samples of 20 units (usually soft gels) were repackaged, organized into batches and shipped to laboratories for the analysis of ALA, EPA, DHA, octadecatetraenoic acid, docosapentaenoic and eicosatrienoic acid. Results for octadecatetraenoic acid, docosapentaenoic and eicosatrienoic acid are not given in this report.

Laboratories were instructed to homogenize the material from all 20 units before sub-sampling for analysis (per US Pharmacopeia recommendations for analysis of dietary supplements). Two qualified analytical contract laboratories analyzed the sample sets using validated sample-handling protocols and appropriate methods to obtain analytical information about fatty acid levels. Gas chromatography methods were used by both laboratories and the data from both laboratories were combined. The method used by one of the laboratories included a combination of AOAC official method 991.39 (fatty acids in encapsulated fish oils methyl and ethyl esters) and AOAC official method 996.06 (fat total, saturated and unsaturated). The other laboratory used a method that combined AOAC official method 983.23 (fat in foods: chloroform-methanol extraction method) and AOAC official method 996.06.

Quality control (QC) materials were added to each batch of omega-3 fatty acid products for evaluation of laboratory precision and accuracy throughout the study. NIST Standard Reference Materials (SRMs), including NIST botanical oil SRM 1588c, NIST botanical oil SRM 3274 (flaxseed oil), NIST botanical oil SRM 3274 (borage oil) and NIST cod liver oil SRM 1588b were sent in each batch. Also, each batch included a set of product duplicates (2 sets of 20 soft gels of the same omega-3 fatty acid product but with different test sample identification numbers) and at least two in-house control materials that were analyzed for all ingredients in the study. A case of a single lot of an omega-3 fatty acid supplement with a matrix similar to that of the tested supplements was purchased to provide a sufficient amount for each in-house control material.

Analytical retests for ingredients in specific products were conducted to check unusually high or low results, high variability among product lots, or questionable data from batches where QC results showed a bias.

5. Statistical Analysis

Initial statistical analysis focused on the analytical results for ALA, EPA and DHA in products that had a label level for at least one of these fatty acids (71 of 84 products, or 85%). The analytical data, which were reported in mg/g, were converted to mg/serving and mg/day and compared to label levels. The maximum recommended number of servings per day was used to calculate mg/day value. Percent differences from label levels were calculated using the following formula: ((analytical value – label value)/label value) × 100%.

To identify overly influential supplement observations, a jackknife technique was used to calculate Cook's distances and the restricted likelihood distances. Using a SAS mixed model procedure, weighted regression analysis was performed to estimate relationships between the label levels and percent differences from label level for ALA, EPA and DHA. For each fatty acid, the label value was the independent variable and the percent difference from the label value was the dependent variable. Three models (mean, linear and quadratic) were used to fit the data for all three fatty acids. The most complex and statistically significant model was selected. Laboratory, supplement within label level and lot within supplement were modeled as random sources of variation. The accuracy of the models' predictions was assessed with validation techniques.

Predicted analytical values were calculated from the predicted percent difference from the label level using the following formula: label value × (1 + predicted percent difference/100). In the DSID files, these mean predictions are reported as predicted mean percent differences from the label level or as predicted mean values. In addition, the standard error of the mean (SEM), 95% confidence intervals (CI) for the mean, and the standard error (SE) of an individual observation were calculated at each labeled level. Because the regression equation could be used to predict ingredient values of independent supplement samples, SE were adjusted to reflect this expected greater prediction variability.

6. Summary of Results

Based on regression analysis, predicted mean percent differences from label levels for the three major omega-3 fatty acids (ALA, EPA and DHA) are reported per serving (Table 1) and per day (Table 2).

Table 1. Predicted Mean Percent Differences from Label Levels: Per Serving Amounts

Omega-3 Fatty Acid	Range of Predicted Mean % Difference	Most Common Label Level	Mean % Difference at Most Common Label Level	Predicted SEM at Most Common Label Level
ALA	-14.1 to 6.3%	450 mg	3.6%	3.3%
EPA	-5.4%	180 mg	-5.4%	1.1%
DHA	-1.7%*	120 mg	-1.7%*	1.3%

* Not statistically significantly different from label level

Table 2. Predicted Mean Percent Differences from Label Levels: Per Day Amounts

Omega-3 Fatty Acid	Range of Predicted Mean % Difference	Most Common Label Level	Mean % Difference at Most Common Label Level	Predicted SEM at Most Common Label Level
ALA	0.25%*	1350 mg	0.25%*	2.7%
EPA	-5.5%	360 mg	-5.5%	1.1%
DHA	-1.7%*	240 mg	-1.7%*	1.3%

* Not statistically significantly different from label level

Regression estimates for the mean predicted percent differences from label amounts varied by fatty acid. For the per-serving label amounts, the mean percent differences from the most common label level were 3.6% for ALA, -5.4% for EPA and -1.7% for DHA. For the per-day label amounts, the mean predicted percent differences from the most common label levels were 0.3% for ALA, -5.5% for EPA and -1.7% for DHA.

The per-serving results have been applied to products reported in NHANES DS files and the results have been released in the DSID. The per-day results are the best data for comparing ingredient levels among products because many product labels (including 65% of products analyzed in this study) recommend multiple servings per day.

7. Use of DSID data

The regression equations for omega-3 fatty acids released in the DSID predict the mean percent differences from label levels for ALA, EPA and DHA in dietary supplements consumed in the United States. The predicted amounts are linked to labeled levels for three omega-3 fatty acids and are not specific to any brand or supplement. They are included in the DSID for research purposes and are not meant to provide analytical estimates for omega-3 fatty acid levels in individual products.

Measures of variability are reported with predicted means, as discussed previously. The SE and 95% CI for an individual observation are much larger than the SEM and 95% CIs of the means because they represents the error of prediction for an individual product vs. the error of prediction of a mean value for many products. Results predicted by regression for the mean percent difference from label level and SE have been assigned linking codes that may be applied to NHANES DS data files or used for other studies of DS intake.

Documentation about the DSID data files and instructions for appropriate use of the files are described in the report, *DSID-4 Data File Documentation,* available on the "Data Files" page of the website. Please refer to that report for additional information on how best to interpret and use each data file.

An online, interactive, Omega-3 Fatty Acid DS Calculator is now provided on the DSID website. This calculator allows the user to enter ingredient information from products with claimed levels of omega-3 fatty acids and generate the appropriate predicted mean values, SE and 95% CI for those label levels.

9. References

1. Bailey RL, Gahche JJ, Miller PE, Thomas PR, Dwyer JT. Why US adults use dietary supplements. JAMA Intern Med. 2013 Mar 11;173:355-61.
2. Pehrsson PR, Haytowitz DB, Holden JM, Perry CR, Beckler DG. USDA's national food and nutrient analysis program: food sampling. J Food Comp Anal 2000;13:379-89.
3. Nutrition Business Journal. Global Supplement & Nutrition Industry Report. 2008.
4. Nutrition Business Journal. Global Supplement & Nutrition Industry Report. 2007.
5. Nutrition Business Journal. Global Supplement & Nutrition Industry Report. 2006.