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

Children's Multivitamin/mineral (MVM) Dietary Supplement Study

Research Summary

Prepared by

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1. Introduction

Nearly a third of US children use dietary supplements, and multivitamin/minerals (MVMs) were most frequently used (1);(2). 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 Children's MVM Study

A study of children's MVMs (dietary supplements sold for use by children and containing three or more vitamins with or without minerals or other bioactive components) was conducted to estimate the relationship between label values and analytical values for priority vitamins and minerals. Protocols established in the DSID adult MVM study were applied where appropriate.

Products identified as representative of the US market were purchased from nationwide retail outlets and through direct-to-consumer sales channels. Samples of multiple lots of these products were sent to qualified laboratories for the analysis of specified vitamins and minerals using validated methods and appropriate quality assurance measures. The final analytical dataset was statistically analyzed using regression techniques to estimate relationships between label claims and analytically measured ingredient content and to analyze sources for predicted content variability. These study results and their National Health and Nutrition Examination Survey (NHANES) application tables were originally released in DSID-2 (2012) and were updated in DSID-3 (http://dsid.usda.nih.gov). For more information, please visit the DSID "Release History" page.

The purpose of the children's MVM study was to provide nationally representative estimates and measures of variability for specific vitamins and minerals in this dietary supplement product category.

3. Sampling Plan

A national sampling of children's MVMs was conducted for two purposes:

- To provide representative estimates for ingredients in products most commonly consumed by children in the United States (top market share [TMS] products).
- To obtain additional data for lower-market share (LMS) products identified as representative and purchased in different regions of the country.

Information on frequency of intake (market share estimate) for reported supplements was derived from the 2003-04 NHANES dietary supplement data files, which are population weighted to indicate reported usage trends. This information was compared to preliminary reports from NHANES 2005-06 and information from store surveys conducted in 2008. The 21 TMS products identified represented approximately 50% of the estimated market share from the NHANES infant/pediatric MVM subset.

MAFCL consulted with statisticians to set up a sampling frame and product-specific plans for the collection of dietary supplement product samples to ensure that samples purchased were representative of the US market. The purpose of each sampling plan was to select sample units from multiple geographic areas of the United States that, when analyzed, could provide reliable and representative estimates of means and variability for nutrient content.

Using a multistage probability-proportional-to-size approach with US Census data (3), locations for product sampling were selected. For the children's MVM study, purchase locations were identified in six US counties in Alabama, California, Minnesota, Missouri, New York, and Oklahoma. Samples of retail products were purchased from a variety of retail channels, including mass merchandisers (e.g., Safeway, Target, and Wal-Mart) and natural/specialty stores (e.g., Whole Foods and GNC). In addition, some products were purchased from direct marketers (e.g., Amway, Herbalife, or internet-based retailers).

LMS children's MVM products were initially identified using NHANES 2003-2004 and 2005-2006 information. Subsequently, market channel information from the supplement industry and survey results from dietary supplement researchers, local stores and internet searches were used to identify newer products, ensure product availability and to identify any product name changes. Multiple lots of 45 representative LMS products were obtained from the same market channels as the TMS products. Retail products were purchased in the same six geographic locations as the TMS products.

The TMS and LMS products analyzed for this study were purchased in 2008. The analytical content of vitamins and minerals for up to six lots of each TMS product and up to three lots of each LMS product were measured in 2008-10.

4. Laboratory Analysis and Quality Control

The purchased products were sent to MAFCL for processing. Relevant information on each product purchased (e.g., ingredient names and levels, lot number, purchase location and date, and expiration date) was recorded in MAFCL's in-house database. Samples were repackaged and sent for laboratory analysis in defined batches. Each product sample sent to labs contained at least 20 units (tablets, capsules or liquid serving amounts) of the MVM product. Labs were instructed to homogenize at least 20 sample units before sub-sampling for analysis (per the United States Pharmacopeia) recommendations for the analysis of dietary supplements).

Qualified analytical contract laboratories analyzed the sample sets using validated sample-handling protocols and appropriate methods to obtain analytical information about ingredient levels (Table 1).

Table 1. Analytical Methods

Nutrients	Analytical Method(s) Used
Calcium Copper Iron Magnesium Manganese Phosphorus Potassium Zinc	Multi-element inductively coupled plasma spectrometry (ICP) after wet ashing
Chromium	Atomic absorption spectroscopy (AAS), with a matrix-matched standard
Iodine	Three methods: Thiosulfate titration, colorimetry and ICP-mass spectrometry (MS)
Selenium	Hydride generation with AAS
Beta-carotene Retinol Riboflavin Thiamin Vitamin B-6 Vitamin D	High-performance liquid chromatography (HPLC) with ultraviolet detection (UV)
Niacin	Two methods: HPLC with UV detection and microbiological
Folic acid Vitamin B-12	Microbiological methods
Vitamin E	HPLC with fluorescence detection

In some cases, more than one method of analysis was used to accurately measure unusual ingredient forms or low ingredient levels or to replace older methods with newer technologies. For example, iodine levels were determined by three different methods. Thiosulfate titration was initially used for supplements with high levels of iodine and colorimetric measurement for supplements with low levels of iodine. Subsequently, an inductively coupled plasma mass spectrometry (ICP-MS) method was validated in this matrix. ICP-MS results were more consistent than colorimetric and titration results. For this reason, data generated with the ICP-MS method was used to verify or replace the data obtained using the other two methods.

Results for nine vitamins and 11 minerals are reported in this study. For comparison to label levels, the major components of vitamin A (retinol and beta-carotene) were measured separately, converted to international units (IU) and combined to calculate total vitamin A. The methods used to measure the vitamins and minerals for this study are listed in Table 1. Vitamin C was measured, but the results are not reported here due to high variability within lots for specific matrices and other methodology issues that were not resolved until after the expiration date for some products.

Quality control (QC) materials were added to each batch of children's MVM products to evaluate laboratory precision and accuracy on an ongoing basis. NIST Standard Reference Material (SRM) 3280, an MVM matrix with certified values for vitamins and minerals, was sent in each batch to monitor laboratory accuracy. Each batch also included a set of product duplicates (two sets of 20 tablets of the same MVM product with different test sample identification numbers) that were analyzed for all ingredients in the study and at least two in-house control materials. For each in-house control material, we purchased a case of a single lot of an MVM product with a matrix similar to the study samples. A sample of these control materials was analyzed with each batch of product samples to evaluate the precision of the laboratory methods over time in a similar product matrix.

Analytical retests for ingredients in specific products were conducted to check unusually high or low results, high variability among product lots, and questionable data in batches where QC results showed a bias. For each sample analyzed, laboratory results reported in mg/g or µg/g were compared to label levels and a percent difference from the label level was calculated.

5. Statistical Analysis

The labels of many of the children's MVMs products in this study listed more than one age group or serving size (infants 0 to less than 1 year, children 1 to <4 years, and/or children 4 years and older). For some products, the serving size was the same for two age groups; for other products, the serving size was different for each age group.

To evaluate the effect of different serving sizes on the results, two datasets were created and analyzed independently using regression techniques. One dataset contained products with serving size information for children aged 4 years and older (n=59 products), and the other dataset was for products with serving size information for children 1 to <4 years (n=50 products). The labels of the few products for infants also included nutrition information for ages 1 to <4 years using the same serving size. As a result, the infant products are represented in the dataset of MVMs for children aged 1 to <4 years.

To prepare the datasets for analysis, data for each ingredient were identified by supplement, lot, sample and repeated laboratory analysis. All data were weighted using estimated market share information, which was calculated for each product.

To identify overly influential supplement observations, a jackknife technique was used to calculate Cook's distances and restricted likelihood distances. Relationships between the label and percent difference from label were estimated by regression with SAS® mixed model procedures. For each supplement ingredient, the label value was the independent variable and the percent difference from the label level (based on the laboratory analysis) was the dependent variable. Percent differences from label were calculated: ((analytical value – label value)/label value) × 100%. The two regression equations (for serving sizes for children aged 4 years and older and for those aged 1 to <4 years) were derived for each supplement ingredient. Three models (mean, linear, and quadratic) were used to fit the data, and the most complex statistically significant model was selected. The accuracy and stability of the models' predictions were assessed using validation techniques. The selected regression equations were used to predict mean analytical levels for each ingredient in children's MVMs: label value × (1 + predicted percent difference/100). In the DSID files, these mean predictions are shown in application tables as predicted percent differences from the label level or as predicted values in international units (IU), mg, or µg per serving.

In addition, the standard error of the mean (SEM), 95% confidence intervals (CI) for the mean, and the standard error (SE) were calculated at each label level. Because the regression equations could be used to predict ingredient values of independent supplement samples, SE were adjusted to reflect this expected greater prediction variability using sums of squares.

Data on 15 or fewer supplements were available for four ingredients (chromium, potassium, manganese, and selenium), and so these were not analyzed by regression techniques. Descriptive statistics are provided for these nutrients.

6. Results

For this study, regression results are reported for the following 16 vitamins and minerals: folic acid, niacin, riboflavin, thiamin, total vitamin A, vitamin B-12, vitamin B-6, vitamin D, vitamin E, calcium, copper, iodine, iron, magnesium, phosphorus and zinc. Weighted descriptive statistics are provided for chromium, manganese, potassium and selenium.

Regression results for the mean predicted percent differences from label values and the associated SE and 95% CI varied by ingredient and, in many cases, by ingredient level. Detailed results for this study, including regression equation parameters and predicted values, are listed in the DSID "Data Files" page.

For some ingredients, the predicted results for the two datasets (serving sizes for ages 4 years and older and for ages 1 to <4 years) were similar, but the predicted results for other ingredients had significant differences. Results are reported separately for the two serving sizes.

A. <u>Serving Sizes for Ages 4 Years and Older</u>

Data on children's MVMs with label information for children aged 4 years and older (n=59 products) made up the primary dataset for the application of regression analysis results to products in population studies due to these observations:

- The most common age group for children's MVMs purchased for the study was 4 years and older.
- Most products in the study (45/64) contained recommended serving sizes for both children aged 4 years and older and those aged 1 to <4 years. The analytical results for all 45 of these products are in both datasets.
- The NHANES DS files record only one serving size per product. In most cases, the larger serving size is used.

In the DSID, these regression equations are listed as product category 02. The regression results for ingredients in the children's MVM study (for serving sizes for ages 4 years and older) are summarized in Tables 2 and 3 below. Table 2 lists the predicted mean percent difference from the label value for vitamins, and Table 3 does the same for minerals. If a linear or quadratic regression model was used, a range of label levels was predicted. If a means model was used, the predicted mean percent difference was not dependent on the label level. Table 4 provides weighted descriptive statistics for four minerals that were not analyzed by regression techniques.

Table 2. Predicted Means for Vitamins in Children's MVMs (Serving Sizes for Ages 4 and Older)

Ingredient	Range of Predicted Mean Percent Differences from Label Levels	Most Common Label Level per Serving	Predicted Mean Percent Differences at Most Common Label Level	Predicted SEM at Most Common Label Level
Folic acid	17.5%	400 mcg	17.5%	3.7%
Niacin	6.5%	13.5 mg	6.5%	1.0%
Riboflavin	6.1%	1.7 mg	6.1%	1.7%
Thiamin	8.6%	1.5 mg	8.6%	2.1%
Vitamin A	2.0% to 26.8%	2,500 IU	19.7%	4.0%
Vitamin B-12	15.4%	6 mcg	15.4%	2.7%
Vitamin B-6	5.1% to 16.9%	2 mg	6.5%	2.3%
Vitamin D	36.3%	400 IU	36.3%	3.8%
Vitamin E	-2.8% to 0.4%	30 IU	10.5%	4.0%

Table 3. Predicted Means for Minerals in Children's MVMs (Serving Sizes for Ages 4 and Older)

Ingredient	Range of Predicted Mean Percent Differences from Label Levels	Most Common Label Level per Serving	Predicted Mean Percent Difference at Most Label Common Level	Predicted SEM at Most Common Label Level
Calcium	5.0% to 30.4%	100 mg	19.1%	2.2%
Copper	6.4%	2 mg	6.4%	1.5%
Iodine	-1.1% to 65.0%	150 mcg	24.9%	5.7%
Iron	2.0% to 12.8%	18 mg	2.0%	1.3%
Magnesium	4.5%	20 mg	4.5%	0.8%
Phosphorus	3.0% to 15.5%	100 mg	3.0%	1.9%
Zinc	-0.1% to 19.8%	12 mg	0.5%	2.2%

Predicted percent differences from label levels were between 0% and 10% above the label level for five ingredients (niacin, riboflavin, thiamin, magnesium, and copper), between ~15% and 18% above label for two vitamins (vitamin B-12 and folic acid, respectively), and ~36% above label across the entire regression ranges for vitamin D (results were independent of the label levels). However, the mean predicted percent differences from label for the remaining eight ingredients were more variable and depended on label level. The predicted percent differences from label level ranged from -0.1% to ~17% above label for three ingredients (vitamin B-6, iron and phosphorus) and from 2% to ~30% for vitamin A and calcium. For vitamin E and iodine, the mean predicted percent difference from label level ranged from -3% to ~ 40% and from -1.0% to 65% above label, respectively.

Table 4. Descriptive Statistics for Four Minerals Not Analyzed by Regression Techniques in the Children's MVM Study (Serving Sizes for Ages 4 and Older)

Ingredient	Number of Supplements Analyzed	Mean Percent Difference from Label Level	SE for Mean Percent Difference from Label Level
Chromium	10	43.6%	4.0%
Manganese	14	24.0%	5.9%
Potassium	5	76.5%	16.7%
Selenium	9	44.1%	7.3%

Table 4, provides weighted descriptive statistics (mean percent differences from label levels) for four minerals that were not analyzed by regression techniques. These results include data from a number of label levels. These limited results indicate that levels of chromium, manganese, potassium, and selenium in children's MVMs were, on average, substantially higher than the levels listed on the label.

B. Serving Sizes for Ages 1 to <4 Years

Regression estimates for ingredients in children's MVMs with serving sizes for ages 1 to <4 years (n=50 products) were calculated and reported in the DSID separately. The results for the different serving sizes may be useful for certain types of studies. The regression equations for ages 1 to <4 years are reported as product category 02A in DSID Table 1 on the "Data Files" page.

The regression results for serving sizes for ages 1 to <4 years are summarized in Tables 5 and 6 below. Table 5 lists the mean predicted percent difference from label levels for vitamins, and Table 5 does the same for minerals. Table 7 provides descriptive statistics weighted by relative estimated market share for four minerals not analyzed by regression techniques.

Table 5. Predicted Means for Vitamins in Children's MVMs (Serving Sizes for Ages 1 to <4)

Ingredient	Range of Mean Predicted Percent Differences from Label Levels	Most Common Label Level per Serving	Mean Predicted Percent Difference at Most Common Label Level	Predicted SEM at Most Common Label Level
Folic acid	19.4%	200 mcg	19.4%	4.5%
Niacin	6.0%	7.5 mg	6.0%	1.3%
Riboflavin	4.3%	0.85 mg	4.3%	2.2%
Thiamin	11.2%	0.75 mg	11.2%	2.5%
Vitamin A	13.9%	2,500 IU	13.9%	3. 9%
Vitamin B-12	15.8%	3 mcg	15.8%	3.2%
Vitamin B-6	-6.6% to 13.9%	1 mg	8.8%	2.0%
Vitamin D	38.7%	200 IU	38.7%	4. 5%
Vitamin E	20.3	15 IU	20.3%	3.5%

Table 6. Predicted Means for Minerals in Children's MVMs (Serving Sizes for Ages 1 to <4)

Ingredient	Range of Mean Predicted Percent Differences from Label Levels	Most Common Label Level per Serving	Mean Predicted Percent Difference at Most Common Label Level	Predicted SEM at Most Common Label Level
Calcium	4.1% to 31.5%	50 mg	20.3%	2.5%
Copper	5.1%	1 mg	5.1%	1.5%
Iodine	8.3% to 40.3%	75 mcg	18.3%	3. 6%
Iron	3.4%	9 mg	3.4%	1.4%
Magnesium	3.9%	10 mg	3.9%	0.8%
Phosphorus	2.8% to 17.0%	50 mg	2.8%	1.9%
Zinc	0.3% to 8.6%	6 mg	1.0%	1.3%

The results for mean percent differences from label levels in Tables 5 and 6 were similar to the results in Tables 2 and 3, especially at the most common label levels.

However, for some nutrients, there were differences for some label levels due to linear vs. quadratic relationships (e.g., for iodine) or linear vs. mean relationships (e.g., for iron and vitamin A). The labeled serving sizes for children aged 1 to <4 years tended to be lower than those for older children. Therefore, predicted values for the lower label levels may be valuable for intake studies.

Table 7. Descriptive Statistics for Four Minerals Not Analyzed by Regression Techniques in the Children's MVM Study (Serving Sizes for Ages 1 to <4)

Ingredient	Number of Supplements Analyzed	Mean Percent Difference from Label Level	SE for Mean Percent Difference from Label Level
Chromium	8	44.1%	4.3%
Manganese	10	24.5%	7.0%
Potassium	3	87.0%	20.0%
Selenium	5	23.9%	5.6%

Table 7 shows mean percent differences from label levels that were calculated using market share estimates for each product containing one or more of the four minerals not analyzed with regression techniques. The results are based on data from a number of label levels. These limited results indicate that levels of chromium, manganese, potassium, and selenium in children's MVMs were, on average, substantially higher than the levels listed on the supplement label. Results from the descriptive statistics for the mean percent difference from label levels for the four minerals found occasionally in children's MVMs were similar for the two age groups studied (Tables 4 and 7).

7. Use of DSID data

The regression equations for children's MVMs released in DSID-3 predict the mean percent differences from label levels for 16 ingredients in children's MVM supplements sold in the United States. The predicted amounts are linked to label levels for each ingredient and are not specific to any brand or supplement product. These predictions are estimates of the mean ingredient levels per serving and are applicable to children's MVMs reported in *large* population surveys of supplement use. The predicted SEM is the SE for this mean prediction.

The regression equations also estimate the SE for an individual product at specific label levels. This SE is much larger than the SEM because it represents the error of prediction for an individual product vs. the error of prediction of a mean value for many products.

Results predicted by the regression equations for serving sizes for children aged 4 years and older are reported in Table 1 on the DSID-3 website, and these data are linked to NHANES products at the label level reported for those ingredients. The predicted results from the DSID can be used to replace information from labels to more accurately assess ingredient intakes from dietary supplements. Regression equations identified for ingredients in products with serving sizes for ages 1 to <4 years old are also reported in Table 1 on the DSID "Data Files" page but are not linked to NHANES products because multiple serving sizes are not reported in the NHANES DS files.

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, children's MVM calculator is provided on the DSID website. This calculator allows the user to enter ingredient information from MVM labels and generate the appropriate predicted mean values, SE and 95% CI for those label levels.

8. Future Research

Additional DSID studies are underway to evaluate ingredient quantities in prescription prenatal MVMs and green tea dietary supplements.

9. References

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