A collaborative study was conducted to determine total iodine in infant formula and adult/pediatric nutritional formula by inductively coupled plasma-MS (ICP-MS) using AOAC First Action Official Method\textsuperscript{SM} 2012.15. The purpose of this study was to evaluate the method’s intralaboratory and interlaboratory performance and submit the results to AOAC INTERNATIONAL for adoption as a Final Action Official Method for the determination of total iodine in infant formula and adult/pediatric nutritional formula. Upon providing acceptable results for practice samples National Institute of Standard and Technology (NIST) Standard Reference Material (SRM) 1849a and a low-fat adult nutritional powder, 13 laboratories analyzed seven various infant and adult nutritional products including a blind duplicate of each. Products were chosen with varying levels of iodine and included low-fat, soy-based, and milk-based formulas and NIST SRM 1849a. Random identification numbers were assigned to each of the seven fortified test materials. Digestion of the test samples occurred using a potassium hydroxide solution in an oven or open-vessel microwave system. Iodine was stabilized with ammonium hydroxide and sodium thiosulfate after digestion. The solutions were brought to volume followed by filtration. The filtrates were then analyzed by ICP-MS after dilution. Results for all seven test samples met all the AOAC Standard Method Performance Requirements (SMPR\textsuperscript{®} 2012.008) guidelines. The RSD,
ranged from 0.77 to 4.78% and the RSDR from 5.42 to 11.5%. The Horwitz ratio (HorRat) for each result was excellent, ranging from 0.35 to 1.31%. The results demonstrate that the method is fit-for-purpose to determine iodine in infant formula and adult/pediatric nutritional formula.

iodine plays a very important role in maintaining a healthy thyroid gland in humans. Hormones produced by the thyroid are essential for ensuring a healthy body. Benefits include maintaining appropriate metabolism and reproductive function. Perhaps the most critical time for regulation of thyroid hormone production is prenatal, infancy, and childhood when proper growth and development is imperative. Several sources providing optimal amounts of iodine to ensure a well-functioning thyroid gland include fortified infant, pediatric, and adult nutritional formulas. Due to the nutritional benefits provided by iodine, a method for accurate quantification of iodine in these products is of the utmost importance (1).

While a matrix-focused method (AOAC Official Method**SM** 992.24 Iodide in Ready-to-Feed Milk-Based Infant Formula, Ion-Selective Electrode) was available, a dispute resolution method capable of very low and accurate determination of iodine in a variety of infant and adult/pediatric nutritional formula was needed. In 2012 the AOAC Expert Review Panel (ERP) on Nutrient Methods approved and assigned First Action status for AOAC INTERNATIONAL Official Method 2012.15 (2). In August 2013, based on the results of a single-laboratory validation (SLV; 3), AOAC Official Method 2012.15 was chosen by the AOAC ERP as the most appropriate method for the determination of total iodine in infant formula and adult/pediatric nutritional formula to be subjected to a full collaborative study in 2014. Upon successful completion and review of the data, in March 2015 the AOAC ERP approved AOAC Official Method 2012.15 for Final Action.

**Collaborative Study**

Invitations to participate in the collaborative study of AOAC First Action Official Method 2012.15 were sent to 38 laboratories. Twenty-four laboratories expressed interest in participating. Qualification samples were sent to 20 laboratories after four laboratories made the decision not to participate for various reasons.
Six laboratories did not meet acceptance criteria. The remaining 14 laboratories went on to analyze seven test samples (13 laboratories submitted test sample data). Test samples used in this study were obtained from commercial sources and provided by AOAC INTERNATIONAL.

Upon successful completion of two qualification samples, individually prepared test kits, including seven test samples and their blind duplicates, were provided to each collaborator. All powdered samples, with the exception of National Institute of Standards and Technology Standard Reference Material (NIST SRM) 1849a, were required to be analyzed on a reconstituted basis where approximately 25 g of material was diluted with approximately 200 g of deionized water resulting in a total weight of approximately 225 g. Once the test sample was in solution and well mixed, an accurately weighed aliquot of approximately 6 or 12 g (depending on final transfer volume) was subsampled (while continuously stirring) for analysis. This reconstituted solution was discarded after 24 h. Approximately 0.5 or 1 g (depending on final transfer volume) of the NIST SRM 1849a was weighed for analysis. For ready-to-feed (RTF) samples, the laboratory weighed approximately 1 or 2 g (depending on final transfer volume) for analysis. The remaining RTF solutions were transferred to a sealed, brown polypropylene container and held at refrigerated conditions between 2 and 8°C. These solutions were discarded after 5 days.

The test samples were shipped at ambient temperature. Collaborators were asked to store the samples at room temperature before and during analysis with the exception of the RTF samples, which were refrigerated after the initial sampling.

Bulk standards were to be stored as directed on the certificate of analysis/receipt paperwork. Laboratories were directed to follow instructions in the method for storage and shelf life of solutions.

Once analysis of the test samples was successfully completed, study participants were asked to complete and submit a spreadsheet summarizing an abundance of information, including (but not limited to) aliquot (sample weight subjected to analysis), digestion technique used, oven or microwave used, instrument make/model used, solution preparation codes, curve information, analysis batch codes, checklist of 10 different QC/study checks, and results as µg/100 g reconstituted final product. Study participants were asked to record comments (positive or negative) and to provide deviations (if any) from
the protocol.

All test sample data were subjected to statistical analysis per AOAC requirements, which included overall average, RSD₀, RSDᵣ, and Horwitz ratio (HorRat). Cochran’s maximum variance ratio test (2.5% significance level) and Grubbs’ outlier test (single and double, 2.5% significance level) were used to determine outliers.

The method protocol sent to the collaborating laboratories was as described in AOAC First Action Method 2012.15 but with a significantly greater amount of detail. The method below appears as presented in the protocol but now includes improvements and/or additional information as suggested by the AOAC ERP. It also includes minor modifications taken from comments provided by several collaborators, as well as incorporation of components requiring clarification as suggested by the Study Director.

**AOAC Official Method 2012.15**

**Determination of Total Iodine**

**in Infant Formula and Adult/Pediatric**

**Nutritional Formula**

**Inductively Coupled Plasma-MS (ICP-MS)**

**First Action 2012**

**Final Action 2015**

[Applicable to the measurement of total iodine in infant formula and adult/pediatric nutritional formula from 0.5 to 1500 µg/100 g reconstituted final product and for RTF products from 2.5 to 1000 µg/100 g using ICP-MS. This method is not applicable to products containing FD&C Red Dye No. 3 (erythrosine). The iodine from erythrosine is also quantitatively determined by this method; thus, accurate quantification of fortified levels of iodine is not possible.]

*See Table 2012.15A for results of the interlaboratory study supporting acceptance of the method.*

*Caution:*

Refer to Material Safety Data Sheets (MSDS) for safety precautions when using chemicals. Use personal
A. Principle

Digestion occurs using a potassium hydroxide (KOH) solution in an oven or open-vessel microwave system. Iodine is stabilized with ammonium hydroxide and sodium thiosulfate after digestion. The solution is brought to volume followed by filtration. The filtrate is analyzed directly or after dilution by ICP-MS.

B. Safety Considerations

Use only ovens and microwave ovens specifically designed for laboratory use.

The method involves the use of strong bases and concentrated acids. Avoid spills, inhalation, and exposure to human tissues.

Oven and microwave digestion procedures involve moderately elevated temperatures. Carefully remove samples and allow cooling before removing the lids from the digestion vessels.

C. Chemicals and Reagents

(a) **KOH pellets.**—Certified ACS grade (Fisher Scientific, Fairlawn, NJ). *(Note: KOH may contribute background levels of iodine.)*

(b) **Ammonium hydroxide 28–30% (NH₄OH).**—Certified ACS PLUS (Fisher Scientific).

(c) **Sodium thiosulfate (Na₂S₂O₃).**—≥99.99% metal basis (Fisher Scientific).

(d) **Surfactant (e.g., Triton® X-100).**—Sigma (St. Louis, MO).

(e) **Nitric acid concentrated (HNO₃).**—OPTIMA (high purity; Fisher Scientific).

(f) **Perchloric acid 70% (HClO₄).**—Reagent ACS (Fisher Scientific).

(g) **Purified water.**—18 MΩ/cm.

*Note: Equivalent chemicals and reagents may be substituted.*
D. Apparatus

(a) Polypropylene (PP) tubes.—Assorted sizes, use as received; 50 mL PP DigiTUBES® (Part No. 010-500-261), 100 mL PP DigiTUBES (Part No. 010-501-263); SCP Science (Montreal, Canada).

(b) Oven (i.e., warming/drying oven).—Isotemp oven Model 6921 (Fisher Scientific).

(c) Open-vessel microwave digestion unit (optional).—MARS 5 or MARS 6 (CEM Corp., Matthews, NC).

(d) Analytical and top-loader balances.—Sensitive to 0.0001 and 0.01 g, respectively (Sartorius, Goettingen, Germany).

(e) ICP-MS system.—ELAN DRC II (PerkinElmer, Waltham, MA).

(f) Autosampler for ICP-MS.—SC4-DX (Elemental Scientific, Inc., Omaha, NE).

(g) Adjustable (electronic or manual) volumetric pipets.—Eppendorf (Hamburg, Germany). Capable of volumes 100–5000 μL.

(h) Re-pipet volumetric dispensers.—Adjustable volume.

(i) PP or Teflon bottles for storage of reagents.

(j) Disposable plastic syringes.—e.g., 10 mL with LuerLok.

(k) Syringe filters with 1 μm membrane.—Non-sterile glass fiber B (Part No. SLPBDZ5NK; EMD Millipore, Corp., Billerica, MA).

(l) Beakers.—Assorted sizes.

(m) Stir bars.—7.9 × 50 mm, assorted sizes (VWR, Chester, PA).

(n) Stir plate.—Adjustable speed, Corning (Corning, NY) or equivalent.

(o) Pump tubing.—Peristaltic, black/black two-stop polyvinyl chloride (PVC), 0.76 mm id (SCP Science, Champlain, NY), used for introducing carrier solution.

(p) Pump tubing.—Peristaltic, orange/green two-stop PVC pump tubing, 0.38 mm id (SCP Science), used for introducing internal standard (IS) solution.

Notes: Equivalent apparatus may be substituted.

All laboratory plasticware should be single-use whenever possible. If reuse is necessary, wash using
10% HNO₃, then rinse thoroughly with purified water prior to use. When needed, general laboratory acid-washed glassware may also be used.

Filter membranes <1 µm (e.g., 0.25 or 0.45 µm) may be used.

Adherence as close as possible to the recommended ids of the pump tubing is critical. The ratio of the pump tubing id (0.76 mm) used for the carrier solution to the pump tubing id (0.38 nm) used for the IS solution may be used as a guideline (0.76/0.38 = 2). For best performance, the ratio should remain as close to 2 as possible. Vast differences in id between the carrier solution pump tubing and the IS solution (e.g., 1.02/0.19, respectively) may result in poor accuracy.

E. Instrument and Parameters

*Instrument.*—ICP-MS PerkinElmer ELAN DRC II, or equivalent.

*Mode.*—Standard (STD).

*Gas.*—Argon (≥99.998%, high purity).

*Rinse.*—0.1% Triton/1% NH₄OH in purified water.

*Sweeps/reading.s.*—20.

*Readings/replicate.*—One.

*Replicates.*—Three.

*Nebulizer gas flow.*—Optimized daily.

*Auxiliary gas flow.*—1.2 L/min.

*Plasma gas flow.*—15.00 L/min.

*Lens voltage.*—Optimized daily.

*ICP radio frequency power.*—1500 watts.

*(m) Peristaltic pump.*—Rate optimized.

*Notes:* Parameters of other manufacturer’s instruments may be optimized accordingly to ensure the instrument’s minimum daily performance requirements are met.

All analyses must be performed using the STD mode. (Use of a reaction or collision gas is not required
or allowed.)

F. Reference Standards

Iodide 1000 ppm standard solution in H₂O.—SPEX CertiPrep (Metuchen, NJ.)

(b) Iodide 1000 ppm standard solution in 1% triethanolamine (TEA).—Inorganic Venture (Christiansburg, VA.)

Notes: Either stock iodide reference solutions may be used for intermediate and working standard solutions preparation. The remaining source may be used as a continuing calibration verification (CCV) standard.

Equivalent reference standards may be substituted.

“Iodide” may be referred to as “iodine” throughout this method.

G. Internal Standard

Praseodymium 10 ppm standard solution in 5% HNO₃.—Inorganic Ventures.

Notes: Individual values of iodine will be reported for each test sample using praseodymium as the IS. Equivalent stock IS solutions may be substituted.

H. Procedure

(a) Reagent solutions preparation.—Note: Prepare all reagent solutions as recommended by either weight/volume (w/v) or volume/volume (v/v). Adjusting for purity and/or concentration is not required.

(1) 5% KOH solution.—Dissolve 25 g KOH pellets in an appropriate amount purified water, then dilute to 500 mL with purified water. This solution may be added using a re-pipet volumetric bottle top dispenser. Store this solution at room temperature. Reagent expires 6 months after preparation date.

(2) Stabilizer concentrate.—Dissolve 5 g Na₂S₂O₃ in an appropriate amount purified water, add 50 mL NH₄OH, then dilute to 500 mL with purified water. The resulting concentration is 10% NH₄OH and 1% Na₂S₂O₃ in purified water. Store this solution at room temperature. Reagent expires 6 months after preparation date.
(3) **Wash solution (rinse).**—Dissolve 2 g Triton X-100 in an appropriate amount of purified water, add 20 mL NH₄OH, then dilute to 2 L with purified water. The resulting concentration is 1% NH₄OH and 0.1% Triton X-100 in purified water. This solution may be added using a re-pipet volumetric bottle top dispenser. Store this solution at room temperature. Reagent expires 6 months after preparation date.

(4) **Diluent.**—Dissolve 10 g KOH pellets and 0.4 g Na₂S₂O₃ in an appropriate amount of purified water, add 4 mL NH₄OH, then dilute to 2000 mL with purified water. Store this solution at room temperature. Reagent expires 6 months after preparation date. Alternatively, for a smaller volume, dilute 50 mL 5% KOH and 10 mL stabilizer concentrate to 500 mL with purified water. Store this solution at room temperature. Reagent expires 6 months after preparation date.

*Note:* The resulting concentrations for both preparations are 0.5% KOH, 0.2% NH₄OH, and 0.02% Na₂S₂O₃ in purified water.

(5) **Conditioning solution.**—Prepare by aliquoting 25 mL 5% KOH solution, then diluting to 250 mL with purified water. This solution is used to prepare the instrument for analysis. The resulting concentration is 0.5% KOH. Store this solution at room temperature. Reagent expires 6 months after preparation date.

(6) **Carrier solution.**—Equivalent to the wash solution. The carrier solution is used to deliver the sample solution to the nebulizer through the ICP-MS autosampler introduction system. The carrier solution is introduced via a peristaltic pump using black/black two-stop PVC pump tubing (0.76 mm id). Store this solution at room temperature. Reagent expires 6 months after preparation date.

(b) **Standard solutions preparation.**—*Notes:* Stock solutions are stable until the date indicated on the certificate of analysis. Intermediate, calibration, continuing calibration verification, and IS solutions are stable at room temperature until the earliest expiration date of all components used to prepare the solution.

All calibration standards, continuing calibration verification, continuing calibration blank, and IS solutions are analyzed as prepared. Do not carry these solutions through sample preparation or digestion.
(1) **Stock iodine and praseodymium solutions.**—Purchase of stock iodine and praseodymium standard solutions with accompanying certificates of analysis is recommended.

(2) **Intermediate stock standard (ISS) iodine solutions.**—Prepare the ISS iodine solutions according to Table 2012.15B.

(3) **Calibration standard (CS) iodine solutions.**—Prepare the solutions according to Table 2012.15C.

(4) **Intermediate continuing calibration verification (ICCV), continuing calibration verification (CCV) iodine solutions, and continuing calibration blank (CCB).**—Prepare the ICCV, CCV standard solutions, and CCB blank according to Table 2012.15D.

*Note:* A CCV must be prepared from a second source stock solution (e.g., purchased from another vendor) other than that used for the CS solutions.

(5) **IS solutions.**—Prepare the IS solution according to Table 2012.15E. The IS concentration typically used for analysis is 30 ppb praseodymium (Pr).

*Notes:* Ideally, the intensity generated for the IS should be similar to the intensity of iodine standard at the mid-point of the standard curve.

As some ICP-MS instruments provide greater sensitivity, the concentration of Pr may be adjusted accordingly to provide intensities similar to the intensity generated by the 50.0 ppb iodine standard.

(c) **Reconstitution.**—*Note:* All powdered samples, with the exception of NIST SRM 1849a, are required to be analyzed on a reconstituted basis. Do not reconstitute RTF samples.

Accurately weigh approximately 25 g powdered test sample into an appropriate vessel (e.g., 400 mL beaker) and record the weight. Without zeroing the balance, add water to make approximately 225 g. Record the sample + water weight. Place a stir bar in the mixture and stir on a stir plate to form a homogeneous slurry/suspension. Proceed to **Sample preparation (d).**

*Note:* This reconstituted solution should be discarded after 24 h.

(d) **Sample preparation.**—Weighing (after weighing all materials, proceed to **Addition of reagents (e)).**

(1) **Reconstituted material.**—Accurately weigh an aliquot of approximately 6 g reconstituted test
sample into a 50 mL DigiTUBE® or 12 g into a 100 mL DigiTUBE.

(2) *NIST SRM 1849a.*—Accurately weigh approximately 0.5 g NIST SRM 1849a into a 50 mL DigiTUBE or 1 g into a 100 mL DigiTUBE.

(3) *RTF material.*—Accurately weigh approximately 1 g of the RTF test sample into a 50 mL DigiTUBE or 2 g into a 100 mL DigiTUBE.

*Note:* The remaining RTF material should be transferred to a sealed, brown PP container and held at refrigerated conditions between 2 to 8°C. These solutions should be discarded after 5 days.

(4) *Blank.*—Designate at least one 50 mL or 100 mL DigiTUBE digestion vessel as the digest blank. The digestion blank(s) should be treated in the same manner as the samples.

(e) *Addition of reagents (after adding all reagents and mixing, proceed to Oven digestion (f), or Open vessel microwave digestion (g)).*—(1) *Water.*—Add 10 mL purified water to each 50 mL DigiTUBE or 20 mL to each 100 mL DigiTUBE.

(2) *5% KOH.*—Add 5 mL 5% KOH if material was weighed into a 50 mL DigiTUBE or add 10 mL of 5% KOH if material was weighed into a 100 mL DigiTUBE.

(3) *Mixing.*—Seal the vessels and swirl or use a vortex apparatus to mix. Avoid inverting as this may allow sample to adhere to the inner walls of the vessel above the level of the digestion solution.

(f) *Oven digestion.*—(1) *Digestion/extraction.*—Digest samples in an oven set to maintain 105 ± 5°C until the dissolution of iodine is complete, approximately 1 h.

*Notes:* The digestion vessels may either be tightened completely or loosened slightly while in the oven. Carefully swirl by hand each digestion vessel approximately halfway through the digestion/extraction procedure.

(2) *Addition of stabilizer.*—After removal of samples from the oven, add 1 mL of stabilizer concentrate to the 50 mL DigiTUBE samples or add 2 mL if material was weighed into a 100 mL DigiTUBE. Allow samples to cool to room temperature.

*Note:* Alternatively, allow samples to cool to room temperature first, and then add the stabilizer.
concentrate.

(3) Final volume.—If 50 or 100 mL vessels were used for digestion, bring samples to a final volume of 50 or 100 mL respectively, with purified water.

(4) Capping/mixing.—Cap all vessels, and then invert to mix thoroughly.

(g) Open vessel microwave digestion.—(1) Digestion/extraction.—Place the digestion vessels into the carousel of the open-vessel microwave digestion unit. If less than the maximum capacity is to be digested, distribute the vessels evenly throughout the carousel. Digest the samples in the microwave unit until the dissolution of iodine is complete. See Table 2012.15F for suggested open-vessel microwave digestion parameters.

Note: Vessel caps should be loosened slightly (from fully tightened) during the digestion procedure. Use caution: Ensure vessels do not completely seal (bursting hazard) or overheat (melting may occur). Alternatively, instead of just loosening the caps, drill small holes (approximately 3 mm) in the caps. This way the caps can be tightened, but venting (thus the “open” vessel) can occur. Caps may be reused after acid washing.

(2) Addition of stabilizer.—After removal of samples from the oven, add 1 mL stabilizer concentrate to the 50 mL DigiTUBE samples or add 2 mL if material was weighed into a 100 mL DigiTUBE. Allow samples to cool to room temperature.

Note: Alternatively, allow samples to cool to room temperature first, and then add the stabilizer concentrate.

(3) Final volume.—If 50 or 100 mL vessels were used for digestion, bring samples to a final volume of 50 or 100 mL, respectively, with purified water.

(4) Capping/mixing.—Cap all vessels, and then invert to mix thoroughly.

(h) Sample filtering.—(1) Filtering.—Filter each sample solution by filling a disposable syringe with the digested sample solution, attach a 1 μm membrane filter, and then filter an adequate amount (e.g., at least 5 mL) into an appropriate vessel (e.g., 15 mL PP centrifuge tube or autosampler vial) to be used for analysis.
Notes: Samples may be difficult to filter. Use of multiple filter membranes may be required. To ease filtration, allow the inverted sample digestates to rest for a period of time (e.g., 1 h) before filtering.

Digested sample solutions may be stored at ambient temperature. Samples may be stored at ambient temperature indefinitely, as long as the results for the applicable digest blank(s) and/or control sample(s) are acceptable when analyzed.

(i) Sample dilution.—Aliquot 5 mL of each sample’s filtrate into an appropriate volumetric vessel and then bring to a final volume of 10 mL with diluent.

Note: Analyze all samples diluted 5 to 10 mL as directed above.

I. Determination (Instrument and Parameters see Section E)

Notes: All analyses must be performed using the STD mode. (Use of a reaction or collision gas is not required or allowed.)

Prior to conditioning, calibration, and sample analysis, ensure the instrument is optimized to meet the manufacturer’s minimum daily performance requirements.

Conditioning.—Condition the ICP-MS sample introduction system. Analyze the conditioning solution while concomitantly introducing IS solution online (e.g., through a mixing block or T) until conditioned (approximately 1 h). The IS solution is introduced via a peristaltic pump using orange/green two-stop PVC pump tubing (0.38 mm id). After conditioning, begin to aspirate carrier solution while continuing to add IS. Analyze samples using ICP-MS. Ensure the wash solution (rinse) is available and ready for use to rinse out the sample lines and introduction system between each analysis.

Notes: If acidic sample solutions are typically analyzed on the ICP-MS system, perform a thorough cleaning of the entire sample introduction system prior to conditioning. Background counts for both iodine and the IS should be relatively stable (e.g., not ascending or descending).

A dedicated set of cones ( sampler and skimmer), if possible, is recommended. Analysis of acid-type (e.g., HNO₃) matrixes with the same set of cones used for iodine analysis may increase conditioning time or produce elevated background levels.
Analyzing several (e.g., at least six) digested samples prior to calibration is recommended. Introducing and analyzing actual digested sample solutions increases conditioning efficiency.

Possible additional maintenance: Due to the nature of the digestion/extraction solution (i.e., KOH) and the amount of organic material in the sample solutions, additional maintenance may be required (as compared to typical acid matrix digestions/analysis). Lenses in instruments and/or lens stack assemblies may require more frequent cleaning. Once cleaned, a period of reconditioning may be required.

Calibration.—In addition to a calibration blank, working standards of 0.250, 0.500, 1.00, 10.0, 50.0, and 100 ppb are used. Calibrate the ICP-MS system using an autosampler or manually.

Notes: The curve type used should be linear, forced through the calibration blank.

All standards must be included in the calibration curve.

The 0.250 ppb signal must be ≥1.5 times the calibration blank signal. Consistent background throughout the entire analytical run is imperative for a successful analysis. This will be evident based on the results obtained for the CCB.

Sample analysis.—Analyze a 5 to 10 mL dilution of each digested filtered sample using ICP-MS.

Notes: A 5 to 10 mL dilution is preferable and required in order to achieve a reporting limit of 0.5 µg/100 g as reconstituted final product or the limit of 2.5 µg/100 g for RTF samples.

Diluting the samples reduces the matrix load on the plasma and may reduce frequency of maintenance (e.g., cleaning cones).

For other applications, samples digested with 5% KOH solution may be analyzed directly or diluted (if necessary) so that the iodine concentration will fall within the calibration range. Alternative volume aliquots may be prepared by placing an aliquot of the filtrate into an appropriate volumetric vessel, and then diluting to an appropriate final volume with diluent. Greater dilutions, such as 1 to 18 mL, would achieve a higher upper reporting limit (e.g., 1500 µg/100 g reconstituted final product).

Data acceptability.—The calibration curve must include a calibration blank (as a calibration point). The calibration curve must have a correlation coefficient \( r \) ≥ 0.998 to be acceptable.

The individual back-calculated calibration standard concentrations must be within 90–110% of the
theoretical concentrations to be acceptable.

The 0.250 ppb signal must be ≥1.5 times the calibration blank signal. Consistent background throughout the entire analytical run is imperative for a successful analysis. This will be evident based on the results obtained for the CCB.

A CCB is analyzed after calibration, at least every 10 samples, and after the last sample in the analysis batch to monitor background. A CCB should be of the same matrix as the standards used for calibration. Iodine levels ≤30% of the lowest calibration standard are considered acceptable.

With each batch of samples, at least one digest blank should be prepared in the same manner as the samples. An iodine result of ≤30% of the lowest calibration standard is considered acceptable.

A CCV standard solution containing iodine from a source other than that of the calibration standards is used to verify acceptable calibration and to evaluate the ongoing performance of the instrument. The CCV should be analyzed after calibration, at least every 10 samples, and after the last sample in the analysis. A CCV should be of the same matrix as the standards used for calibration. A CCV result is considered acceptable when the result is within 90–110% of theoretical.

**J. Calculations**

If a reconstitution was performed, use the following equation:

\[
\frac{[(C \times V) \times D]}{\text{WRA}}/10 = S
\]

where \( C = \) sample concentration (ng/mL, sample solution reading on the curve); \( V = \) volume (mL, final volume after digestion); \( D = \) dilution factor (if not applicable, enter 1); \( \text{WRA} = \) weight (g) of reconstitution aliquoted during *sample preparation* (d); and \( S = \) sample concentration of iodine (µg/100 g reconstituted “as fed” basis).

If a reconstitution was not performed, use the following equation:

\[
\frac{[(C \times V) \times D]}{W}/10 = S
\]

where \( C = \) sample concentration (ng/mL, where sample solution reads on the curve); \( V = \) volume (mL,
Results and Discussion

Seven samples were analyzed by 13 independent laboratories. These laboratories were from industry, contract research organizations, and government institutions. Laboratories were located in North America, Europe, and Asia. The seven samples for the collaborative study were selected to represent varying levels of iodine in a variety of applicable matrixes. The matrixes included an SRM, two different lots of milk-based infant formula RTF, a child powder formula, an adult nutritional low-fat powder, soy-based infant formula powder, and milk-based infant formula powder. Table 1 presents the diversity of ICP-MS instrument makes and models used by collaborating laboratories to generate data for the study. This table also attests the versatility of the method by showing that either of two digestion options provides the same results.

Laboratories were asked to record any deviation from the method protocol and to provide comments in general about the method. Of the 13 laboratories, three did not provide any comments. A significant majority of the remaining 10 study participants comments were related to the QC/study check criteria included on the test sample data summary spreadsheet. One of the QC/study check questions asked of participants was whether the analysis was performed on the same day as digestion, and if not, what was the length of time between digestion and analysis. Many participants responded yes or within 24 h. The amount of time from digestion to analysis for the remainder of the laboratories typically ranged from 2 to 7 days. One laboratory stated a period of 17 to 50 days between digestion and analysis. Additional QC/study check questions asked of participants included:

1. Did you perform the analysis in standard (STD) mode?

2. Were all individual back-calculated calibration standard concentrations within 90–110% of theoretical?

3. Was the signal of the lowest calibration standard $\geq 1.5$ times the blank signal?
(4) Were all CCB results run before, during, and after samples within ≤30% of the lowest calibration standard's nominal concentration?

(5) Were all digest blank results ≤30% of the lowest calibration standard's nominal concentration (≤0.075 ng/mL)?

(6) Were all CCV results (before, during, and after samples) within 90–110% of standard's nominal concentration (9.00–11.0 ppb)?

(7) Were all RSD values for iodine and praseodymium ≤5%?

Very few comments were provided pointing out values that exceeded these criteria. All participants indicated the analysis was performed in the STD mode. When limits were breached, exceedance was not significant. In three instances, digest blank or CCB results were 31.2, 32.4, and 34.4% of the lowest calibration standard. There were three occurrences where the individual back-calculated lowest calibration standard concentration (0.250 ppb) exceeded the assigned acceptance range exhibiting recoveries of 81.1, 83.3, and 113% of theoretical. One laboratory commented that the RSD of one sample analysis exceeded the assigned ≤5% criteria. This same laboratory commented “The last CCV (at end of run) was 8.84 ppb (ideally no lower than 9.00 ng/mL).” Other deviations noted by two laboratories were minor. One laboratory used sealed 55 mL digestion vessels and then transferred the samples “...to a final volume of 50 mL in another container.” This same laboratory also used 0.25 µm syringe filters instead of the recommended 1 µm syringe filters. One laboratory altered the calibration standard scheme. Instead of using the recommended 0.250, 0.500, 1.00, 10.0, 50.0, and 100 ppb calibration standard curve points, a 5.00 ppb was added and the 100 ppb was deleted resulting in 0.250, 0.500, 1.00, 5.00, 10.0, and 50.0 ppb points. One participant mentioned issues with RSDs and IS drift when the method had not been performed on their instrument for a period of time but commented that adequate conditioning resolved the issues. The Study Director thoroughly reviewed all deviations and was confident, based on an overall assessment of the QC check information provided and statistical analysis of the results, that no impact to the data was evident.

All of the laboratories’ results are presented in Table 2. Table 2012.15A shows the statistical
evaluations for all the samples analyzed in this multilaboratory testing study. The RSD, ranged from 0.77 to 4.78%, and the RSD ranged from 5.42 to 11.5%. The HorRat values for all results ranged from 0.35 to 1.31%. Repeatability and reproducibility for all seven samples were below the limits set forth in AOAC SMPR 2012.008 (4). All 13 laboratories’ data were included for statistical analysis for both RTF samples. Outliers for the powdered reconstituted samples and NIST SRM 1849a were removed prior to performing statistical analysis based on Cochran’s and Grubbs’ outlier tests.

Upon completion of the collaborative study, comparison of data for the reconstituted powders revealed five laboratories’ results (Laboratories C, E, H, I, and L) were approximately 9 to 10 times higher than the other eight laboratories’ data. The other eight laboratories’ data agreed with values obtained during the SLV. The consistent factor of 9 to 10 suggested a calculation error, which agreed with the reconstitution factor (e.g., 225 g ÷ 25 g = 9). After correspondence with the five laboratories whose data were in question, it was evident that a misunderstanding of the calculation requirements for the reconstituted powders had occurred. The five laboratories had calculated the reconstituted powdered sample results on a dry basis instead of on an “as fed” basis. Laboratories C, I, and L submitted recalculated results prior to the collaborative study report submission due date, allowing inclusion of their data in the results table. Laboratory H submitted acceptable data but only after the due date. Laboratory E did not submit recalculated data. Since laboratories E and H recalculated reconstituted powder data were not received in time to include in the report, their original data were reported.

Several comments to strengthen the method were provided during the SPIFAN ERP meeting in March 2015:

Clarify in the method that it is not applicable to samples containing FD&C Red Dye No. 3 (erythrosine).

Point out the possible need for increased instrument maintenance when using the method. Include precautions about the lens and/or lens stack possibly requiring additional maintenance and that analysis would benefit from thoroughly conditioning the instrument.

Clarify the use and/or preparation of second source standards for CCV standard solutions.
If acidic sample matrixes are typically analyzed on the ICP-MS instrument, perform a thorough cleaning of the entire sample introduction system and appropriate conditioning prior to analyzing basic matrixes.

Clarify the importance of adhering to the peristaltic pump tubing sizes recommended for introducing IS and carrier solutions.

If possible, maintain a dedicated set of cones and/or lens.

These suggestions have been accepted and incorporated into the method. Also incorporated were minor modifications taken from comments provided by several collaborators as well as incorporation of components requiring clarification as suggested by the Study Director.

The overall results demonstrated that the method is fit-for-purpose to determine iodine levels in infant formula and adult/pediatric nutritional formula, and the Study Director recommended that it be adopted Official Final Action.

**Recommendations**

It was the recommendation of the Study Director that the method is fit-for-purpose in determining total iodine in infant formula and adult/pediatric nutritional formula by ICP-MS and that it be adopted as an AOAC Final Action *Official Method*. The AOAC ERP evaluated the data presented in the final report for the collaborative study of AOAC First Action *Official Method 2012.15* in March 2015 after which the method was recommended Final Action status. Subsequently, the Official Methods Board approved the method for Final Action in June 2015.

**Acknowledgments**

We are very grateful to all laboratories that participated in the study, especially to the following collaborators (listed alphabetically based on their institution name):

Angela Song and Fang Tian (Abbott Nutrition International ANRD, China)

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Grant Fulford and Pee Yang (Covance, Madison, WI)
Yirong Xu, Sook Yain Chen, and KienHeng Lee (Covance, Singapore)
Marvin Boyd Jr, and Cheryl D. Stephenson (Eurofins Central Analytical Laboratories, New Orleans, LA)
Josh Messerly (Eurofins Nutrition Analysis Center, Des Moines, IA)
Anders K. Svaneborg and Per K.B. Nilsson (Eurofins Steins Laboratorium A-S, Denmark)
Sudhakar Yadlapalli (First Source Laboratory Solutions LLP, Hyderabad, India)
Cai Weihong [Guangzhou Quality Supervision and Testing Institute (GZ GQT), China]
Raymond Yu (National Center of Supervision and Inspection on Food Stuff Product Quality, Shanghai, China)
Xiaojun Deng (Shanghai Exit and Entry Inspection and Quarantine Bureau, China)
Wu Bolong (Test Center of Chinese Academy of Inspection and Quarantine, China)
Qin Xu and Lei Bao (The Food and Agricultural Products Testing Agency of Shandong CIQ, China).

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References

http://dx.doi.org/10.5740/jaoacint.11-350

(2) AOAC Official Method 2012.15 (2012) Official Methods of Analysis of AOAC INTERNATIONAL,
19th Ed., AOAC INTERNATIONAL, Rockville, MD

(3) AOAC SPIFAN (February 2013) Single-Laboratory Validation for Iodine Analysis in Infant
   Formula and Adult Nutritionals, Covance Laboratories, Inc., Madison, WI

(4) AOAC SMPR 2012.008, *Iodine in Infant Formula and Adult/Pediatric Nutritional Formula*
Table 2012.15A. Statistical data

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Average</th>
<th>$S_r^a$</th>
<th>RSD$_r$</th>
<th>$S_R^b$</th>
<th>RSD$_R$</th>
<th>No. of outlier laboratories$^c$</th>
<th>HorRat</th>
<th>No. of laboratories used</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIST SRM 1849a, mg/kg</td>
<td>1.24</td>
<td>0.010</td>
<td>0.77</td>
<td>0.067</td>
<td>5.42</td>
<td>1</td>
<td>0.35</td>
<td>12</td>
</tr>
<tr>
<td>Infant formula RTF, milk based-1$^d$</td>
<td>5.48</td>
<td>0.262</td>
<td>4.78</td>
<td>0.507</td>
<td>9.25</td>
<td>0</td>
<td>1.06</td>
<td>13</td>
</tr>
<tr>
<td>Infant formula powder, soy based$^d$</td>
<td>12.4</td>
<td>0.313</td>
<td>2.53</td>
<td>0.945</td>
<td>7.62</td>
<td>2</td>
<td>0.98</td>
<td>11</td>
</tr>
<tr>
<td>Infant formula powder, milk based$^d$</td>
<td>18.5</td>
<td>0.693</td>
<td>3.75</td>
<td>1.39</td>
<td>7.54</td>
<td>2</td>
<td>1.03</td>
<td>11</td>
</tr>
<tr>
<td>Infant formula RTF, milk based-2$^d$</td>
<td>5.45</td>
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<td>0.626</td>
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<tr>
<td>Child formula powder$^f$</td>
<td>3.47</td>
<td>0.135</td>
<td>3.87</td>
<td>0.278</td>
<td>8.01</td>
<td>2</td>
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<tr>
<td>Adult nutritional powder, low fat$^d$</td>
<td>7.03</td>
<td>0.137</td>
<td>1.94</td>
<td>0.503</td>
<td>7.15</td>
<td>2</td>
<td>0.85</td>
<td>11</td>
</tr>
</tbody>
</table>

$^a$ $S_r$ = SD for repeatability.
$^b$ $S_R$ = SD for reproducibility.
$^c$ Values from laboratories with outliers were not used in statistical calculations.
$^d$ Results expressed as µg/100 g reconstituted final product.
### Table 2012.15B. Preparation of intermediate stock standard (ISS) iodine solutions

<table>
<thead>
<tr>
<th>Iodine standard solution ID</th>
<th>ID of solution used for preparation</th>
<th>Initial iodine concentration, ng/mL</th>
<th>Aliquot volume, mL</th>
<th>Final volume, mL</th>
<th>Final iodine concentration, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>10000 (ISS)</td>
<td>Stock</td>
<td>1000000</td>
<td>0.5</td>
<td>50</td>
<td>10000</td>
</tr>
<tr>
<td>1000 (ISS)</td>
<td>10000 (ISS)</td>
<td>1000</td>
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<td>50</td>
<td>1000</td>
</tr>
<tr>
<td>10.0 (ISS)</td>
<td>1000 (ISS)</td>
<td>1000</td>
<td>0.5</td>
<td>50</td>
<td>10.0</td>
</tr>
</tbody>
</table>

Aliquot the appropriate amount of iodine standard solution into a single use 50 mL DigiTUBE® and add 5 mL of stabilizer concentrate, fill to the 50 mL mark on the tube with water, cap the tube, and then mix thoroughly. The resulting matrix concentration is 1% NH₄OH and 0.1% Na₂S₂O₃ in water.

*ISS solutions are used for calibration standard preparation and are typically prepared according to the table. The ISS concentrations presented are nominal. Using the stock iodine concentration found on the certificate of analysis, determine the exact concentration of each ISS. The use of an electronic adjustable volume pipet, capable of delivering 100 to 5000 μL, is recommended.*
### Preparation of calibration standard (CS) iodine and calibration blank (CB) solutions

<table>
<thead>
<tr>
<th>Iodine standard solution ID</th>
<th>ID of solution used for preparation</th>
<th>Initial iodine concentration, ng/mL</th>
<th>Aliquot volume, mL</th>
<th>Final volume, mL</th>
<th>Final iodine concentration, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 (CS)</td>
<td>1000 (ISS)</td>
<td>1000</td>
<td>5</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>50.0 (CS)</td>
<td>1000 (ISS)</td>
<td>1000</td>
<td>2.5</td>
<td>50</td>
<td>50.0</td>
</tr>
<tr>
<td>10.0 (CS)</td>
<td>1000 (ISS)</td>
<td>1000</td>
<td>0.5</td>
<td>50</td>
<td>10.0</td>
</tr>
<tr>
<td>1.00 (CS)</td>
<td>10.0 (ISS)</td>
<td>10.0</td>
<td>5</td>
<td>50</td>
<td>1.00</td>
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<tr>
<td>0.500 (CS)</td>
<td>10.0 (ISS)</td>
<td>10.0</td>
<td>2.5</td>
<td>50</td>
<td>0.500</td>
</tr>
<tr>
<td>0.250 (CS)</td>
<td>10.0 (ISS)</td>
<td>10.0</td>
<td>1.25</td>
<td>50</td>
<td>0.250</td>
</tr>
<tr>
<td>Blank (CB)</td>
<td>NA*</td>
<td>NA</td>
<td>NA</td>
<td>50</td>
<td>0</td>
</tr>
</tbody>
</table>

Aliquot the appropriate amount of iodine standard solution into a single-use 50 mL DigiTUBE and add 5 mL of 5% KOH and 1 mL of stabilizer concentrate, fill to the 50 mL mark on the tube with water, cap the tube, and then mix thoroughly. The resulting matrix concentration is 0.5% KOH, approximately 0.2% NH₄OH, and approximately 0.02% Na₂S₂O₃ in water.

*Typical CS standard concentrations are nominally 0.250, 0.500, 1.00, 10.0, 50.0, and 100 ppb iodine and are typically prepared according to the table. The CB is the zero point of the curve. The curve type used, if using a PerkinElmer ICP-MS system with ELAN software, should be linear through zero. If using an Agilent or Thermo ICP-MS system, force the curve through the calibration blank. The calibration curve must have a correlation coefficient (r) of ≥0.998 to be acceptable. Determine the exact concentration of each CS (traceable back to the certificate of analysis) and assign these values to the curve points used to generate final results. The use of an electronic adjustable volume pipet, capable of delivering 100 to 5000 μL, is recommended.

*NA* = Not applicable.
Table 2012.15D. Preparation of intermediate continuing calibration verification (ICCV) and continuing calibration verification (CCV) iodine solutions and continuing calibration blank (CCB) solution

<table>
<thead>
<tr>
<th>Iodine standard solution ID</th>
<th>ID of solution used for preparation</th>
<th>Initial iodine concentration, ng/mL</th>
<th>Aliquot volume, mL</th>
<th>Final volume, mL</th>
<th>Final iodine concentration, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>10000 (ICCV)</td>
<td>Stock</td>
<td>1000000</td>
<td>0.5</td>
<td>50</td>
<td>10000</td>
</tr>
<tr>
<td>1000 (ICCV)</td>
<td>10000 (ICCV)</td>
<td>10000</td>
<td>5</td>
<td>50</td>
<td>1000</td>
</tr>
<tr>
<td>10.0 (CCV)</td>
<td>1000 (ICCV)</td>
<td>1000</td>
<td>0.5</td>
<td>50</td>
<td>10.0</td>
</tr>
<tr>
<td>Blank (CCB)</td>
<td>NA&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NA</td>
<td>NA</td>
<td>50</td>
<td>0</td>
</tr>
</tbody>
</table>

Aliquot the appropriate amount of iodine standard solution into a single-use 50 mL DigiTUBE, fill to the 50 mL mark on the tube with diluent, cap the tube, and then mix thoroughly. The resulting matrix concentration is 0.5% KOH, approximately 0.2% NH₄OH, and approximately 0.02% Na₂S₂O₃ in water. For the blank (CCB), fill a single-use 50 mL DigiTUBE to the 50 mL mark on the tube with diluent, cap the tube, and then mix thoroughly.

ICCV solutions are used for preparation of the CCV standard solution and are typically prepared according to the table. The ICCV and CCV concentrations presented are nominal. Using the stock iodine concentration found on the certificate of analysis (from the second source), determine the exact concentration of each ICCV. With this information, determine the exact concentration of the CCV standard. The use of an electronic adjustable volume pipet, capable of delivering 100 to 5000 μL, is recommended.

<sup>b</sup> NA = Not applicable.
Table 2012.15E. Preparation of internal standard (IS) solution

<table>
<thead>
<tr>
<th>Standard solution ID</th>
<th>ID of solution used for preparation</th>
<th>Initial concn, ng/mL</th>
<th>Aliquot volume, mL</th>
<th>Final volume, mL</th>
<th>Final concn, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>30.0 (Pr)</td>
<td>Stock</td>
<td>10000</td>
<td>1.5</td>
<td>500(^b)</td>
<td>30.0</td>
</tr>
</tbody>
</table>

\(^a\) The IS concentration typically used for analysis is 30 ppb. The table outlines a typical preparation scheme.

\(^b\) After aliquoting the 10000 ppb Pr into the 500 mL vessel, add approximately 100 mL water, 10 mL HNO\(_3\), 0.5 mL HClO\(_4\), 0.05 g Triton\(^{\circledR}\) X-100, and then bring to volume with water and mix thoroughly. The resulting concentration is 2% HNO\(_3\), 0.1% HClO\(_4\), and 0.01% Triton\(^{\circledR}\) X-100 in water.
Table 2012.15F. Open-vessel microwave digestion parameters

<table>
<thead>
<tr>
<th>Wattage</th>
<th>Power, %</th>
<th>Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>400</td>
<td>20</td>
<td>6</td>
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<tr>
<td>400</td>
<td>20</td>
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<td>400</td>
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<td>10</td>
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<tr>
<th>Wattage</th>
<th>Power, %</th>
<th>Minutes</th>
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<td>10</td>
</tr>
<tr>
<td>400</td>
<td>65</td>
<td>10</td>
</tr>
</tbody>
</table>

* Microwave used: CEM MARS 5 or CEM MARS 6. Use caution: Ensure vessels do not completely seal (bursting hazard) or overheat (as melting may occur). Note: Using AOAC Method 2012.15, the parameters, with the corresponding number of vessels, produced acceptable results for NIST SRM 1849a infant/adult nutritional formula. For each number of vessel’s range, if fewer vessels than the minimum are placed in the microwave, overheating may occur resulting in loss of sample or injury. If greater than the suggested number of vessels is placed in the microwave, the digestion may not be complete.
<table>
<thead>
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<th>Laboratory code</th>
<th>Oven</th>
<th>Microwave</th>
<th>Instrument</th>
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<tr>
<td>A</td>
<td>Yes</td>
<td>No</td>
<td>Thermo iCAP Q</td>
</tr>
<tr>
<td>B</td>
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<td>No</td>
<td>Thermo iCAP Q</td>
</tr>
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<td>C</td>
<td>Yes</td>
<td>No</td>
<td>Agilent 7700 x</td>
</tr>
<tr>
<td>D</td>
<td>Yes</td>
<td>No</td>
<td>Agilent 7500 ce</td>
</tr>
<tr>
<td>E</td>
<td>No</td>
<td>Yes</td>
<td>PE Elan DRC-e</td>
</tr>
<tr>
<td>F</td>
<td>No</td>
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<td>PE Elan DRC-e</td>
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<td>G</td>
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<td>PE Elan DRC II</td>
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<td>H</td>
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<td>No</td>
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<td>I</td>
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Table 2. Laboratory results

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<th>NIST SRM 1849a</th>
<th>Infant formula RTF milk based-1</th>
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<th>Infant formula powder milk based</th>
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<th>Child formula powder</th>
<th>Adult nutritional powder low fat</th>
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<td>1.17</td>
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<td>11.9</td>
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<table>
<thead>
<tr>
<th>Lab</th>
<th>Iodine results, mg/kg&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Iodine results, µg/100 g&lt;sup&gt;b&lt;/sup&gt;</th>
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<tr>
<td>J</td>
<td>1.33</td>
<td>19.9</td>
</tr>
<tr>
<td>K</td>
<td>1.28</td>
<td>18.6</td>
</tr>
<tr>
<td>L</td>
<td>1.22</td>
<td>19.9</td>
</tr>
<tr>
<td>M</td>
<td>1.25</td>
<td>18.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> NIST SRM 1849a results presented as mg/kg.

<sup>b</sup> µg/100 g reconstituted final product.

<sup>c</sup> Statistical outliers, data not included for statistical analysis.