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Validation of a Method for the Determination of Total Chromium in Rat Feces by Inductively Coupled Plasma Optical Emission Spectrometry

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Abstract

The validation of a method for the determination of total chromium in Fischer-344 rat feces by inductively coupled plasma optical emission spectrometry following a rapid, atmospheric-pressure microwave digestion is described. The performance of the method was evaluated over the concentration range of 5.00 to 200 µg Cr/g feces. Data for method linearity, accuracy, precision, digest stability, and storage stability are presented along with limit of detection and limit of quantitation data. Data from a cross-validation method for B6C3F1 mouse feces are also presented. Following validation, the method was applied to analyze samples collected in support of two chronic toxicological investigations.

Keywords

Chromium; feces; inductively coupled plasma optical emission spectrometry

INTRODUCTION

Chromium compounds are employed in a wide variety of industrial processes. Because of its resistance to oxidation, metallic chromium is heavily utilized by metallurgical industries for the production of steel and other alloys (Reilly 2004). Chromium compounds are also used in the production of chromium pigments and in metal finishing, leather tanning, and wood preservation (Barnhart 1997). This high degree of industrial utility has resulted in the presence of chromium in manufactured products and ultimately in the release of some of its compounds into the environment (Pellerin and Booker 2000).

Chromium has six oxidation states; the most stable and most biologically and industrially important of these are the trivalent, Cr(III), and hexavalent, Cr(VI), states. Cr(III) and Cr(VI) have very different chemical and biological properties (ATSDR 2000). Cr(VI) is a water

pollutant, a human carcinogen by the inhalation route, and a carcinogen in rats and mice (IARC 1990; Cohen et al. 1993; NTP 1998, 2008b). In contrast, Cr(III) is an essential nutrient that appears to participate in carbohydrate and lipid metabolism by improving insulin sensitivity (Anderson 1998) and is consumed in a wide variety of foods (NIH 2007). There is very little evidence of toxicity or carcinogenicity following exposure to Cr(III) (ATSDR 2000; NTP 2008a).

Although both Cr(III) and Cr(VI) are poorly absorbed following oral exposure, absorption and tissue uptake of chromium has been shown to be greater following exposure to Cr(VI) than with Cr(III) (ATSDR 2000; Costa 1997; Costa and Klein 2006). This difference is thought to occur because of different mechanisms of tissue uptake, as Cr(VI) structurally resembles sulfate and phosphate and is thought to be taken up through the transporters of these ions, whereas Cr(III) is not a substrate and enters cells by diffusion or phagocytosis (ATSDR 2000). In an attempt to increase the absorption and tissue uptake of Cr(III) over nonchelated forms such as chromium chloride, Cr(III) was chelated with three molecules of picolinic acid to form chromium picolinate (CP) (Evans and Pouchnik 1993); CP exposure did not improve the absorption of Cr(III) over chromium chloride in one study (Olin et al. 1994) but did result in higher tissue chromium concentrations compared to those resulting from chromium chloride exposure in another study (Anderson et al. 1996).

Both extracellular and intracellular reduction of Cr(VI) to Cr(III) occur (ATSDR 2000). Because Cr(III) is less bioavailable than Cr(VI), extracellular reduction is thought to be a protective mechanism. In contrast, intracellular reduction is thought to be a mechanism of carcinogenesis, because DNA damage occurs when Cr(VI) is reduced to Cr(III); the pathways of DNA damage resulting from chromium have been recently reviewed (O'Brien, Ceryak, and Patierno 2003).

Because of the low absorption and contrasting biological and toxicological properties of Cr(VI) and Cr(III), and because Cr(VI) is reduced to Cr(III), the determination of chromium in feces an essential analytical component of toxicity and carcinogenicity studies on chromium. Recently, the concentration of chromium has been determined in elk fecal pellets and livestock animal manures by graphite furnace atomic absorption spectrometry (GFAAS) following extensive digestion procedures with aqua regia (Parker and Hamr 2001; Nicolson et al. 1999). The chromium concentration of the stomach contents of marine mammals has also recently been determined by inductively coupled plasma mass spectrometry (ICP-MS) following a closed-vessel, elevated pressure microwave digestion (Hung et al. 2007).

The objective of the current investigation was to validate an analytical method for the determination of total chromium in feces from Fischer-344 rats and to cross-validate this method in B6C3F1 mice. To measure total chromium in fecal samples of animals used in toxicology and carcinogenesis studies, an analytical method must be sensitive enough to detect the analyte of interest in the study matrix, while allowing for high throughput to process the large number of collected samples. The method must also demonstrate accurate, precise, and consistent analytical performance throughout the study. Samples were prepared using a rapid, atmospheric-pressure, microwave-digestion technique. The digested samples were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES) in

the radial viewing mode, which offered adequate detection limits and minimal matrix impact (Sengoku and Wagatsuma 2006). Method validation data as well as quality control sample data from two chronic studies are presented here. This method measures total chromium, regardless of oxidation state, and was used in the determination of Cr in feces of F344/N rats and B6C3F1 mice exposed to Cr(VI) (as sodium dichromate dihydrate; NTP 2008b) or Cr(III) (as chromium picolinate monohydrate; NTP 2008a) as part of chronic toxicity and carcinogenicity studies by the National Toxicology Program (NTP).

EXPERIMENTAL

Apparatus

A Thermo Jarrell Ash (Franklin, MA, USA) Atomscan-16 ICP-OES was used to determine the concentration of chromium in sample digests. This radial plasma, sequential instrument was equipped with an autosampler to facilitate analyses. A polypropylene spray chamber, high-flow torch assembly, glass injector, and polymer cross-flow nebulizer were obtained from the instrument manufacturer and used throughout this investigation.

A CEM MDS-2000 microwave digestion system with a 630-W maximum power output was used for sample decomposition (Matthews, NC, USA). A direct-drive alternating turntable allowed for the rotation of a 36-position polypropylene sample carousel. Constant temperature monitoring and feedback for a control vessel was achieved with a fiber-optic temperature probe (in a hydrofluoric acid-resistant thermowell) obtained from the instrument manufacturer. Rodent fecal samples were digested in graduated 50-mL polypropylene centrifuge tubes from Corning (Acton, MA, USA). The caps from these centrifuge tubes were replaced with polypropylene digestion caps obtained from CEM. These caps had a hole in the center 3 mm in diameter and allowed for the venting of gases generated during sample digestion.

Prior to digestion, control rodent fecal tissue was lyophilized using a VirTis freeze dryer equipped with a 3.3-L condenser (Gardiner, NY, USA). A Mettler AT 261 Deltarange analytical balance was used to determine sample masses (Hightstown, NJ, USA). The lyophilized fecal material used for method validation was manually homogenized with a mortar and pestle prior to aliquot removal. Sample preparation procedures were carried out under high-efficiency particulate air (HEPA) filters to minimize potential contamination. Sample digests were passed through 0.45- μ m syringe filters from PALL Corporation (East Hills, NY, USA) prior to analysis.

Reagents

Reagents were carefully selected to minimize the potential for chromium contamination. Trace metal-grade nitric acid (HNO_3) and hydrofluoric acid (HF) from Fisher Scientific (Pittsburgh, PA, USA) and Ultrex II grade 30% hydrogen peroxide from J.T. Baker (Phillipsburg, NJ, USA) were used for sample digestion. Nominal 1,000 $\mu\text{g/mL}$ standard solutions of chromium and yttrium, traceable to the National Institute of Standards and Technology (NIST), were purchased from High Purity Standards (Charleston, SC, USA). Ultra-grade TritonX-10 from Sigma (St. Louis, MO, USA) and HNO_3 were used to prepare

the ICP-OES rinse solution. All of the deionized (DI) water used in this investigation (approximately 18-M Ω quality) was obtained from a Pure Water Solutions deionization system (Hillsborough, NC, USA). Rodent fecal control tissue from male Fisher-344 rats and male B6C3F1 mice was purchased from BioChemed (Winchester, VA, USA). A dogfish muscle (DORM-2) certified reference material (CRM) sample was obtained from the National Research Council of Canada and was used to monitor method performance. This CRM had a certified chromium value of 34.7 ± 5.5 $\mu\text{g/g}$.

Labware Preparation

Throughout this investigation, labware was cleaned to minimize the potential for contamination. All pipette tips and centrifuge tubes were rinsed with DI water and dried under HEPA-filtered air, then stored in sealed containers until use. Reusable labware was soaked in a phosphate-free detergent solution, rinsed with tap water, and soaked in a 20% (v/v) HNO₃ bath for a minimum of 24 h. After removal from the bath, labware was rinsed three times with DI water and dried under HEPA-filtered air. If not used immediately, the labware was sealed in plastic storage bags.

Stock Solutions and Diluent Solution

Two individual 200 $\mu\text{g Cr/mL}$ (WS200-A and B) and 10.0 $\mu\text{g Cr/mL}$ (WS10-A and B) stock solutions were prepared by transferring aliquots of the 1,000 $\mu\text{g/mL}$ NIST-traceable Cr stock solution to polypropylene volumetric flasks. These solutions were brought to volume with DI water and contained 2% (v/v) HNO₃. A diluent solution was prepared to contain a final concentration of 5 $\mu\text{g/mL}$ of the yttrium internal standard and 10% (v/v) HNO₃. All of the solutions described in this section were stored in a refrigerator (approximately 2–8°C) when not in use.

Preparation of Fecal Matrix Pools

Control fecal tissue was pooled from five undosed male Fischer-344 rats. The control material was lyophilized; the water loss was 13.5% of the original wet mass. The dried material was then manually homogenized with a mortar and pestle and used for the preparation of matrix standards, quality control samples, and stability samples. Aliquots were based on a nominal 0.250-g wet mass (0.216 g of dried, homogenized material). When not in use, the control material was stored in a freezer (approximately –20°C).

Control fecal tissue from six undosed B6C3F1 male mice was lyophilized; the water loss was 46.9% of the original wet mass. The dried material was manually homogenized with a mortar and pestle and used for the preparation of cross-validation matrix standards. Aliquots were based on a nominal 0.250-g wet mass (0.133 g of dried, homogenized material). When not in use, the control material was stored in a freezer (approximately –20°C).

Preparation of Matrix Standards

Fischer-344 rat fecal matrix standards were prepared by weighing out approximately 0.216 g of the lyophilized, homogenized control feces (equivalent to 0.250 g of the wet material) into digestion tubes and adding 2.5 mL of HNO₃. Matrix standard preparations were fortified with the chromium working stock solutions as described in Table 1. Each tube was

capped with a vented microwave digestion cap and placed in a HEPA-filtered environment for a minimum of 2 h. After this predigestion period, the caps were removed, and 2.0 mL of DI water were added to each tube. The tubes were then recapped and subjected to the microwave digestion program presented in Table 2. After the digestion tubes cooled to room temperature, H₂O₂ (0.500 mL) and HF (0.125 mL) were added to each tube. The tubes were returned to the microwave and were subjected to the program presented in Table 2 once more. Sample digests were transferred to 25-mL polypropylene volumetric flasks (each containing 0.125 mL of 1,000 µg/mL yttrium internal standard solution) and were brought to volume with DI water. Each sample was filtered through a 0.45-µm filter into a new 30-mL, low-density polyethylene storage bottle.

Method Validation

Rat fecal validation experiments to assess method linearity, accuracy, precision, detection limits, specificity, dilution accuracy, and matrix impact were conducted over 3 analysis days. Two sets of matrix standards were prepared on the first validation day, and one set of matrix quality control (QC) samples was prepared at the same matrix standard concentration levels. The two sets of matrix standards were used to establish linearity, whereas the matrix QC samples were used with the matrix standards to establish accuracy and precision on the first validation day (intra-assay) and interassay accuracy and precision on all 3 validation days.

For each validation day, three matrix blanks were prepared by weighing out the lyophilized, homogenized control feces into digestion tubes. These samples were then prepared as the matrix standards described previously with the exception of fortification with chromium. In addition, a specificity matrix blank was prepared for 1 of 3 validation days. This sample was prepared as a matrix blank without the addition of internal standard.

Dilution check matrix standards were prepared in triplicate on 1 of the 3 validation days to demonstrate that samples with concentrations exceeding the validation range could be successfully diluted to the validation range and analyzed. These samples were prepared using the digestion procedure described previously for matrix standards, except that they were fortified with the 1,000 µg/mL NIST-traceable Cr solution to contain 13,000 µg Cr/g feces (equivalent to 130 µg Cr/mL in the digested samples). Before analysis, the digested samples were diluted 100 × with the previously described diluent solution. To establish an experimental limit of quantitation (ELOQ) and a limit of detection (LOD), six replicate matrix samples were prepared to contain 2.00 µg Cr/g feces (equivalent to 0.0200 µg Cr/mL in the digested samples) on 1 of the 3 validation days.

B6C3F1 mouse fecal cross-validation matrix standards were prepared by weighing out approximately 0.133 g of the lyophilized, homogenized control feces (equivalent to 0.250 g of the wet material) into digestion tubes and adding 2.5 mL of HNO₃. Triplicate matrix standards were fortified to contain 10.0, 20.0, and 100 µg Cr/g feces (equivalent to 0.100, 0.200, and 1.00 µg Cr/mL feces, respectively, in the digested samples). Samples were digested using the procedure for rat matrix standards previously described in detail. Matrix blanks were also prepared for the mouse fecal cross-validation.

Preparation of Quality Control Samples

Method blank and CRM samples were prepared for each validation and cross-validation analysis day. Method blank samples were used to monitor the background contribution from the reagents and the analytical procedure. These samples were prepared in an identical manner to the matrix blanks described previously, except that fecal material was not added to the tubes. CRM samples were prepared in an identical manner to the matrix blanks, except 0.100 g of the DORM-2 material was weighed into the tubes instead of 0.250 g of feces.

Preparation of Stability Samples

During this validation, several experiments were conducted to assess analyte stability in the Fischer-344 rat fecal matrix. Freeze-thaw stability samples were prepared by transferring nominal 0.216-g aliquots of the lyophilized, homogenized control feces to digestion tubes. These samples were then fortified with chromium to contain 10.0, 50.0, and 100 mg Cr/g fecal concentrations (equivalent to 0.100, 0.500, and 1.00 mg Cr/mL, respectively, in the digested samples). Three replicates were prepared at each nominal concentration level, for a total of nine samples. After fortification, the tubes were capped and placed in freezer storage (approximately -20°C). They were removed from the freezer on 3 consecutive days, allowed to thaw completely over several hours, and returned to the freezer. After three freeze-thaw cycles, samples were digested using the procedure previously described for the rat fecal matrix blanks.

Analysis-period stability samples were prepared by transferring nine nominal 0.216-g aliquots of the homogenized, lyophilized control tissue to digestion tubes. These samples were fortified (in triplicate) and digested as described before for the freeze-thaw stability samples. After digestion, each sample was split into three analytical cohorts. Aliquots from the first cohort were analyzed immediately after digestion and served as the reference (day 0) time point, and the aliquots from the second cohort were analyzed approximately 3.5 h later to assess autosampler residence time stability. Aliquots from the third cohort were stored in a refrigerator (approximately $2-8^{\circ}\text{C}$) for 32 days and analyzed to assess digest storage stability.

To assess long-term storage stability, nominal 0.216-g aliquots of the fecal control tissue were transferred to digestion tubes. Thirty-six replicates were fortified with chromium to contain nominal concentrations of 10.0, 50.0, and 100 $\mu\text{g Cr/g feces}$ (equivalent to 0.100, 0.500, and 1.00 $\mu\text{g Cr/mL}$, respectively, in the digested samples). These samples were stored for up to 239 days at freezer temperature (approximately -20°C). Three digestion tubes from each nominal concentration were removed from freezer storage and were prepared for analysis using the previously described procedure for matrix blanks on days 0, 15, 30, and 239.

ICP-OES Analysis

Solvent standards were prepared and used to calibrate the ICP-OES instrument on each validation, cross-validation, or stability-assessment analysis day. These standards were prepared in polypropylene volumetric flasks using DI water as the diluent in an acid matrix

that approximated that used for the samples (10% v/v HNO₃ and 5 µg/mL yttrium). During validation, a solvent standard was prepared at each matrix standard concentration level using the pattern of alternating chromium working stock solutions described in Table 1. Solvent standards used to calibrate the instrument for cross-validation or stability-assessment analyses were prepared in the same matrix and spanned the linear validation range. An ICP-OES rinse solution [5% (v/v) HNO₃ and 0.0010% (v/v) TritonX-100 in DI water] was also prepared. The ICP-OES instrumental parameters used throughout this investigation are presented in Table 3.

RESULTS AND DISCUSSION

Matrix Linearity and Recovery

On the first validation day, two sets of matrix standards were analyzed to establish linearity. The resulting regression equation was $y = 0.09368(x) + 0.003240$, where y was the internal standard corrected chromium-emission intensity and (x) was the chromium concentration, expressed as µg Cr/mL in analysis solution. The correlation coefficient for this matrix regression was 0.9992, indicating acceptable linearity. The regression was then applied to the internal standard corrected emission intensity data for the matrix standards to determine the concentration of each preparation. These found total chromium concentration data are presented in Table 4 along with the analyte recovery calculated at each nominal matrix standard concentration level. Recoveries ranged from 86.2 to 116% across the validation range, indicating acceptable analyte recovery in the presence of the rat fecal matrix.

The internal standard corrected responses for the matrix standards and the solvent standards prepared at the same nominal concentrations are also presented in Table 4. These data were used to assess the impact of the fecal matrix on the determination of chromium. The recovery at each concentration level was calculated by dividing the matrix blank corrected matrix standard intensity by the solvent blank corrected solvent standard intensity from the same nominal concentration level. The recoveries ranged from 91.9 to 116%, and the mean recovery across the entire validation range was 101%. These data indicated that the impact of the digested rat fecal matrix on the determination of chromium was not significant, so solvent standards were used for all subsequent analyses. The solvent regressions for the second and third validation analyses over the same 5.00 to 200 µg Cr/g fecal range (equivalent to 0.0500 to 2.00 µg/mL) were $y = 0.07836(x) - 0.0004179$, $r = 0.9999$, and $y = 0.08672(x) - 0.0002622$, $r = 0.9999$, respectively.

Accuracy and Precision

Data from the intra-assay (same day) triplicate matrix QC preparations are presented in Table 5. The mean recoveries for these samples ranged from 92.3 to 110%, indicating acceptable accuracy. Precision for these preparations, expressed as percent relative standard deviation (%RSD), ranged from 1.4 to 21%. A preparation error was suspected for one of the replicate samples at the 20.0 µg Cr/g feces level, which resulted in 21% RSD. Accuracy and precision data from the inter-assay (different day) matrix standard preparations are also presented in this table. The recovery and precision data for these samples were calculated using one matrix standard preparation that was analyzed on each of the 3 validation analysis

days. Mean recoveries ranged from 95.7 to 112%, and % RSD data ranged from 0.52 to 16%.

Limits of Detection

The experimental limit of quantitation (ELOQ) was determined based on the analysis of six replicate matrix samples, which were prepared to contain a nominal chromium concentration of 0.0200 µg/mL in the sample digests. The average determined concentration of these samples was 0.0178 µg/mL (with a 12% RSD). Based on these performance data, the ELOQ was conservatively established as 0.0200 µg/mL (equivalent to 2.00 µg Cr/g feces). The limit of detection (LOD), defined as three times the standard deviation of these matrix samples, was determined to be 0.00621 µg/mL (equivalent to 0.621 µg Cr/g feces).

Dilution Check Accuracy

To evaluate the accuracy of the method for samples outside the validated concentration range, three matrix samples were prepared to contain a nominal concentration of 13,000 µg Cr/g feces (equivalent to 130 µg Cr/mL in the sample digests). These samples were diluted by a factor of 100 prior to analysis to bring the nominal concentration within the validation range. Recoveries for the three replicates were 95.5, 93.2, and 92.1%, verifying the accuracy of the analytical method when diluting samples containing up to 13,000 µg Cr/g feces.

Specificity Check

One matrix blank was prepared without the yttrium internal standard as a specificity check. This blank had an analyte response within the range of the responses for the three matrix blanks prepared on the same day, indicating that the internal standard did not interfere with the determination of the analyte.

Analysis Period and Freeze–Thaw Stability

Nine matrix samples were fortified with chromium to contain nominal concentrations of 10.0, 50.0, and 100 µg Cr/g feces (equivalent to 0.100, 0.500, and 1.00 µg Cr/mL, respectively, in the sample digests) and digested using the previously described procedure. These samples were then split into three cohorts corresponding to samples analyzed immediately after digestion (reference), samples analyzed approximately 3.5 h later to assess autosampler storage stability, and samples stored in a refrigerator for 32 days to assess digest stability. Recoveries for the autosampler residence time and refrigerator storage stability samples were calculated against the reference concentrations. Recoveries for the fortified samples subjected to three freeze-thaw cycles prior to digestion were also calculated against the reference samples. Recovery and precision data for these samples, presented in Table 6, indicate that the analyte is stable in the digested samples at both 3.5 h and 32 days and that analyte recovery is not impacted by three freeze-thaw cycles.

Freezer Storage Stability

Long-term storage stability was assessed in control rat fecal samples fortified with chromium to contain nominal concentrations of 10.0, 50.0, and 100 µg Cr/g feces (equivalent to 0.100, 0.500, and 1.00 µg Cr/mL, respectively, in the sample digests). Samples

were stored in a freezer and were prepared for analysis on days 0, 15, 30, and 239. Recovery and precision data for these long-term storage samples are presented in Table 7. Recoveries across all three concentration levels and all time points ranged from 93.4 to 119%, indicating that the analyte at the tested concentrations was stable when stored under freezer temperature (approximately -20°C) conditions.

Mouse Fecal Cross-Validation

A cross-validation study was conducted to demonstrate the suitability of the validated method for the determination of total chromium in male B6C3F1 mouse feces. This cross-validation consisted of the digestion and analysis of test matrix samples fortified with chromium at three nominal concentration levels of 10.0, 20.0, and 100 $\mu\text{g Cr/g feces}$ (equivalent to 0.100, 0.200, and 1.00 $\mu\text{g Cr/mL}$, respectively, in the sample digests). Data from this cross-validation study are summarized in Table 8. A linear regression was performed on the internal standard corrected responses for the mouse fecal matrix standards, which gave a best fit line of $y = 0.09368(x) + 0.003240$; $r = 0.9992$. The mean recoveries for the matrix standard replicates calculated with this equation ranged from 97.3 to 105%, indicating acceptable analyte recovery in the presence of the mouse fecal matrix. The internal standard corrected responses for the matrix standards and the solvent standards prepared at the same nominal concentrations were used to calculate matrix recovery. These recoveries ranged from 93.0 to 103%, indicating that the digested mouse fecal matrix did not significantly impact the determination of total chromium for the cross-validated method.

Application of the Method

DORM-2 certified reference material aliquots were processed with each batch of study samples and were also prepared during the method validation experiments. Overall, DORM-2 samples were processed with 20 digestion batches spanning a period of approximately 1 year. The average recovery for each batch of samples calculated against the certified value for DORM-2 total chromium value ($34.7 \pm 5.5 \mu\text{g/g}$) is plotted in Figure 1 as a function of the digestion batch. The error bars correspond to the standard deviation for the determined batch DORM-2 total chromium concentrations. Data from 63 preparations are presented across the 20 analysis days. Data from four additional replicates are not presented (two were lost during sample preparation, and two were rejected as outliers at the 99% confidence level using the Dixon-Q test).

The validated method for the determination of total chromium in rat feces and the cross-validated method for the determination of chromium in mouse feces were used to analyze approximately 360 samples from F344/N rats and B6C3F1 mice exposed to sodium dichromate dihydrate [Cr(VI)] in drinking water (NTP 2008b) or chromium picolinate monohydrate [Cr(III)] in feed (NTP 2008a). Determining the concentration of total chromium recovered in the feces collected from study animals was a critical component used to assess the extent of absorption of chromium following exposure to these chemicals.

CONCLUSION

A method for the determination of total chromium was validated in F344/N rat feces and cross-validated in B6C3F1 mouse feces. Samples were analyzed by ICP-OES following a rapid, atmospheric-pressure, microwave-digestion technique. This method demonstrated the necessary accuracy, precision, and throughput to be used for the analysis of fecal samples from two NTP chronic bioassays.

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REFERENCES

- Agency for Toxic Substances and Disease Registry (ATSDR). 2000 Toxicological Profile for Chromium. U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry Atlanta, GA, USA.
- Anderson RA 1998 Chromium, glucose intolerance and diabetes. *J. Am. Coll. Nutr.* 17: 548–555. [PubMed: 9853533]
- Anderson RA, Bryden NA, Polansky MM, and Gautschi K 1996 Dietary chromium effects on tissue chromium concentrations and chromium absorption in rats. *J. Trace Elem. Exp. Med.* 9 (1): 11–25.
- Barnhart J 1997 Chromium, chemistry, and implications for environmental fate and toxicity. *J. Soil Contam.* 6: 561–568.
- Cohen MD, Kargacin B, Klein CB, and Costa M 1993 Mechanisms of chromium carcinogenicity and toxicity. *Crit. Rev. Toxicol.* 23: 255–281. [PubMed: 8260068]
- Costa M 1997 Toxicity and carcinogenicity of Cr(VI) in animal models and humans. *Crit. Rev. Toxicol.* 27: 431–442. [PubMed: 9347224]
- Costa M, and Klein CB 2006 Toxicity and carcinogenicity of chromium compounds in humans. *Crit. Rev. Toxicol.* 36: 155–163. [PubMed: 16736941]
- Evans GW and Pouchnik DJ 1993 Composition and biological activity of chromium-pyridine carboxylate complexes. *J. Inorg. Biochem.* 49: 177–187. [PubMed: 8433089]
- Hung CLH, Lau RKF, Lam JCW, Jefferson TA, Hung SK, Lam MHW, and Lam PKS 2007 Risk assessment of trace elements in the stomach contents of indo-pacific humpback dolphins and finless porpoises in Hong Kong waters. *Chemosphere*, 66: 1175–1182. [PubMed: 16979696]
- International Agency for Research on Cancer (IARC). 1990 Chromium (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, vol. 49), 49–256. Lyon, France: IARC.
- National Institutes of Health (NIH). 2007 Dietary Supplement Fact Sheet: Chromium. Office of Dietary Supplements, NIH Clinical Center Available at http://ods.od.nih.gov/factsheets/Chromium_pf.asp
- National Toxicology Program (NTP). 1998 Eighth Report on Carcinogens: 1998 Summary, 29–31 Research Triangle Park, NC: U.S Department of Health and Human Services, Public Health Service, National Institute of Environmental Health Sciences.
- National Toxicology Program (NTP). 2008a Toxicology and Carcinogenesis Studies of Chromium Picolinate Monohydrate (CAS No. 27882-76-4) in F344/N Rats and B6C3F1 Mice (Feed Studies) (Technical Report Series No. 556, NIH Publication No. 08–5897) Research Triangle Park, NC: National Institutes of Health, Public Health Service, U.S. Department of Health and Human Services.
- National Toxicology Program (NTP). 2008b Toxicology and Carcinogenesis Studies of Sodium Dichromate Dihydrate (CAS No. 7789-12-0) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies) (Technical Report Series No. 546, NIH Publication No. 08–5887) Research Triangle Park, NC: National Institutes of Health, Public Health Service, U.S. Department of Health and Human Services.

- Nicholson FA, Chambers BJ, Williams JR, and Unwin RJ 1999 Heavy metal contents of livestock feeds and animal manures in England and Wales. *Biosource Tech*, 70: 23–31.
- O'Brien TJ, Ceryak S, and Patierno SR 2003 Complexities of chromium carcinogenesis: Role of cellular response, repair, and recovery mechanisms. *Mutat. Res*, 533: 3–36. [PubMed: 14643411]
- Olin KL, Stearns DM, Armstrong WH, and Keen CL 1994 Comparative retention/absorption of 51chromium (51Cr) from 51Cr chloride, 51Cr nicotinate and 51Cr picolinate in a rat model. *Trace Elem. Electroly*, 11: 182–186.
- Parker GH and Hamr J 2001 Metal levels in body tissues, forage, and fecal pellets of elk (*Cervus elaphus*) living near the ore smelters at Sudbury, Ontario. *Env. Poll*, 113: 347–355.
- Pellerin C and Booker SM 2000 Reflections on hexavalent chromium: Health hazards of an industrial heavyweight. *Env. Heal. Persp.*, 108 (9): A402–407.
- Reilly C 2004 *The Nutritional Trace Metals*. Oxford, UK: Blackwell Publishing.
- Sengoku S and Wagatsuma K 2006 Comparative studies of spectrochemical characteristics between axial and radial observations in inductively coupled plasma optical emission spectrometry. *Anal. Sci*, 22: 245–249. [PubMed: 16512416]

Figure 1. DORM-2 CRM recovery for 20 analyses over an approximately 1-year period; error bars represent $\pm$ standard deviation for triplicate preparations on each analysis day.

Table 1.

Preparation of rat fecal matrix standards

Matrix standard	Spiking solution	Nominal [Cr] ^a (µg/mL)	Equivalent tissue [Cr] ^b (µg/g)
Matrix low	WS10-A	0.0500	5.00
Matrix mid-1	WS10-B	0.100	10.0
Matrix mid-2	WS10-A	0.200	20.0
Matrix mid-3	WS10-B	0.500	50.0
Matrix mid-4	WS200-A	1.00	100
Matrix high	WS200-B	2.00	200

^aNominal Cr concentration in final analysis solution.^bCalculated as (concentration of Cr (µg/mL) × 25-mL digest volume)/nominal 0.250-g wet tissue mass.

Table 2.

Microwave sequence for sample digestion

Stage	Power (%)	Temperature (°C)	Ramp time (min)	Hold time (min)
1	60	60	1	5
2	80	85	2	10
3	100	100	2	20
4	100	110	5	10

Table 3.

ICP-OES operating parameters

Parameter	Value
Instrument	Thermo Jarrell Ash Atomscan 16
Software	ThermoSPEC ver. 6.20
Analyte wavelength	Cr 205.552 nm
Internal standard wavelength	Y 371.030 nm
RF power	1350 W
Torch gas flow	High
Auxiliary gas flow	Medium
Peristaltic pump rate	100 rpm
Rinse time	60 s
Scans per analysis	4
Integration times	5 s (Cr); 2 s (Y)
Nebulizer pressure	Optimized for each analysis

Table 4.

Rat fecal method linearity and recovery

Nominal [Cr] (µg/mL)	Response ^a		Found [Cr] ^b (µg/mL)	Recovery ^c (%)	Matrix recovery ^d (%)
	Matrix	Solvent			
0.0500	0.007296	0.004410	0.04330	86.2	91.9
(5.00 µg/g) ^e	0.007263		0.04294		
0.100	0.01156	0.009747	0.08881	101	97.1
(10.0 µg/g)	0.01390		0.1138		
0.200	0.02133	0.01865	0.1931	116	116
(20.0 µg/g)	0.02877		0.2725		
0.500	0.05026	0.04609	0.5019	98.0	99.5
(50.0 µg/g)	0.04806		0.4784		
1.00	0.09399	0.09333	0.9687	96.7	97.0
(100 µg/g)	0.09360		0.9646		
2.00	0.1914	0.1858	2.009	101	102
(200 µg/g)	0.1928		2.023		

^a Internal standard corrected emission intensity for matrix and solvent standards.^b Calculated with matrix regression equation: $y = 0.09368(x) + 0.003240$; $r = 0.9992$.^c Calculated as (mean found [Cr] (mg/mL)/nominal [Cr] (µg/mL)) $\times$ 100.^d Calculated as (mean blank corrected matrix response/blank corrected solvent response) $\times$ 100. Mean matrix blank response = 0.002600; solvent blank response = -0.0006811.^e Calculated as (concentration of Cr (µg/mL) $\times$ 25-mL digest volume)/nominal 0.250-g wet tissue mass.

Table 5.

Intra-assay and interassay accuracy and precision

Nominal [Cr] (µg/g)	Mean found [Cr] (µg/g)	Mean recovery ^a (%)	Recovery precision (% RSD)
Intra-assay accuracy and precision (n = 3 at each level)			
5.00	4.616	92.3	11
10.0	9.696	97.0	15
20.0	21.91	110	21
50.0	48.50	97.0	3.0
100	97.42	97.4	1.4
200	200.1	100	1.4
Interassay accuracy and precision (n = 3 at each level)			
5.00	5.583	112	16
10.0	9.566	95.7	2.7
20.0	20.52	103	6.0
50.0	48.80	97.6	0.38
100	99.81	99.8	2.1
200	199.0	99.5	0.52

^a Calculated as (mean found [Cr] (µg/g)/nominal [Cr] (µg/g)) × 100.

Table 6.

Analysis period and freeze-thaw stability

Nominal [Cr] (µg/mL)	Mean found [Cr] (µg/mL)	Mean reference [Cr] (µg/mL)	Mean recovery ^a (%)	Precision (% RSD)
Autosampler residence stability: 3.5 h				
0.100 (10.0 µg/g)	0.09631	0.09794	98.3	6.0
0.500 (50.0 µg/g)	0.4767	0.4775	99.8	0.35
1.00 (100 µg/g)	0.9922	0.9975	99.5	2.3
Refrigerator storage digest stability: 32 days				
0.100 (10.0 µg/g)	0.09967	0.09794	102	2.7
0.500 (50.0 µg/g)	0.4785	0.4775	100	1.8
1.00 (100 µg/g)	0.9892	0.9975	99.2	1.9
Freeze-thaw stability: three cycles				
0.100 (10.0 µg/g)	0.1010	0.09794	103	3.5
0.500 (50.0 µg/g)	0.4904	0.4775	103	1.0
1.00 (100 µg/g)	0.9955	0.9975	99.8	1.3

^aCalculated as (mean found [Cr] (µg/mL)/mean reference [Cr] (µg/mL)) × 100.

Table 7.

Freezer temperature storage stability

Nominal [Cr] (µg/mL)	Time point	Mean recovery ^a (%)	Recovery precision (% RSD)
0.100 (10.0 µg/g)	Day 15	101	17
0.100 (10.0 µg/g)	Day 30	116	8.0
0.100 (10.0 µg/g)	Day 239	119	3.0
0.500 (50.0 µg/g)	Day 15	104	8.3
0.500 (50.0 µg/g)	Day 30	93.4	4.1
0.500 (50.0 µg/g)	Day 239	98.3	NA ^b
1.00 (100 µg/g)	Day 15	105	11
1.00 (100 µg/g)	Day 30	101	2.2
1.00 (100 µg/g)	Day 239	106	0.54

^a Mean recovery for three preparations calculated against mean reference concentration.^b Not applicable; two of three replicates lost during sample preparation.

Table 8.

Mouse fecal cross-validation method linearity and recovery

Nominal [Cr] (µg/mL)	Response ^a		Found [Cr] ^b (µg/mL)	Recovery ^c (%)	Matrix recovery ^d (%)
	Matrix	Solvent			
0.100 (10.0 µg/g) ^e	0.02010	0.009777	0.1000	105 (4.2% RSD)	99.9
	0.02087		0.1083		
	0.02071		0.1066		
0.200 (20.0 µg/g)	0.02911	0.01946	0.1968	97.3 (1.6% RSD)	93.0
	0.02857		0.1910		
	0.02901		0.1957		
1.00 (100 µg/g)	0.1048	0.09076	1.009	100 (0.73% RSD)	103
	0.1036		0.9964		
	0.1036		0.9964		

^aInternal standard corrected emission intensity for matrix and solvent standards.^bCalculated with matrix regression equation $y = 0.09368(x) + 0.003240$; $r = 0.9992$.^cCalculated as (mean found [Cr] (µg/mL)/nominal [Cr] (µg/mL)) × 100.^dCalculated as (mean blank corrected matrix response/blank corrected solvent response) × 100. Mean matrix blank response = 0.01093; solvent blank response = 0.001610.^eCalculated as [concentration of Cr (µg/mL) × 25-mL digest volume]/nominal 0.250-g wet tissue mass.