

Cadmium Contamination in Cereal-Based Diets and Diet Ingredients

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Cereal-based diet and/or diet ingredient cadmium levels were determined by graphite furnace AAS. Cadmium contamination was 88.3 and 447 ppb in two cereal-based diets, 44.6 and 48.9 ppb in two purified diets, and ranged from less than 1.1 to 22 900 ppb in the ingredients of one cereal-based diet. The major source of cadmium contamination was attributed to the calcium supplement used for diet formulation. Comparative analyses of two purified diet samples and one cereal-based diet by the National Institute of Standards and Technology (NIST, formerly the National Bureau of Standards) and the National Center for Toxicological Research (NCTR) gave virtually identical results for Cd. A comparative study of Cd levels determined by flame and furnace AAS was also made by the NCTR and the NIST.

INTRODUCTION

Research at the National Center for Toxicological Research (NCTR) involves the toxicological evaluation and human risk assessment of various naturally occurring and/or artificial biological or chemical agents. This research requires that laboratory animal diets be monitored for known hazardous contaminants that could prejudice experimental results (Bowman, 1979; Coleman et al., 1979). It has been shown that cadmium accumulates in the kidney and has been implicated in connection with numerous deleterious health effects (Fowler et al., 1975). This study was necessitated by elevated levels of Cd detected in NIH-31 cereal-based diet, which the supplier asserted contained acceptable Cd levels. Acceptable Cd levels in diets for use at the NCTR had been established at 250 ppb. It was determined that the manufacturer used flame AAS to establish the diet Cd levels for certification, while the NCTR employed furnace AAS (Blakemore and Billedeau, 1981). The purposes of this study were to (1) validate the reliability of Cd measurement at the NCTR, (2) identify the source of Cd contamination in the diet supplied to the NCTR, and (3) determine the comparability of flame AAS to furnace AAS for quantitation of Cd at levels occurring in animal diets used at the NCTR.

MATERIALS AND METHODS

Diet. NIH-31 and Masoro diets were obtained from supplier A; Emory Morse diet was obtained from supplier B. For parallel analyses by the National Institute of Standards and Technology (NIST) and the NCTR 1 kg of each diet type was blended for 1 h in a Model LV twin-shell blender (Patterson-Kelley, East Stroudsburg, PA), split into equal portions, and transferred to Zip-loc plastic bags. Samples of NIH-07 cereal-based diet were obtained from the National Institutes of Health.

Diet Ingredients. Samples of each NIH-31 diet ingredient and a sample of the finished diet produced from those specific ingredients were obtained from supplier A.

Digestion Procedures. *Diet.* Prior to digestion, at the NCTR or the NIST, diet samples were dried for a minimum of 24 h at ambient temperature in a vacuum oven equipped with a liquid nitrogen trap to assure that diet samples were analyzed on a dry weight basis. The NCTR digestion procedure involved dry ashing 5.00 g of diet at 500 °C, leaching the residue with 1 N HNO₃, and adjusting the final volume to 50 mL (Bowman, 1979). For the comparative flame and furnace AAS analysis the digestion was modified to mimic the diet suppliers' procedure by increasing the weight of diet digested to 12.5 g and decreasing the final leachate volume to 25 mL. A wet digestion procedure was utilized by the NIST; 1.00-g diet samples

were digested with concentrated nitric, hydrofluoric, and perchloric acids to effect total dissolution.

Diet Ingredients. The organic constituents of diet were digested in the same manner as the finished diet. The inorganic constituents were solubilized with 1 N HNO₃ prior to analysis.

AAS Instrumentation. The AAS instrument used by the NCTR was an IL Model 957 Video 22 (Thermo Jarrell-Ash, Franklin, MA) with a Model 755 furnace atomizer and Fastac II autosampler operated by utilizing Smith-Heiftje background correction. The specific conditions used were a slit width of 320 μm, a bandpass of 1 μm, a signal current of 2.5 mA, and a background current of 1.5 mA. The NIST used a graphite furnace AAS instrument operated with Zeeman background correction. Additional instrumental conditions appear in Table I. For the flame AAS analysis at the NCTR a single-slot burner head was used with an air-acetylene fuel lean flame.

Recovery Determination. Experiments were performed to determine the percent of Cd recovered for each of the digestion procedures. At the NCTR triplicate 5.00-g diet samples were fortified with 1.00 μg of Cd as the nitrate prior to digestion. Recovery was determined by comparison to unfortified samples of the same diet. Also, NIST Standard Reference Materials (SRMs) 1567a wheat flour and 1577a bovine liver were analyzed for additional recovery data. At the NIST, duplicate samples of each diet type were fortified with NIST/SRM 3108 Cd prior to digestion for recovery determination.

RESULTS AND DISCUSSION

Recoveries. Cd recovery averaged $90.4 \pm 4.5\%$ for the diets analyzed by the NCTR. The level quantitated in NIST/SRMs was consistently within the statistical limits of the certified values. Recovery data for experiments conducted at the NCTR appear in Table II. Recovery of Cd at the NIST averaged $100 \pm 5\%$ for the three diet types.

Diet Cd Content. Results of furnace AAS analyses by the NCTR and the NIST appear in Table II. The percent difference in mean levels of Cd detected in the three diets analyzed by NCTR compared to the NIST results averaged $1.7 \pm 1.4\%$. These data, when combined with data resulting from analyses of diet ingredients and finished diet, led to the conclusion that valid Cd measurement techniques were employed at the NCTR.

The NCTR determined the Cd level in a NIH-31 diet by flame and furnace AAS using the same diet sample leachate for the separate analyses. The flame analysis resulted in a quantitation of 240 ± 6 ppb of Cd, while the furnace assay yielded 398 ± 37 ppb. A matrix spike addition to the leachate gave 95% recovery in both cases so that the mechanism for the lower quantitation by flame was not completely understood. The NIST stated that the Cd level

Table I. Instrumental Conditions for Analysis of Cd at 228.8 nm by Graphite Furnace AAS

special conditions	temp program, temp (°C)/time (s)		
	drying	charring	atomization
NIST			
L'vov platform, 1% (NH ₄) ₂ HPO ₄	120/40	750/20	2350/5
NCTR			
delayed atomization cuvette	125/10	225/10 - 350/20	2400/10

Table II. Cadmium Content (Micrograms per Kilogram) of Various Diet Types As Determined by Graphite Furnace AAS

diet type	NIST ^a	NCTR ^b	% difference	% recovery
NIH-07	NR ^c	88.3 ± 18		89.7 ± 5.2
NIH-31	448 ± 3	447 ± 20	0.22	88.6 ± 6.1
Masoro	46.0 ± 4.2	44.6 ± 2.0	3.0	96.8 ± 1.5
Emory Morse	48.0 ± 13	48.9 ± 1.3	1.9	86.4 ± 0.9

^a NIST mean ± SD are the result of duplicate analyses. ^b NCTR mean ± SD are the result of triplicate analyses. ^c This diet type not submitted to NIST for analysis.

in the NIH-31 diet sample was too near the detection limit to be determined by flame AAS even when they increased the sample weight to 5 g. These experiments suggest that furnace AAS is required to accurately quantitate Cd at levels which occur in these types of laboratory animal diets. Additionally, these data make it evident that strong QA/QC practices are essential to assure that analyses are reliable. The routine analysis of NIST/SRMs or similar certified reference materials is one practice that would allow the analyst to maintain better control of analytical results.

Diet Ingredient Cd. Results of furnace AAS analysis of NIH-31 diet ingredients and their contribution to Cd levels in the finished diet appear in Table III. From the data in Table III, it was apparent that the source of Cd contamination was due mainly to the dicalcium phosphate and, to a lesser extent, the ground wheat. The finished diet derived from the diet ingredients was also analyzed by furnace AAS and found to contain 386 ± 11 ppb of Cd. The calculated diet Cd level derived by summing the Cd contribution from each ingredient was comparable (within 10%) to the level determined by analysis of the finished diet. Discussion with the NIH-31 diet supplier indicated that technical rather than food grade dicalcium phosphate had been used for the diet formulation. It was also revealed that food grade dicalcium phosphate was used for formulation of both purified diets. A sample of the food

Table III. Cadmium Content (Micrograms per Kilogram) of NIH-31 Diet Ingredients

diet ingredient	Cd	% of formulation	contribution to diet, ppb
brewers' yeast	61.0	1.0	0.61
calcium carbonate	350	0.5	1.8
corn gluten meal	<mdl ^a	2.0	
dehulled soybean meal	120	5.0	6.0
dehydrated alfalfa	140	2.0	2.8
dicalcium phosphate	22 900	1.5	344
fish meal	58.0	9.0	5.2
ground corn	8.40	21.0	1.8
ground oats	66.0	10.0	6.6
ground wheat	110	35.5	39.1
sodium chloride	<mdl	0.5	
wheat midds	150	10.0	15.0
total		98.0 ^b	423

^a The minimum detectable level (mdl) was determined to be 1.09 µg/kg. ^b The diet soybean oil and vitamin premix which accounted for 1.5 and 0.5% of the formulation, respectively, were not submitted for analysis.

grade dicalcium phosphate was found to contain 66.7 ± 1.7 ppb of Cd when analyzed. Remediation of the elevated Cd levels in the NIH-31 diet was accomplished by specifying the use of food grade dicalcium phosphate for future diet formulations. The initial five lots of NIH-31 diet, formulated with food grade dicalcium phosphate, were analyzed by furnace AAS and found to contain 83.0 ± 1.3 ppb Cd.

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