Bull. Environm. Contam. Toxicol. 27, 810–815 (1981)

An Extraction Procedure May Not Be Feasible for Cadmium Analysis of Tissues, Such as Horse Kidney Cortex, Having A Very High Cadmium Content

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Cadmium is a wide-spread environmental contaminant attaining considerable scientific interest and concern. Normal values for cadmium in various biological materials have been presented in several reports (see review by FRIBERG et al. 1974; CEC 1978). PENUMARTHY et al. (1980) recently reported on lead, cadmium and mercury tissue residues in healthy swine, cattle, dogs and horses from the Midwestern United States. Lead and cadmium in tissues were determined by flame atomic absorption spectrophotometry (AAS) after wet digestion, dissolving of the residue in water, complexing with APDC and extraction into MIBK as originally described by YAEGER et al. (1971 and 1973). This method makes it possible to measure cadmium and lead in various biological materials at comparably low concentrations. The extraction procedure may, however, be less efficient when utilized for analysis of tissues with very high cadmium and lead content. YEAGER et al. (1971 and 1973) state that the total amount of cadmium or lead should not exceed 100 ug.

PENUMARTHY et al. (1980) present data on lead, cadmium and mercury concentrations in liver, kidney and muscle samples obtained from swine, cattle dogs and horses. Reported metal concentrations agree very well with other reports (BECKMAN et al. 1974; KREUZER et al. 1978) with one important exception - horse kidney. In the report by PENUMARTHY et al. (1980), the average cadmium concentration in 93 2-gram kidney samples obtained from yearlings and older horses was 2.5 ug Cd/g (range 0.84-5.0 ug

0007-4861/81/0027-0810 $01.20

Cd/g) wet weight. This is in the same range as cadmium in liver (0.83-4.1 ug Cd/g). These two organs had higher concentrations of cadmium than any other material analyzed in the study. The values from horse kidney are, however, considerably lower than what has been reported elsewhere. In adult Swedish horses slaughtered for meat production the average cadmium concentration in kidney cortex is in the order of 50 to 70 ug Cd/g wet weight (BECKMAN et al. 1974; PISCATOR 1975; ELINDER & PISCATOR 1978; NORDBERG et al. 1979). It should be mentioned in this context that kidney cortex generally have about 50% higher cadmium concentration in comparison to whole kidney, i.e. medulla and cortex FRIBERG et al. 1974). Similar results on cadmium concentration in horse kidney cortex have also been reported from Wales (U.K.) (GOODMAN & ROBERTS 1971), Finland (PEKKANEN et al. 1974; KORKEALA et al. 1976), Germany and Poland (ANKE et al. 1976; HOLM 1979) and Italy (RENON et al. 1980). There is a lack of data on cadmium in horse kidneys from the U.S. Two reports, each on two horses only, indicate, however, that also horses in the U.S. accumulate considerable amounts of cadmium in kidney (SASS et al. 1972; LEWIS 1972).

The extraordinary high content of cadmium in normal horse kidney cortex induced MARGOSHES & VALLEE (1957) for the first time to isolate a cadmium-binding protein, which was later given the name metallothionein (KÄGI & VALLEE 1960).

In the light of the discrepancy between PENUMARTHY'S et al. (1980) data on cadmium in horse kidney and what has been reported from Europe, the present authors have attempted to test the analytical procedure employed by PENUMARTHY et al. (1980) on kidney samples with low and high cadmium concentrations.

MATERIALS AND METHODS

Lyophilized and pulverized kidney cortex was obtained from three different batches with low, intermediate and high cadmium concentrations. The low level sample consisted of reference animal (pig) kidney (H.7) obtained from the International Atomic Energy Agency (WHO/IAEA 1980) having a certified cadmium concentration of 1.99 ± 0.19[a)] ug Cd/g dry weight. Intermediate and high level samples were taken from reference material prepared from horse kidney cortex by our department in connection with a quality control programme within the WHO/UNEP Pilot project on assessment of human exposure through biological monitoring (WHO 1979). The cadmium concentration in these samples was found to be 117 ± 2 ug Cd/g dry weight, n = 4, and 368 ± 6 ug Cd/g dry weight, n = 4, respectively, using the previously described method (ELINDER et al. 1976). These values have been confirmed not only by AAS analysis in several different participating laboratories, but also by neutron activation analysis (NA). The intermediate sample had been measured as 110 ± 15 ug Cd/g by four laboratories using NA and as 126 ± 13 ug Cd/g in six laboratories using AAS. The high level samples had been measured as 344 ± 47 ug Cd/g by three

a) Average $\pm$ S D

laboratories using NA, and as 355±15 ug Cd/g in three laboratories employing AAS (unpublished results within the WHO/UNEP Biological Monitoring Programme).

Cadmium analysis were carried out on these three samples and on a blank with and without additions of about 0.5 ug Cd. The analyses were performed exactly as described by YEAGER et al. (1971 and 1973) and by PENUMARTHY et al. (1980). About 0.36 g of lyophilizied kidney, corresponding to about 2 g of fresh weight, was digested in 25 ml concentrated nitric acid and perchloric acid in 2:1 ratio. Digestion was performed to complete dryness. The residue was subsequently dissolved in 5 ml of deionized water. Two drops of phenol red indicator were added and pH adjusted to 8.5 by addition of 5 ml ammonium citrate buffer and ammonium-hydroxide drop wise. One ml of 1% w/v potassium cyanide solution and 1 ml of 2% w/v APDC was added. The volume was adjusted to about 40 ml by addition of deionized water. Four ml of water saturated MIBK ketone was added. The APDC complex was extracted into the ketone phase by vigorous shaking for more than 30 sec. Deionized water was then added to bring the MIBK phase up to the neck of the volumetric flask. The flask was shaken again, and subsequently kept still for some minutes before AAS analysis. The MIBK phase was aspirated into a Perkin Elmer 403 AAS instrument, equipped with a three-slot Boling burner head, and a Perkin Elmer recorder Model 56. A system for deuterium background correction was available, but not used since this was not done by PENUMARTHY et al. (1980). The nebulizer of the AAS instrument had previously been optimized to give a maximum absorption when using a copper standard solution extracted into the MIBK phase as an APDC complex. A solution of 1.25 ug Cu/ml MIBK gave an absorption of 0.250 absorbance units. Cadmium analyses were performed with an electrode-less discharge lamp (EDL) at standard conditions for flame analysis in MIBK. The sensitivity for cadmium in the MIBK solution was 0.008 ug Cd/ml, i.e. the cadmium concentration resulting in an absorption of 0.0044 absorbance units.

RESULTS

Table 1 presents the calculated total amount of cadmium in samples (a) and the amounts measured (b) with the extraction technique. The ratio (in percent), between the measured amount minus blank and the calculated amount of cadmium in samples, was 86% for the blank with addition of 0.5 ug Cd and 91% for the low level pig kidney with and without additions of 0.5 ug Cd. The ratio was however unacceptably low for the intermediate and high level horse kidney samples. The extracted amount of cadmium did not differ markedly between the intermediate and the high level kidney cortex sample in spite of the fact that the cadmium content was more than 3 times higher in the high level sample compared with the intermediate. Due to the very high content of cadmium in the MIBK phase in some of the samples after extraction, one portion of the ketone phase was diluted 5 to 20 times before measuring the concentration in the AAS. This did not markedly influence the measured amount. Thus, the non-linearity between absorption and concentration was not the cause of the low recovery in the high level samples.

TABLE 1

Estimated total amount of cadmium in samples and measured amount of cadmium after extraction.

Sample	Weight (g)	Concentration (ug Cd/g)	Cd added (ug)	(a) Total amount of Cd (ug)	(b) Measured amount (ug)	Ratio $\frac{b\text{-blank}}{a}$ (%)
Blank	-	-	-	-	0.02	-
Blank	-	-	0.511	0.511	0.46	86
Animal kidney (IAEA ref)	0.370	1.99	-	0.736	0.69	91
Animal kidney (IAEA ref)	0.353	1.99	0.501	1.203	1.11	91
Horse kidney	0.357	117	-	41.8	11.2	27
Horse kidney	0.372	117	0.501	44.0	13.2	30
Horse kidney	0.359	368	-	132.1	9.2	6.9
Horse kidney	0.354	368	0.501	130.8	6.4	4.9

DISCUSSION

Extraction procedure for cadmium analysis in biological materials has been shown to be efficient and accurate for analysis of cadmium at low concentration in different biological materials (e.g. YEAGER et al. 1971 and 1973). For cadmium determination in materials with a comparably high concentration, it is usually sufficient to use dry or wet ashing followed by dissolvement of the ashes in acid, and analysis of the metal concentration in the acid by ordinary flame AAS, preferably with a system compensating for nonspecific absorption. The present report indicates that extraction procedure, as performed by PENUMARTHY et al. (1980) may not be feasible for cadmium analysis in samples such as horse kidney which have very high cadmium content. Our results give evidence that such analysis can give rise to far too low measured amounts. The measurable amount of cadmium far less than the actual amount in kidney cortex having a cadmium concentration of about exceeding 117 ug Cd/g dry weight, corresponding to about 22 ug Cd/g wet weight. Cadmium concentrations of these magnitudes are, apart from horse kidneys, also found in human kidney and in some cases also in human and horse liver. However, at cadmium concentrations below 2 ug Cd/g dry weight, the method appears to be sufficient.

Apart from difficulties encountered when using the extraction procedure for cadmium analysis in high level samples described in this report, and the discrepancy between data on cadmium in horse kidney presented by PENUMARTHY et al. (1980) in comparison with data from other reports, it should also be mentioned that the ratio between horse kidney and horse liver concentration presented by PENUMARTHY et al. (1980) is unusual. The average cadmium concentration was 3.4 ug/g wet weight in horse liver and 2.5 ug/g in horse kidney. As a rule, cadmium concentration in kidney exceeds that in liver by a factor 2-15 (FRIBERG et al. 1974; ELINDER et al. 1981). This is also the case for swine, dogs and cattle as presented in the report by PENUMARTHY et al. (1980). These latter samples had lower and probably more correctly measured cadmium concentrations.

In conclusion, on the basis of our data, and data from the literature, we believe that results on cadmium concentration in horse kidney presented by PENUMARTHY et al. (1980) are erroneous. There is, however, no reason to mistrust the other analytical results in that report.

It is not possible, based on this limited study, to conclude which part of the analytical procedure that has failed and resulted in a very low ratio of measured cadmium from high level samples. The satisfying ratio of measurable cadmium in the low level samples could indicate that the capacity of the extraction procedure has been insufficient.

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