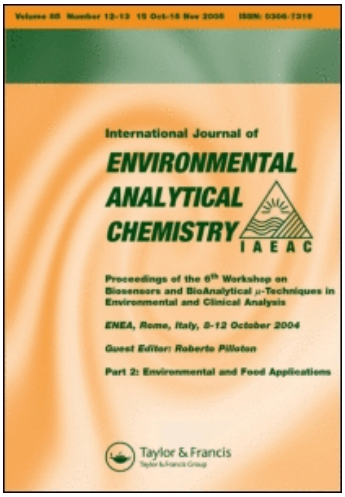

International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

An Analytical Procedure for the Determination of Cadmium in Human Placentae

B. Van Hattum[ab]; P. De Voogt[ab]; J. W. Copius Peereboom[c]
[a] Department of Obstetrics and Gynaecology, Wilhelmina Hospital, Amsterdam [b] Institute for Environmental Studies, Free University, Amsterdam, The Netherlands [c] Institute for Environmental Studies, Amsterdam

To cite this Article Van Hattum, B. , De Voogt, P. and Peereboom, J. W. Copius(1981) 'An Analytical Procedure for the Determination of Cadmium in Human Placentae', International Journal of Environmental Analytical Chemistry, 10: 2, 121 — 133
To link to this Article: DOI: 10.1080/03067318108071537
URL: http://dx.doi.org/10.1080/03067318108071537

Intern. J. Environ. Anal. Chem., 1981, Vol. 10, pp. 121–133
0306-7319/81/1002-0121 $06.50/0

Printed in Great Britain

An Analytical Procedure for the Determination of Cadmium in Human Placentae

B. VAN HATTUM, P. DE VOOGT,

Wilhelmina Hospital, Department of Obstetrics and Gynaecology, Amsterdam. Present address: Institute for Environmental Studies, Free University, P.O. Box 7161, Amsterdam, The Netherlands

and

J. W. COPIUS PEEREBOOM

Institute for Environmental Studies, Free University, Amsterdam

(*Received 3rd September, 1980*)

Cadmium was determined in human placental tissue by flameless atomic absorption spectrometry (AAS). Several sampling, homogenizing and decomposition procedures were tested with regard to their suitability for flameless AAS. Main criteria involved recovery, representativity, contamination, accuracy and precision. Analysis of biological reference materials yielded results in agreement with reported certified values or grand means.

A sampling strategy was developed based on expected placental distribution patterns of the metal. The sampling method used appeared to be satisfactorily representative of the organ as a whole.

During 1978 and 1979 placentae were collected from mothers living in the Amsterdam area in the Netherlands. Mean placental cadmium levels of smokers (66 ± 33 ng/g dry weight) appeared to be slightly elevated compared to those of non-smokers (51 ± 20 ng/g).

KEY WORDS: Cadmium, analysis, biological tissue, placenta, smoking

INTRODUCTION

In recent years, the accumulation of certain trace elements in the human body has given rise to an increased concern. Amongst the heavy metals, cadmium is considered as one of the most hazardous, since environmental levels of this element appear to approach existing guideline values[1–3]. In particular some vulnerable groups of the general population may be at

risk, e.g. pregnant women and their fetuses. Reduced birth weights of new borns from women occupationally exposed to cadmium have been reported.[4] Moreover, in a few studies, cadmium was found to be embryotoxic to animals.[5-7] Effects on reproductive organs have been shown.[5,8] To establish body burdens of environmental pollutants, the trace element analysis of human body fluids and human tissues has become of particular interest.[9,10] Since the kidney is considered as the critical organ for cadmium exposure, monitoring should be focused, by preference, on easy obtainable tissues and/or body fluids that show a distinct relation to the cadmium burden of the kidney.

From epidemiological studies Lauwerijs et al.[13] concluded that cadmium in blood appears to reflect mainly recent exposure, whereas cadmium in urine either reflects integrated exposure (= body burden) at low exposure levels or recent exposure at high levels of exposure. Recently, the in vivo measurement of metals in kidney and liver by prompt γ-ray spectroscopy has been used in monitoring occupational exposure.[11,12] In a recent workshop of the CEC/WHO/IUPAC the analysis of cadmium in blood, tissue and hair was recommended for monitoring purposes.[14] The human placenta has been advocated as a suitable indicator for environmental exposure to heavy metals "because of its unique lifetime of several months, thus reflecting an integrated environmental exposure".[15]

Several authors have reported on the determination of cadmium in human placentae. Data up to January 1980 are summarized in Table I. The rather divergent results cannot be explained only by different exposure levels. A variety of procedures for sampling, sample handling and analysis has been used. Except for the study of Thieme *et al.*[19] no systematic evaluations of accuracy and precision of the analytical procedures were presented. Special emphasis was therefore laid on the quality control of the analytical procedure used in our study.

We developed a simple, low-cost and relatively rapid method for the routine determination of cadmium in human placental tissue by flameless AAS. A sampling procedure including homogenizing was developed in order to get representative aliquots, since cadmium may be inhomogeneously distributed within the organ. Two wet-decomposition procedures were tested on their suitability for the flameless AAS determination. The reliability of the results of this study was evaluated by analyzing standard biological reference materials and by recovery determinations. Furthermore some of the results of the placenta determinations were compared with those of two other analytical methods, viz. destructive neutron activation analysis and dithizone-extraction-UV spectrophotometry.

TABLE I
Cadmium in human placentae

Country	Concentrations as ng Cd/g (ppb) (wet weight)	(dry weight)	n	Ref.
BRD Ruhrgebied (Dortmund)	176 ± 51	1051 ± 284	53	16
Mittel Franken	129 ± 39	854 ± 287	34	16
Bayerische Wald	152 ± 52	861 ± 293	61	16
Berlin	43 ± 10	252	20	17
Ruhrgebied (Essen)		120 ± 80	23	18
Altötting		18 ± 1	29	19
München		21 ± 4	45	19
Bayerische Wald		28 ± 3	30	19
USA ?	72†		125	9
Texas	8000	47000	554	20
Georgia	53 ± 4		19	21
Alabama	30 ± 5		22	21
North Carolina	28 ± 4		17	21
Tennessee	17 ± 12	102 ± 77	135	15
UK	20		—	22
Belgium smokers	16 ± 9		109	23
non-smokers	12 ± 9		333	23
Sweden	< 10		44	24

†probably wet weight, not properly specified by authors.

We applied this procedure to determine cadmium levels in placentae from non-smokers and smokers (smoking 15 or more cigarettes/day). Smoking has been shown to contribute considerably to the daily intake of cadmium[1-3], of which a small portion is accumulated in the placenta[23,25].

MATERIALS AND METHODS

Sample Collection

During 1978 and 1979 61 term placentae were obtained from the Department of Obstetrics and Gynaecology of the Wilhelmina Gasthuis in Amsterdam. The placentae were collected in two separate series, indicated as series A (n = 23) and series B (n = 38). The results of series A were used to optimize sampling, pretreatment and chemical analysis of series B. All women that were selected lived in the Amsterdam area. Smoking habits were examined by means of questionaires. For series A this was performed only once at a prenatal visit, whereas for series B the questionaires were filled in at three different stages during pregnancy (at month 3, at mid-pregnancy and after delivery). The umbilical cord was cut at least 3 min after delivery. Placentae were collected in acid cleaned stainless steel dishes. After weighing the placentae of series A were put in a 6%

formaldehyde solution during 2–5 days, as this was a standard procedure in the clinic, including also routine examination of the organ on infarcts. Then several blocks were cut and prepared for routine light microscopy.

Subsequently the placentae were transferred to polyethylene bags, which were previously examined on contamination, and stored in a deep-freezer at −20°C. Regular samples were taken from the formaldehyde solutions to examine possible interfering adsorption and/or migration processes.

Placentae of series B were sampled directly after delivery, using acid cleaned carbon steel scalpels. Approximately one half of each placenta was collected and stored in polyethylene bags at −80°C in a Format 8107 ultra-freezer.

For both series code numbers were given to the samples at the clinic in order to perform genuine blind analysis. The placentae were transported to the laboratory in insulated boxes on dry ice and stored at −40°C until the final analysis.

Sample Preparation

After recording the as received weight the placentae were thawed overnight at 4°C. No significant weight changes were observed during thawing for placentae of series A. For placentae of series B the blood losses during thawing varied from 13–33% of the as received weight (mean, 21%, standard deviation, 6%; n=38). To avoid contamination and metal losses sample preparation was kept limited. Adhering blood clots were removed. Easily removable membranes were cut off. This resulted in a mean weight loss of 3.2% with regard to the as received weight.

Radial samples (see Fig. 1 below) of approximately 80 g were dissected. Carbon steel scalpels and petri dishes (ϕ=25 cm) were applied for the preparation of series A. Materials with lower cadmium contamination potency were used for the preparation of samples of series B: cutting was performed with titanium knives (obtained from IAEA, Vienna), PTFE spatulas and perspex pincets in polyethylene boxes. Directly after preparation the wet weight was determined. Subsequently the samples were lyophilized to constant weight in a freeze-dryer (Virtis, cold trap −60°C, 0.1 Torr). Constant weight was achieved for all samples after a drying period of 120 h.

The dried samples were homogenized with a Retsch-Ultra centrifuge mill (ZM−1, ring sieve 0.12 mm, 12,000 r.p.m.) and stored in polyethylene jars in the dark at room temperature.

Wet Decomposition

From each homogenized placental sample of series A four aliquots of

500 mg were taken with a PTFE spatula and transferred to 30-ml borosilicate Kjeldahl flasks.

Every four aliquots one blank sample was included. Blanks and samples were digested with 2.0 ml HNO_3 (Merck, Suprapur no. 441) and 1.0 ml $HClO_4$ (Baker Analysed, no. 6022) during 2 h on a destruction block, the temperature of which was raised from 120 to 250°C at the end of the destruction. After decomposition the samples were cooled, transferred to volumetric flasks, diluted to 25 ml with twice distilled water (bidist) and stored in polyethylene cups at 4°C.

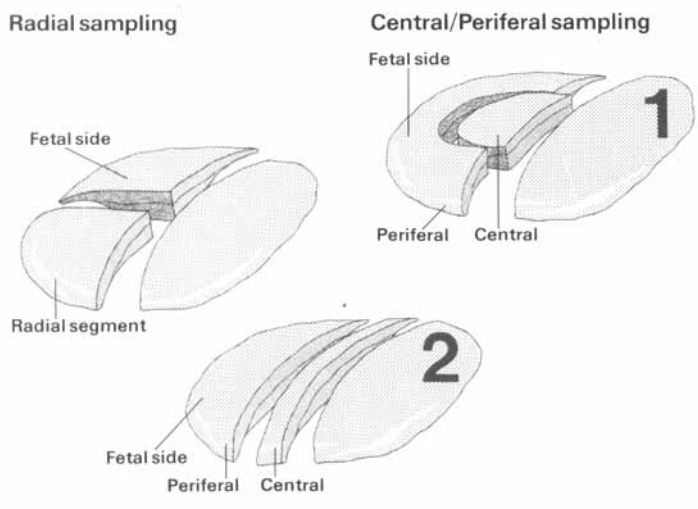

FIGURE 1 Diagram outlining the sampling procedures used for representativity studies.

Pressurized Decomposition

Three aliquots of 150 mg of samples of series B were decomposed with 1.0 ml HNO_3 (Baker Ultrex 4801) in pressurized PTFE vessels (Parr, acid destruction bomb 4746) at 165°C during 2 h in a muffle furnace. Residue formation was avoided by a rapid cooling procedure in a −40°C atmosphere during 45 min. The sample solutions were quantitatively transferred to volumetric flasks, diluted to 10 ml with bidist and stored in polyethylene cups at 4°C. Each series of three aliquots included a blank sample.

Analysis

Analysis was performed by flameless AAS with a Perkin Elmer −403 spectrometer and a HGA–72 graphite atomizer. Deuterium background

correction was applied in order to correct for broad band absorption. From preliminary analysis of standards and samples it was concluded that standard addition calibration had to be applied. A standard stock solution of 1000 ppm Cd was prepared by dissolving MerckTitrisol 9960 in a 0.1 N HNO_3 solution. Standards in the 0–4 ppb range were prepared daily in polyethylene volumetric flasks. Aliquots of 50 μl were injected manually with Eppendorf micropipets for series A. Better precision and a reduction of accidental contamination was achieved for series B, where samples were injected with a Perkin Elmer AS–1 autosampler.

TABLE II
Instrumental settings

HGA-72		PE-403	
Shielding gas	N_2	Wavelength	228.8 nm
Injection	50 μl	Spectral bandwidth	2.0 nm
Replicate firings	4x	Source	EDL,HKL
Drying	90°C–45 sec	Recorder	0.25A = 10 mV
Ashing	325°C–30 sec		
Atomization	1900°C–7 sec		

Instrumental settings (Table II) were chosen according to manufacturers manuals and/or derived from well-known optimizing procedures.[26-28] Absorbance signals were recorded on a Kipp BD-8 recorder. No scale expansion was applied. Peak heights were measured manually. Sample concentrations were calculated from least-squares addition plots. After blank correction dry- and wet-weight placental cadmium concentrations were calculated. As blank levels did not show significant changes with time (2 months) a mean blank level was used for correction. Chauvenet's criterion was used to exclude outlying values.

Cleaning and Contamination Control

As placental cadmium concentrations are usually in the ng/g range, cleaning procedures and contamination control were a prerequisite. Prior to acid cleaning materials were cleaned with a laboratory detergent (Extran, Merck 7550) and rinsed with demineralized water.

All materials used for determination, analysis and storage were rinsed with 6N HNO_3 followed by five fold rinsing with bidist. Sampling materials were rinsed with 1N HNO_3 and bidist (5x). PTFE cups used for pressurized decomposition were placed for 1 h in a hot 6N HNO_3 solution, prior to the above described cleaning procedure. AS-1 PTFE sample cups, micropipet tips, borosilicate Kjeldahl flasks and volumetric

flasks for sample dilution were soaked in a 0.6N HNO_3 solution during at least 24 h before acid cleaning with the 6N HNO_3 solution. All materials were dried at 60°C in a drying oven and stored in closed polyethylene containers.

Reagents and bidist were regularly analysed for cadmium contamination. During all activities Kimguard surgical gloves were worn.

RESULTS AND DISCUSSION

Placental tissue can be regarded as relatively homogeneous; nevertheless one may expect that cadmium (and other metals) are not evenly distributed over the organ. To get a representative sample one has to account for this possibly inhomogeneous distribution of the metal. Samples large enough to include different tissue types[29] should be taken and carefully homogenized before analysis. We designed different sampling procedures, as presented in Fig. 1. Cadmium accumulation may differ between central and periferal parts of the placenta. Consequently the radial sampling should possibly represent an adequate sampling procedure. This was tested by analyzing 5 duplicate radial samples of series B. Differences between central and periferal regions of the placenta were studied by analyzing two groups of 5 related central and periferal samples of series B. The wet weights of radial, central and periferal samples were approximately 80 g each. Differences between related parts were tested on significance with the *t*-statistic for two means.

The results of the comparison between different regions of placentas are shown in Table III. No statistically significant differences were found between the cadmium contents of the different parts of a placenta, irrespective of the sampling procedure used. That is, taking samples either in a radial or a periferal/central way of sufficient size (approx. 80 g) and subsequently homogenizing it according to the present procedure yields representative aliquots for analysis of cadmium.

Spike recovery studies on both HNO_3-$HClO_4$ and pressurized HNO_3 decomposition were performed by volumetric addition of known amounts of dissolved $CdCl_2$ to homogenized samples. After analysis of spiked and non-spiked samples the recovery rate was determined as $100\% \times$ Cd-found/Cd added. The results were $102 \pm 11\%$ $n=9$) for method I and $98 \pm 16\%$ ($n=5$) for method II. The ranges found can be considered as usual in trace element analysis.

Analysis and decomposition of standard reference materials were identical to that of placental samples. The moisture content of the reference materials was determined by freezedrying separate aliquots. NBS bovine liver (SRM-1577) and Bowens kale were digested with both

TABLE III

Verification of the representativity of different parts of a placenta by comparison of cadmium contents (in ng/g dry weight) in these parts

sampling[b]	Cd: mean ± s.d.	mean ± s.d.	df[c]	student's[d] t
radial	left[e]	right[e]		
placenta no. 1	73 18	69 28	4	0.0533†
no. 2	61 11	45 24	4	0.7576[a]
no. 3	59 23	53 19	6	0.1568[a]
no. 4	38 19	43 14	8	−0.4203[a]
no. 5	31 7	36 9	4	−0.7029[a]
central/ periferal 1				
no. 6	78 16	68 27	4	0.3286[a]
no. 7	76 11	90 18	4	−1.4390[a]
no. 8	85 24	73 7	4	0.6239[a]
no. 9	39 22	49 16	5	−0.6860[a]
no. 10	90 5	101 18	4	−1.3109[a]
central/ periferal 2				
no. 11	105 15	80 26	4	1.2920[a]
no. 12	84 25	59 11	3	1.1361[a]
no. 13	51 8	39 12	4	1.1157[a]
no. 14	28 17	22 10	8	0.5463[a]
no. 15	62 13	57 9	4	0.2101[a]

[a] Differences not significant at .20 level (two tailed)
[b] See Fig. 1
[c] Degrees of freedom
[d] Statistics for two means
[e] Arbitrary

procedures. By mediation of the Dutch Interuniversitary Reactor Institute, Delft, single-cell protein (BCR 0006), which will be issued in the future as a reference material with low cadmium content, was obtained from the reference bureau of the CEC. Single-cell protein was only decomposed by the pressurized HNO_3 method. The IAEA Copepoda sample was analyzed by the open digestion method only. The results are presented in Table IV. Determined values are in reasonable agreement with certified or reported values, although in all cases the determined value is somewhat higher. It should be noted however, that most of the available biological reference samples have cadmium contents in the 100–1500 ppb range. Attention should be focused on the single-cell protein determinations, since only this material contains cadmium in a concentration range comparable to placental tissue. Moreover, the definition term of standard reference material does not genuinely apply to Bowen's kale nor to Copepoda since the "certified" value is a grand mean of (several) hundreds of determinations made all

over the world; the "certified" value, in fact, may alter every year. When applied to aliquots of one placenta homogenate, both method I and II yielded results in good agreement, as can be seen from the last column in Table IV.

TABLE IV

Comparison between HNO_3-$HClO_4$ decomposition (I) and pressurized HNO_3 decomposition (II) in the analysis of cadmium in standard reference materials and one placenta sample (concentrations in ng/g cadmium, dry weight)

		Single-cell protein CEC	SRM 1577 bovine liver NBS	Bowen's kale	MA-A-1 Copepoda IAEA	Human placenta
reported value		30 ± 2	270 ± 40	850 ± 150	750 ± 30	
determined value	method I	n.d.	328 ± 24	1060 ± 160	n.d.	78 ± 9
	n =		5	10		4
	method II	37 ± 9	310 ± 11	1030 ± 220	877 ± 95	87 ± 8
	n =	5	4	6	4	3

n.d. = not determined

TABLE V

Cadmium contents (ng/g dry weight) in placenta samples analyzed by different methods.

	AAS			Destructive NAA			Dithizone-UV/Vis.		
Sample	mean	s.d.	n	mean	s.d.	n	mean	s.d.	n
placenta 17	84	17	4	62	11	6			
placenta 18	36	8	4	46	3	5			
placenta 19	37	7	4				40	4	3

In a small-scale intercomparison study two placental samples were analyzed by the Dutch Interuniversitary Reactor Institute, according to a destructive neutron activation analysis method.[30] Another sample was analyzed by the Department of Nuclear Chemistry, Physics Laboratory, Free University, Amsterdam, according to a spectrophotometric method.[31] In Table V the results of the flameless AAS determination using the open digestion method (I) are compared with those of the two other analytical procedures; three samples from the series A were used in this intercomparison experiment.

This set of four accuracy control experiments points out that the

analytical procedures can be considered as practically non-biased, either with the HNO_3-$HClO_4$ or with the pressurized HNO_3 decomposition.

Two series of placentae from smokers and non-smokers living in the Amsterdam area were analyzed according to the above methods. Series A, consisting of 23 placentae (12 from non-smoking women and 11 from women smoking 15–25 cigarettes/day) was determined according to method I, whereas series B (38 placentae, 19 from non-smokers and 19 from women smoking 20–50 cigarettes/day) was determined according to method II.

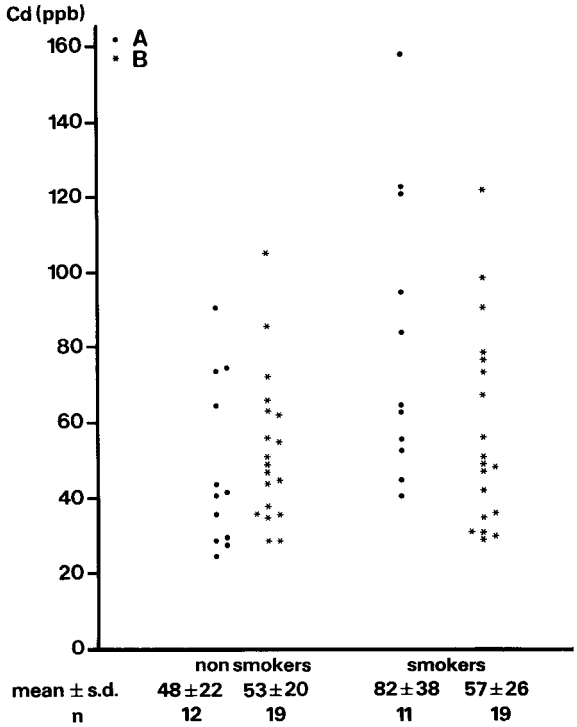

FIGURE 2 Cadmium concentrations (in ppb dry weight) in human placentae from non-smokers and smokers from the Amsterdam area in 1978 (series A) and 1979 (series B).

The repeatability of the analytical procedure was determined by analysis of 4 aliquots of one sample in method I and 3 aliquots of one sample in method II. Mean repeatability using method I was 28 % ($n=23$), whereas method II showed a mean repeatability of 21 % ($n=38$). This decrease can be partly attributed[32] to the reduction of the blank level from 20 ± 9 ng cadmium in method I to 2.7 ± 1.7 ng cadmium in method II. The results are shown in Fig. 2, cadmium concentrations being given in parts per billion (ng/g) dry weight basis.

From accuracy as well as precision data it can be concluded that both methods will yield comparable results, when applied to the same sample material. This is confirmed by the results of the non-smoking groups of series A and series B, which can be regarded with respect to cadmium exposure as samples from the same population. Dry weight placental cadmium levels for the non-smoking women of series A and series B were found to be 48 ± 22 ppb ($n = 12$) and 53 ± 20 ppb ($n = 19$). As women of series B smoked 20–50 cigarettes a day, whereas in series A tobacco consumption was 15–25 cigarettes/day, we expected to find slightly elevated cadmium levels for series B. As can be seen from Fig. 2 we found smokers of series A to have a higher cadmium content (83 ± 38 ppb, $n = 11$) than smokers of series B (57 ± 26 ppb, $n = 19$). Individual consumption habits, sizes of the investigated populations and differences in individual cadmium absorption rates may account for this, as will be discussed elsewhere.

When both series A and series B are combined mean cadmium contents of placentae from smokers (66 ± 33 ppb, $n = 30$) are slightly increased (29%) compared to non-smokers (51 ± 20 ppb, $n = 31$). The difference appeared to be weakly significant at a $p < 0.05$ level (Mann Whitney-U test). Roels *et al.*[23] reported a comparable difference for placentae of smoking and non-smoking mothers in Belgium.

When the results of this study are compared with literature data (Table I) dry/wet weight conversion factors have to be applied. Here the problem arises how to define analytically the wet weight of a placenta. Unfortunately, authors have not always been clear with regard to this aspect, thereby preventing an accurate comparison. One of the main difficulties is the blood loss during thawing of the fresh frozen placental samples. As cadmium blood levels of the general population tend to be rather low[1-3] (<5 ppb) the total amount of cadmium in a placenta will be influenced only slightly by blood losses during thawing. However, blood losses can account for as high as 20% of the total as received weight of a placenta, resulting in an underestimation of the wet-weight concentration. Therefore, in this study, the wet-weight determination was standardized in such a way, that it was measured after thawing and preparation. In this manner a mean dry/wet weight conversion factor of 5.6 was obtained, which corresponds with the value of 6.02 reported by Baglan *et al.*[15] The wet-weight concentrations for placentae of smokers and non-smokers were found to be 12 ± 6 ppb ($n = 30$) and 9 ± 4 ($n = 31$). As can be seen from Table I this agrees very well with data of Roels *et al.*[23], Thieme *et al.*[19] and Baglan *et al.*[15]

Considering the results of the present study, as well as reliable data from the literature (see Table I), we conclude that placental cadmium

concentrations will fall in the range of about 2–75 ppb, wet-weight, for the general population in the western industrialized countries. Higher reported values should probably be attributed to poor analytical procedures and contamination during pretreatment. Occupationally or otherwise heavily exposed individuals may well display higher levels.

CONCLUSIONS

Representative sampling of the human placenta can be performed by cutting a sufficiently large sample (i.e. 70–100 g), either in a radial or central/periferal way and subsequent homogenizing. Both wet digestion by perchloric and nitric acid and pressurized decomposition with nitric acid yield repeatable and accurate results when determining cadmium in placental samples with flameless AAS. The pressurized decomposition method is to be preferred because of its speed and low contamination risk. Careful examination of the accuracy and precision of the results should always be carried out when ppb levels of cadmium are analyzed. There is a need for standard reference materials in the ppb range. Wet weight cadmium concentrations can be severely influenced by preparation conditions.

In the present study, mean placental cadmium levels of smokers (66 ± 33 ppb, dry-weight) appeared to be slightly elevated compared to those of non-smokers (51 ± 20 ppb). These figures and data from the literature point out that cadmium concentrations in human placentae will fall in the range of about 2–75 ppb, wet-weight, considering the general population in Western Europe and the US.

Acknowledgements

We thank Prof. Dr. P. E. Treffers from the Department of Obstetrics and Gynaecology Wilhelmina Gasthuis, Amsterdam and Dr. J. H. J. Copius Peereboom-Stegeman from the Laboratory of Histology and Cell Biology, University of Amsterdam for their valuable assistance and remarks. We also thank Drs. W. van der Velde and Mr. W. Korper for their cooperation in collecting the samples, Dr. J. J. M. de Goeij from the Interuniversitary Reactor Institute (IRI, Delft) and Dr. R. Vis from the Department of Nuclear Chemistry, Physics Laboratory, Free University, Amsterdam, for enabling the comparison analysis and Drs. J. F. Feenstra and Prof. Dr. U. A. Th. Brinkman for reading the manuscript. Finally, we thank Mrs. L. Nassuth and Mrs. A. Jessurun for their typographical assistance.

This investigation was supported by the Dutch Praevention Fund nr. 28–493,6 and the Governmental Steering Group for Environmental Research (LaSOM).

References

1 Commission of the European Communities, Criteria for cadmium (Pergamon Press, Oxford, 1978) 1st ed.

2. L. Friberg, M. Piscator, G. F. Nordberg and T. Kjellström, Cadmium in the Environment (CRC Press, Cleveland, Ohio, 1974) 2nd. ed.
3. P. de Voogt, B. van Hattum, J. F. Feenstra and J. W. Copius Peereboom, *Tox. Environ. Chem. Rev.* **3,** 89 (1980).
4. R. P. Tsvetkova, Gig. Truda. Prof. Zabolevanij **12,** 31 (1970).
5. J. Parizek, J. Reprod. Fert. **7,** 263 (1964)
6. S. Chaube, H. Nishimura and C. A. Swinyard, *Arch. Environ. Health* **26,** 237 (1973)
7. V. H. Ferm and S. J. Carpentier, *Lab. Inv.* **18,** 429 (1968).
8. J. H. J. Copius Peereboom-Stegeman and E. J. Jongstra-Spaapen, *Toxicol.* **13,** 199 (1979)
9. A. V. Colucci, D. I. Hammer, M. E. Williams, T. A. Hinners, C. Pinkerton, J. L. Kent and C. J. Love, *Arch. Environ. Health* **27,** 151 (1973)
10. G. F. Nordberg (ed.), Effects and dose-response relationships of toxic metals (Elsevier Scientific Publishing Company, Amsterdam, New York, Oxford, 1976), 1st. ed.
11. K. J. Ellis, D. Vartsky, I. Zanzi, S. Cohn and S. Yasumura, *Science* **205,** 323 (1979)
12. B. J. Thomas, T. C. Harvey, D. R. Chettle, J. S. McLellan and J. H. Fremlin, *Phys. Med. Biol.* **24,** 432 (1979)
13. R. R. Lauwerijs, H. A. Roels, S. Buchet, A. Bernard and D. Stanescu, *Environ. Health Persp.* **28,** 137 (1979)
14. Workshop Analysis of Cadmium in Biological Materials, CEC/WHO/IUPAC, Amsterdam, June 1980
15. R. J. Baglan, A. B. Brill, A. Schulert, D. Wilson, K. Larsen, N. Dyer, H. Mansour, W. Schafener, L. Hoffman and J. Davies, *Environ. Res.* **8,** 64 (1974)
16. J. Thürauf, K. H. Schaller, E. Engelhardt and K. Gossler, *Int. Arch. Occup. Environ. Health* **36,** 19 (1975)
17. R. Riemschneider, A. F. Martinsand, P. Dietsch, Geburtsh.u.Frauenheilk. **38,** 971 (1978)
18. R. Thieme, P. Schramel and E. Kurz, Geburtsh.u.Frauenheilk. **37,** 756 (1977)
19. R. Thieme, P. Schramel, B. J. Klose and E. Waidl, Geburtsh.u.Frauenheilk. **34,** 36 (1974)
20. E. B. Dawson, H. A. Croft, R. R. Clark and W. J. McGanity, *Am. J. Obstet. Gynec.* **102,** 354 (1968)
21. W. B. Karp and A. F. Robertson, *Environ. Res.* **13,** 470 (1977)
22. A. K. Khera and D. G. Wibberley, *Proc. Anal. Div. Chem. Soc.* **13,** 340 (1976)
23. H. A. Roels, G. Hubermont, J. P. Buchet and R. Lauwerijs, *Environ. Res.* **16,** 236 (1978)
24. M. Piscator in L. Friberg, M. Piscator, G. F. Nordberg and T. Kjellström, Cadmium in the Environment (CRC Press, Cleveland, Ohio 1974) 2nd. ed. p. 30
25. L. Dencker, J. Reprod. Fert. **44,** 461 (1975)
26. B. Welz, Atomic Absorption Spectroscopy (Verlag Chemie-Weinheim New York, 1976) 1st. ed.
27. H. Hein and W. Schrader, Applied Atomic Absorption Spectroscopy (Bodenseewerke Perkin Elmer GMBH, 1976, heft 3)
28. C. Hendrikx-Jongerius and L. de Galan, *Anal. Chim. Acta* **87,** 259 (1976)
29. P. Lievens, thesis, Rijks Universiteit Gent (Belgium) 1977
30. P. S. Tjioe, J. J. M. de Goeij and J. P. W. Houtman, *J. Radioanal. Chem.* **37,** 511 (1977)
31. B. E. Saltzman, *Anal. Chem.* **25,** 493 (1953)
32. T. J. Murphy in P. D. Lafleur, Accuracy in trace analysis, sampling, sample handling, analysis, NBS special publication 422 (U.S. Government Printing Office, Washington, 1976) 1st. ed. p. 509