MONITORING OF TRACE ELEMENTS IN BREAST MILK SAMPLING AND MEASUREMENT PROCEDURES

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SUMMARY

The aims of this study were to test analytical procedures for the determination of Cd, Cu, Mn, Pb, Se and Zn in breast milk and to establish optimum sampling conditions for monitoring purposes. Two population groups were analysed: (1) Seven women from Prague whose breast milk was sampled on days 1, 2, 3, 4, 10, 20 and 30 after delivery; (2) 200 women from four (two industrial and two rural) regions whose breast milk was sampled at defined intervals.

All samples were mineralised in a microwave oven in the mixture of HNO_3 + H_2O_2 and analysed by atomic absorption spectrometry. Conditions for the measurement of the elements under study (i.e. those for the electrothermal atomisation for Cd, Mn and Pb, flame technique for Cu and Zn, and hydride generation technique for Se) were optimized.

Using optimized parameters the analysis was performed and the following conclusion has been made: the concentrations of zinc and manganese decreased very sharply over the first days, that of copper slightly increased within the first two days and then slightly decreased, that of selenium did not change significantly.

Partial "stabilisation" was achieved after the second decade. No correlation among the elements was found. A significant difference between whole and skim milk was only found for selenium (26% rel. higher in whole milk). The majority concentrations of cadmium and lead were below the detection limit of the method (0.3 $\mu g.l^{-1}$ and 8.2 $\mu g.l^{-1}$, respectively, as calculated for the original sample).

To provide biological monitoring, the maintenance of sampling conditions and especially the time of sampling is crucial.

Key words: breast milk, trace elements, AAS, biological monitoring

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INTRODUCTION

The project "Environmental Health Monitoring System in the Czech Republic" has been in operation since 1994 and its subsystems focus on air pollution, drinking water pollution, effects of noise, dietary exposure, soil pollution, biological monitoring and occupational health risks. Biological monitoring plays a substantial role in this context. One of the aims of the biological monitoring is to propose the reference values for selected metals in human body fluids, to study differences between various population groups, areas, and to evaluate long-time trends. The knowledge of reference values is essential for the interpretation of further biomonitoring data. In addition to the analyses of blood, urine, hair, children's teeth and necropsy specimens as indicators of the population health status and exposure carried on since 1994 (1–5), a pilot monitoring study of trace elements in breast milk was launched in 2002.

The aims of this study were to test analytical procedures for the determination of Cd, Cu, Mn, Pb, Se and Zn in breast milk and to establish optimum sampling conditions for further monitoring purposes.

Based on the literature data (6 – 11), the concentrations of different elements vary with time after delivery (within the period between colostrum and transitional milk). This fact and the resulting problem of data comparability had to be taken into account while considering the biological monitoring design. First of all, it was necessary to map the changes in concentrations of zinc, manganese, copper and selenium (essential elements) and cadmium and lead (toxic elements) in breast milk within the first month after delivery.

MATERIAL AND METHODS

Instrumentation

The following Perkin-Elmer instruments were used: 4100 ZL atomic absorption spectrometer equipped with a transversely heated graphite atomizer (THGA) and a longitudinal Zeeman background correction, autosampler AS 70, and 3300 atomic absorption spectrometer with flame or FIAS 400, autosampler 90.

Other instruments used were a microwave oven MEGA 1200 (Milestone) for the mineralization, equipped with an evaporation rotor FAM 40, a unit for producing doubly deionised Millipore Q+ water and a centrifuge Chirana 815 (Czech Republic).

Reagents

Calibration solutions were prepared by dilution of the stock standard solutions (Merck, 1 $g.l^{-1}$) as required. Chemical modifiers

Table 1. *Conditions of the measurement*

Element	Cd	Cu	Mn	Pb	Se	Zn
Technique	ETAAS	FAAS	ETAAS	ETAAS	HGAAS + FIAS	FAAS
Wavelength	228.8	324.7	279.5	283.3	196.0	213.9
Slit nm	0.7 (low)	0.7	0.2 (low)	0.7 (low)	0.7	0.7
Signal	Peak Area	Peak Height	Peak Area	Peak Area	Peak Area	Peak Height
Conditions	t_{pyr} 500 °C t_{at} 1400 °C	C_2H_2-air (Oxid.)	t_{pyr} 1200 °C t_{at} 2000 °C	t_{pyr} 800 °C t_{at} 1500 °C	HCl 10% $NaBH_4$ 2% (read 17s)	C_2H_2-air (Oxid.)
Modifier	$Pd(NO_3)_2$			$NH_4H_2PO_4$		
Type of calibration	analyt addition	standard calibration	analyt addition	analyt addition	standard calibration	standard calibration
LOD (diluted sample)	0.06 µg.l^{-1}	20 µg.l^{-1}	0.6 µg.l^{-1}	1.7 µg.l^{-1}	0.3 µg.l^{-1}	20 µg.l^{-1}

Table 2. *Validation of the analytical method (n=6)*

	Cd	Cu	Mn	Pb	Se	Zn
Repeatability %	7.9	2.0	5.1	4.8	4.5	2.1
Reproducibility %	12.5	2.8	11.9	8.7	11.8	3.0
Recovery %	96.1	98.6	102.6	93.9	95.7	98.8
Uncertainty %	25	5.5	24	18	24	6.8
LOD (original sample)	0.3 µg.l^{-1}	100 µg.l^{-1}	2.9 µg.l^{-1}	8.2 µg.l^{-1}	1.7 µg.l^{-1}	80 µg.l^{-1}
LOQ (original sample)	1 µg.l^{-1}	300 µg.l^{-1}	7 µg.l^{-1}	23 µg.l^{-1}	6 µg.l^{-1}	200 µg.l^{-1}

were prepared by dissolving 1g of the reagent $NH_4H_2PO_4$ (Suprapur Merck) in 100 ml of demineralised water, and by dilution (10 times) of the Palladium matrix modifier solution (10 g.l^{-1}, Merck). Hydrogen peroxide (30% v/v), concentrated nitric acid (65% v/v), hydrochloric acid (30% v/v) Suprapur (Merck), sodium tetrahydroborate p.a. (Merck) and double-demineralised water (18.2 MΩ.cm^{-1}) were used for the sample preparation and treatment. The Certified Reference Material (CRM) Skim Milk Powder 150 (BCR) and Control material EC 26/01 Lyophilized Human Milk (Ekocentrum Ostrava, Czech Republic) were used as controls.

Sampling Procedure

Samples were collected by mothers themselves into preliminary washed polypropylene tubes following the sampling instructions. Sampling tubes had previously been leached for 24 hrs with 10% HNO_3 and then washed 3 times by demineralised water. The nipple and the surrounding area were cleaned with demineralised water. For the time study, seven samples were collected from each of seven women (i.e. 49 samples) on days 1, 2, 3, 4, 10, 20 and 30 after delivery. For the monitoring study, a total of 200 samples from four regions (two industrial and two non-polluted) were collected.

All samples were stored at –20 ^{0}C until analysed. Once thawed, the samples were equilibrated to the room temperature. Aliquots of 1ml were mineralised in a microwave oven with 5 ml of concentrated nitric acid and 1 ml of hydrogen peroxide following the Milestone Application Notes for Microwave Digestion (12). The obtained solution was evaporated in the closed evaporation rotor to the volume of about 0.1–0.2 ml and then diluted by the double-demineralised water to the volume of 5 ml.

To study the difference between skim and whole milk, aliquots of samples were centrifuged at 1000 rpm for 30 minutes. The separated phase was processed the same way as the whole milk was.

Setting the Parameters of Measurement

To determine the influence of the matrix, the slopes of calibration curves for aqueous calibration solutions were compared with those for samples obtained by the standard addition technique. It was found that for zinc, copper and selenium these differences are not significant (less then 10 %) and therefore the calibration curve method was used. As the slopes for cadmium and manganese differed by more than 10% (35 and 45 % respectively) the use of the standard addition technique appeared necessary. The difference for lead (~10 %) was just at the limit of decision.

The parameters for flame technique and hydride generation technique were taken from the Perkin-Elmer Operation Manual (13, 14) and were tested using control samples and certified reference materials (CRM). No biases were found and therefore for further study standard conditions have been used.

The optimum pyrolysis and atomization temperatures for electrothermal atomization were derived from the pyrolysis and atomization curves.

The parameters of the measurement including optimised furnace conditions are given in Table 1.

Validation of the Analytical Method

The performance of the method was tested using real samples, control sample EC 26/01 and certified reference material BCR 150. The following parameters were checked: reproducibility, repeatability, accuracy, recovery, uncertainty, limit of detection (LOD calculated as 3 σ of blank) and limit of quantification (LOQ calculated as 10 σ of blank). The results obtained for 6 measurements of a real sample are presented in Table 2.

Table 3. Test of accuracy

Material	Analyte	Found µg.g^{-1}	Certified/*Indicative value µg.g^{-1}	Z-score/* Bias %
EC 26	Cd	0.0096 ± 0.008	0.0125 (±0.004)	–0.744
	Pb	0.047 ± 0.003	0.069 (±0.017)	–1.294
	Cu	3.14 ± 0.20	3.00 (±0.41)	+0.341
	Zn	41.7 ± 2.1	43.0 (±3.9)	–0.332
BCR 150	Cd	0.0204 ± 0.018	0.0218 (±0.0014)	*–6.4
	Cu	2.23 ± 0.10	2.05 (±0.08)	*+8.8
	Pb	0.94 ± 0.08	1.00 (±0.04)	*6.0
	Mn	0.26 ± 0.6	*0.256 (±0.010)	*1.6
	Se	0.110 ± 0.010	*0.127 (±0.015)	*–13.4
	Zn	48.5 ± 1.0	*49.2 (±1.2)	*1.4

To check conformity with the statistical regulation, Shewhart´s diagrams were constructed for all of the elements under study. All results obtained for control samples were within the range of warning limits.

Accuracy of the measurement was tested using CRM Skim Milk Powder 150 (BCR) and all results were in the tolerance range. Interlaboratory testing organized by Ekocentrum Ostrava, Czech Republic, found Z-score was lower than 1.3 for all checked elements (see Table 3).

RESULTS AND DISCUSSION

Time Study

All samples were analysed after mineralization as specified in Table 1. Analysis of variance ANOVA and Student t-test (95% confidence level) were used for statistical data processing (15) but some limiting factors had to be taken into account:

- limited number of the mothers,
- high interindividual and intraindividual variability in breast milk content of trace elements within the lactation period.

Normal distribution was found for Cu, Mn, Se and Zn after excluding outliers (four outliers for Se and one outlier for Mn and Zn).

The majority of Cd and Pb concentrations were below LOQ, more than 50% of Pb being even below LOD. Therefore no statistic evaluation was made for these elements and the time dependence for Cd and Pb was not evaluated.

Further, the courses of the concentration levels for Cd, Cu, Mn, Pb Se and Zn were studied. The following conclusions can be drawn from the obtained results:

- Zinc and manganese concentrations decrease very sharply within the first 5 days after the delivery to reach 10% and 20% of the day 1 concentration, respectively (Fig. 1 and 2). Pseudo stability is achieved in decades 2 and 3.
- In the very beginning of lactation, the copper concentration increased slowly and then decreased slowly again, remaining without dramatic changes in decades 2 and 3 (Fig. 3).
- The selenium concentration did not change significantly except two outliers (Fig. 4).
- No changes were found for cadmium and lead.
- To estimate an intake of essential elements from breast-feeding, daily breast milk consumption has to be taken into account. To calculate total excreted amount of elements, it is necessary to know the whole volume of breast milk during the day. For this, the child ought to be weighted before and after every feeding. This is possible in the maternity (rarely) but in reality in our study only one mother fulfilled this column in questionnaire. Further, on the beginning of breast-feeding (and also later) there is large variability among mothers and a daily "production" of milk.
- The differences between whole and skim milk were not

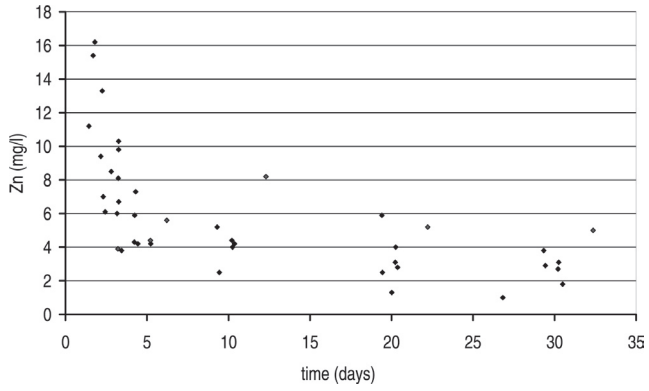

Fig. 1. The time dependence for Zn – concentration of Zn (mg.l^{-1}) in the breast milk in different days after delivery.

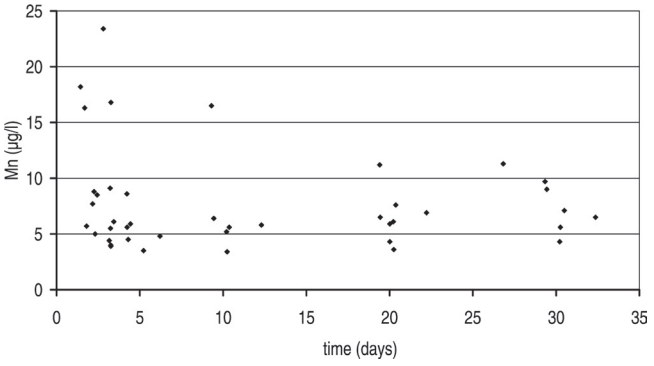

Fig. 2. The time dependence for Mn – concentration of Mn (µg.l^{-1}) in the breast milk in different days after delivery.

significant for Zn, Mn and Cu, significantly higher content in whole milk (26% rel.) was found only for selenium.

- No statistically significant correlation among the elements was found.

Based on these facts, day 20 after the delivery is proposed as the earliest sampling time for monitoring purposes.

Monitoring Study (200 Samples)

The results obtained for the monitoring samples corresponded to the values and trends found for the time study. More than 50% of Cd and Pb concentrations were even below LOD. Medians for all regions together were for Cu, Mn, Se and Zn 461 $\mu g.l^{-1}$, 4.9 $\mu g.l^{-1}$, 11.4 $\mu g.l^{-1}$ and 4,328 $\mu g.l^{-1}$, respectively. The comparison of concentration levels among regions was not possible since sampling at the same time intervals could not be ensured due to the technical reasons (in some regions the samples were collected in the maternity – first days after delivery). In spite of this, the values for Cu and Se did not differ significantly (medians for all regions were for Cu in the range 420 – 500 $\mu g.l^{-1}$ and for Se 10 – 12 $\mu g.l^{-1}$) whereas for Mn and Zn, as expected, the differences were significant (the values of medians were in the ranges 3.3 – 11 $\mu g.l^{-1}$ and 1,995 – 6,590 $\mu g.l^{-1}$ respectively). Lower concentration levels were found in the region where the collection of samples proceeded in the third decade. It has been in good agreement with our conclusion from the time study.

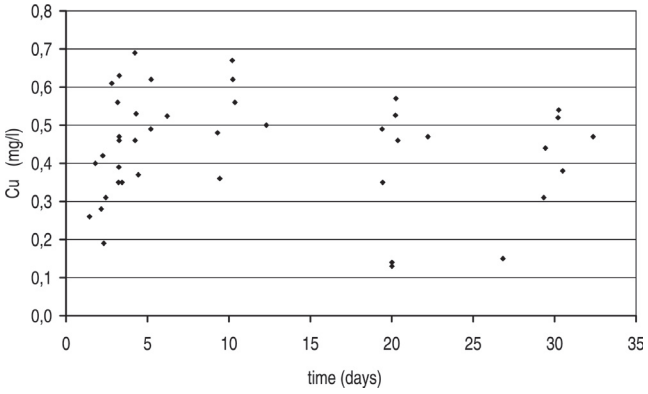

Fig. 3. The time dependence for Cu – concentration of Cu ($mg.l^{-1}$) in the breast milk in different days after delivery.

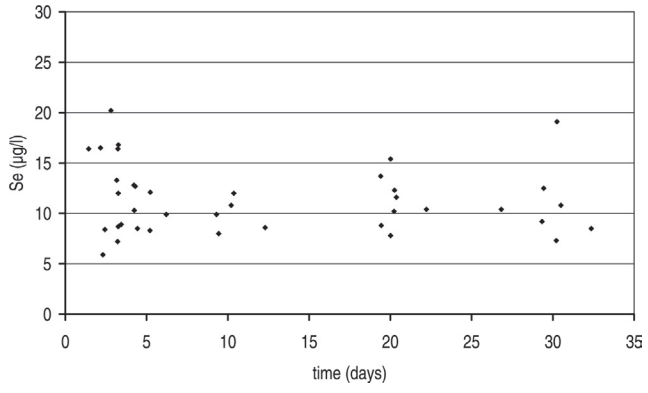

Fig. 4. The time dependence for Se – concentration of Se ($\mu g.l^{-1}$) in the breast milk in different days after delivery.

CONCLUSION

Methods for the determination of Cd, Cu, Mn, Pb, Se and Zn using various AAS techniques aimed at the biological monitoring of trace elements in breast milk were developed and validated. Analysis of the certified reference material BCR 150 (Skim milk) and participation in the interlaboratory testing have shown that the test methods provide accurate results. The sampling interval after delivery appeared crucial for monitoring purposes: sampling should not start earlier than at the end of decade 2. A statistically significant decrease in concentration was observed for zinc and manganese within the first 5 days after delivery; initial concentration increase in the first days followed by a decrease was recorded for copper. Selenium concentration did not show significant variability with the time of sampling, but was lower in skim milk compared to the whole milk. All values for lead and a majority of results for cadmium were below LOQ. The concentration levels found do not differ from the data published in the literature.

To provide biological monitoring, the maintenance of sampling conditions and mainly the time of sampling is crucial.

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Received November 23, 2004
Received in revised form and accepted January 13, 2005