Measurement of cerium in human breast milk and blood

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Abstract

The aim of this study was to evaluate the relationship between cerium content in human breast milk and blood plasma or serum and the influence due to the environmental exposure. Blood samples and breast milk at various stages of lactation, from 5 days to 51 weeks post partum, were donated by 42 healthy breast-feeding mothers from Munich, Germany and by 26 lactating Spanish mothers from Madrid at 4 weeks post partum. Inductively coupled plasma mass spectrometry was applied for the determination of cerium in the biological samples. Cerium concentration in the digested milk samples from Munich showed low values and the arithmetic mean values ranged between the quantification limit of 5 ng/L up to 65 ng/L. The median value amounted to 13 ng/L. The cerium concentrations in the Spanish breast milk samples amounted to similar low values. The data were about a factor of eight lower than values found in a former study of samples from an eastern German province. All cerium concentrations in the German plasma samples, except for two, were at the quantification limit of 10 ng/L. Interestingly, the serum samples of the Spanish mothers

showed cerium values ranging between 21.6 and 70.3 ng/L; these higher data could be explained by an enhanced intake of cerium by humans in Madrid. This could be caused by increased cerium concentrations in particulate matter due to a higher traffic volume in Madrid compared to Munich.

The results obtained in this study contribute to setting reference baseline values of cerium in human breast milk and blood plasma/serum and indicate there a varying cerium amount depending on the cerium environmental pollution. Possibly, the cerium content in plasma/serum could be an indicator for environmental cerium which is not valid for breast milk.

Keywords: Cerium; ICP-MS; Breast milk; Blood plasma; Humans

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Introduction

Cerium (Ce) is a chemical element of the lanthanide series of the rare-earth metals. Among the lanthanides it is the most abundant in the earth crust (about 68 mg/kg) found primarily in monazite and bastnaesite ores in the form of orthophosphate and fluorocarbonate. Cerium is applied for many industrial products (glass additives, lighters, carbon arc lamps, ceramics, etc.). It is an important component of catalytic converters and is used as a diesel fuel additive [1]; it is one of the major component in fertilizers manufactured in China and exported to other countries [2]. Cerium compounds are also used for the treatment of severe skin burns [3]. In addition, there exist two radioactive isotopes of cerium, ^{144}Ce ($t_{½}$=284 days) and ^{141}Ce ($t_{½}$=32.5 days), that are important nuclear fission products contained in the effluents from nuclear power and reprocessing plants [4].

The general population may be exposed to cerium mainly by oral uptake through food ingestion and by inhalation of fine particles. Studies of the daily oral intake of cerium through the ingestion pathway showed that cerium can be taken up in a rate of 5.6-8.6 µg/d [5, 6]. Higher intake values of 83-145 µg/d were also suggested for farmers living near an ore deposit containing about 3% of rare earths [7].

The steady rise of cerium in the environment through its wide industrial and agricultural applications, especially the use of cerium additives in diesel vehicles could cause an increased risk of exposure to cerium in humans. Cerium is considered to be low toxic. However, there are studies that suggested a possible link between elevated levels of cerium in soils, plants and endomyocardial fibrosis as elevated levels of cerium have been reported in the blood of patients with this heart condition [8]. Analysis of lungs and lymph nodes from cases of rare-earth-induced pneumoconiosis developed in workers exposed to fumes from carbon arc lamps for many years, which contained especially cerium, revealed cerium concentrations several orders of magnitude larger than those found in non-exposed persons [9]. Generally, long-term

investigations about health risks and environmental effects of cerium in humans are rare and further studies are needed [10]. Beside the increasing interest in the metabolism and toxicity of cerium in the human body, knowledge of the biokinetics after incorporation of radioactive cerium in case of nuclear accidents is important for assessing the radiation exposure of the population. Due to the scarcely available human data, the currently used biokinetic model for the human cerium metabolism is mainly based on animal experiments and, by *supposed* analogy, on trivalent actinides [11]. Of peculiar interest for radiation protection is the transfer of cerium in human milk of breast-feeding mothers.

Human breast milk is the optimal source of nutrition for the newborn containing appropriate amounts of carbohydrate, protein, lipids, and micronutrients as well as essential and non-essential trace elements required for growth, development and immune protection [12, 13]. However, breast milk can also include some hazards and may contain heavy metals and other pollutants such as polychlorinated biphenyls, DDT and its metabolites in accordance with the environmental contamination and diet of the mothers [14, 15]. In addition, the exposure of a female to radionuclides and the possible transfer to the infant during pregnancy and thereafter during breast-feeding is of special concern in radiation protection for the public. Radiation doses to the newborn and infants through breast feeding resulting from intakes of radioactive cerium by the mother depend on the direct transfer from the mother's blood to the breast tissue and milk. However, there are still inconsistencies in the current biokinetic behaviour of cerium in humans and its transfer rates to breast milk, which are essential for dose calculations in infants and newborns. Therefore, there is a persisting need for further validation of biokinetic parameters of incorporated cerium. Biomonitoring of breast milk represents a preferential way to provide information about the cerium body burden of women as well as the exposure of infants.

Two studies are known in which cerium could be detected in human breast milk [5, 16]. In one of the studies [5] the transfer factor of cerium from food to the milk of the nursing

mothers was determined based on cerium concentration in food and breast milk. In particular, the concentrations of cerium measured in these human milk samples are quite high compared to e.g. cerium concentrations in human blood plasma [17], possibly indicating enrichment from plasma to milk. With respect to radioactive cerium isotopes this could be a matter of concern.

The aim of this study was to assess the relationship between cerium content in human breast milk and blood plasma or serum and the influence due to the environmental exposure, thereby using a simple digestion method directly followed by inductively coupled plasma mass spectrometry (ICP-MS).

Materials and methods

Subjects

In a preliminary study conducted from April to September 2007, ten healthy lactating mothers living in Munich and surroundings (Germany), in an age range of 29 to 40 years, were asked to voluntarily collect some breast milk. This study was repeated and expanded in the time period of August 2008 till January 2009, where further 32 mothers (between 22 to 41 years) agreed to collect breast milk and also blood samples. In addition, 26 Spanish mothers (from Madrid and residential neighbourhood), in the age range of 26 to 44 years were asked to donate paired samples of blood and breast milk; these collections were performed between March and May 2009. All mothers were informed about the aim of the study and written informed consent was obtained from the mothers before the collection started. Each subject obtained code identification (CeMu0-41 for Munich, and CeMa42-67 for Madrid) and had to fill out a personnel questionnaire (Table 1). Several subjects donated samples at various stages of their lactation. Altogether, breast milk samples from 5 days to 51 weeks post partum

were collected in this study. Therefore, the samples comprised both transitory and mature milk. In order to avoid or reduce any contamination risks of the samples, the area around the nipple was carefully cleaned with ultrapure water and a lint-free, soft Kimwipe tissue, and breast milk was extracted into polyethylene bottles by gentle manual pressing on the breast (avoiding any contact to e.g. breast milk pumps). The milk of the Spanish mothers was collected in the container as a mix of milk before and after feeding, and after discarding the first drops, whereas the milk sampling of the German mothers was independent on feeding. The polyethylene containers had been cleaned in advance with 5% nitric acid (suprapur) solution (Merck, Darmstadt, Germany) and rinsed several times with deionised water. Then, all breast milk samples were stored in a freezer at -20^{o}C until analysis. Thirty-one blood samples were collected from the German mothers once from one day before birth to 51 weeks post partum. It was paid attention that at least one blood sample was collected at the same day of a breast milk sampling. After taking the blood samples using heparinised S-Monovettes® (Sarstedt, Germany) they were centrifuged and plasma removed; then the plasma samples were stored frozen until analysis. The blood of the Spanish mothers was collected using trace element-free Vacutainer® tubes (Becton Dickinson, USA); here, serum (and not plasma) was obtained as the residual material had to be utilized in another clinical trial. All biological samples from Madrid (milk and serum) were then shipped frozen to the analytical laboratory at the Helmholtz Zentrum München.

Chemicals and reagents

Chemicals and reagents used were of suprapure grade. Certified cerium and iridium stock standards (1000 mg/L) were purchased from CPI (Santa Rosa, USA). Dilutions of standards and samples were done using deionised water (18.2MΩ cm) prepared by a Milli-Q system (Millipore, Bedford, MA, USA). HNO_3 was bought from Merck, Darmstadt, in *pro analysis* quality and sub-boiling distilled before use. Ar_{liq} was purchased from Air Liquide, Gröbenzell, Germany.

Sample preparation

Human milk samples

For most milk samples two or more specimens were prepared for measurement. After thawing, the samples were gently vortexed for re-homogenization. Typically, two aliquots of 1 mL from each sample were processed. Each sample (aliquot) was mixed with 1 mL concentrated nitric acid (HNO_3, sub-boiling distilled) and subjected to a digestion in precleaned quartz vessels of a Seif apparatus at 170^{o}C, for 10 h. The clear digestion solution was filled up to the mark at 10 mL with Milli-Q-H_2O, 5 mL were taken, then 20 µL ^{193}Ir internal standard were added to a final concentration of 1 µg/L and the solution was directly measured for cerium (2009-campaign); alternatively, 5 mL were taken and diluted 1:2 with Milli-Q-H_2O (2007-campaign), 20 µL ^{193}Ir internal standard were added (final concentration 1 µg/L) and then measured for cerium using an inductively coupled plasma mass spectrometer (ICP-MS; ELAN, DRC II, Perkin Elmer, Sciex Ontario).

Human plasma/serum samples

Human plasma/serum samples were thawed slowly and subsequently gently vortexed for re-homogenization. Aliquots of 500 µL were taken, diluted 1/10 with Milli-Q-H_2O, 20 µL ^{193}Ir

internal standards were added (final concentration 1 µg/L) and then the samples were measured for cerium using ICP-MS.

Instrumental analysis

Instrumental analysis was performed on an ICP-MS ELAN DRC II from Perkin Elmer. Instrumental parameters are given in Table 2.

Analytical quality control

A rigid quality control program was applied to the whole analytical procedure including digestion, blank determination of plastic- and quartz ware, serial and day-to-day precision at two concentrations each, as well as recovery experiments.

1. A set of 20 routinely cleaned sample vessels were filled with 5% HNO_3 solution and allowed to stand for 60 min. Then, they were analyzed for cerium to check whether any contamination might be expected from vessels. All vessels showed blank values between 0.2–0.3 ng/L. No elevated values were monitored, indicating that no contamination from the used vessels should be expected.

2. Blank digestions were carried out regularly with each set of digestions. Digestion blanks showed values as low as instrumental blank values or vessel blanks, proving that no contamination was introduced during the digestion procedure.

3. Serial precision (n = 10) was determined at ca. 1 ng/L by analysis of a digested milk sample (CeMu30, over-all dilution 1/10) or of the same sample but with 10 ng/L standard addition (target concentration ca. 11 ng/L), each ten times in series, resulting in a mean (SD) of 1.01 (0.055) ng/L (RSD/serial precision = 5.4% at ca. 1 ng/L), or in a mean of 11.34 (0.24) ng/L (RSD/serial precision = 2.1% at ca. 11 ng/L).

4. Day-to-day precision was determined by measuring control standards at 2 ng/L or aliquots of a digested milk sample (CeMu32, digestion dilution 1/10; each n = 10). The 2-ng/L control

standards showed a mean of 2.11 (0.16) ng/L (RSD/day-to-day precision = 7.9%) and the digested milk sample had a mean (SD) of 1.45 (0.14) ng/L (RSD/day to day precision = 9.4%).

The detection limit (LOD, 3 σ criterion) was 5.8 ng/L Ce, and the limit of quantification (LOQ, 10 σ criterion) was 10 ng/L Ce for the measurements in 2007, and 2 ng/L (LOD) or 5 ng/L (LOQ) in 2009, each related to native (non-diluted) breast milk samples. The improved LOD and LOQ in 2009 is explained by a methodical change where milk digests could be measured directly without dilution in 2009, but were analyzed 1:2 diluted in 2007.

5. Since no reference material is available for cerium at such low concentration in biological matrices, accuracy was estimated by recovery experiments. For checking the suitability of our method, blanks, digestion blanks, and control standards at 50 ng/L Ce were determined directly before and after samples. Three spiked samples (CeMu1 + 30 ng/L, CeMu2 + 15 ng/L, CeMu5 + 10 ng/L) were determined and recovery rates were calculated. Recovery was 96.9±3.2% (2007-campaign). Further aliquots of milk samples digests were analyzed five (CeMu24) or ten (CeMu30) times before and after the addition of 1 ng/L (CeMu24) or 10 ng/L (CeMu30). The recovery of the 1 ng/l addition was determined at 106%, that of the 10 ng/L addition at 102% (2009-campaign). These quality control results proved that the complete method including digestion and analysis was suitable for the analysis of the human milk and plasma/serum samples.

Results and discussion

In Table 3, the data ranges, the arithmetic mean (SD) values and the median of the cerium concentrations in breast milk and blood samples of the lactating German and Spanish mothers are shown. The results of the participants CeMu18 and CeMu34 were not included as the

sample vessels were not cleaned before. The average value for each German mother was obtained from two or more specimens prepared/digested from the same sample and triplicate measurements of each digest; then, the mean of the mean was formed and expressed as arithmetic mean (SD). The range of the cerium concentration in the human milk samples was between the limit of determination of 5 ng/L and 65 ng/L. In 2007, the median value of the German milk samples amounted to 13 ng/L; and to 12.6 ng/L in the milk of the German study in 2008/9. The cerium concentration in the Spanish human breast milk (from one digestion and triplicate measurements of this digest) showed similar values (median: 10.6 ng/L). In Fig.1, the cerium concentrations of all milk samples collected from the German and Spanish mothers were listed including the multiple data of the mothers who had donated milk on several occasions. It could be seen that all values were in the same range and there was no significant difference between the data of the German and Spanish samples.

Up to now there is only sparse information on concentration of cerium in breast milk in literature. Only two further publications dealing with the measurement of the natural cerium amount in human breast milk [5, 16] are known to the authors. Both working groups applied acid-cleaned breast milk pumps (if needed) for collecting the milk and ICP-MS for measurements. Friel et al. [16] stated that the cerium concentration in human milk was "extremely low". They could scarcely detect any cerium in their samples due to the insufficient sensitivity of their analytical procedure. Cerium concentrations were below LOD (reported LOD = 5 µg/L) which is two to three orders of magnitude higher than the one obtained in the present study. In the work of Wappelhorst et al. [5], the presentation of the results of cerium content in human milk is partly confusing. In their Table 3, the concentration range is given as 60-240 ng/L, with a median value of 100 ng/L; however, in the text one sample is assigned to 1.36 µg/L (approx. 5 times the "maximum" value) whilst in their Fig. 4 other samples are assigned to ca. 0.000075 µg/L (by a factor 1000 below the given "minimum" value). The results presented in our work were below the range indicated by

Wappelhorst et al. in their table [5]; and the median value obtained in their work is about a factor of eight higher than the one found in our study. The reason for their higher cerium values could be the fact that the preparation of the milk samples for measurement involved too many steps, including freeze drying, pooling of digests and open evaporation that could have increased the risk of contamination. No information is given about blank determinations upon this complicated procedure. In our study, the work schedule of the samples comprised only a pressure digestion step and – after adding iridium as an internal standard - the resulting solution was directly given to ICP-MS for measurements of cerium. Nevertheless, problems arose occasionally with the inhomogenity of our milk samples after thawing and preparing for analysis. This might be the cause that some multiple measurements of one single milk sample varied to a large extent.

It is well known that trace elements concentrations of breast milk can considerably vary during the course of lactation [18]; for instance, the concentrations of the essential elements molybdenum or zinc were decreasing with duration of lactation. In addition, the concentrations of the toxic elements, lead, mercury, and also lanthanum, another rare earth element next to cerium, were higher in colostrum than in mature milk. In the study of Wappelhorst et al. [5] a slight decrease of cerium in human milk with duration of lactation was indicated. However, in the present study there was no significant decline of the cerium amount in the human milk of the German mothers with increasing lactating period from 5 days to 51 weeks post partum (Fig. 2). In addition, the cerium concentration of breast milk of mothers who had collected for several times showed no significant decrease or increase over the lactation period.

All cerium concentrations in the German human plasma samples, except for two, were at the quantification limit of 10 ng/L. However, the serum samples of the Spanish mothers showed cerium values ranging between 21.6 to 70.3 ng/L. These higher cerium concentrations in the serum of the Spanish mothers, compared to the German data, could be explained by a higher

daily cerium intake by ingestion or inhalation. As cerium is employed as a promoter in catalytic converters, the widespread use of catalysts in automobiles is an important source of cerium enrichment in the environment. The higher intake of cerium could be by inhalation of cerium in particulate matter due to a much higher traffic volume in Madrid, compared to Munich. Besides cerium, platinum-group elements (PGE), like platinum, palladium, and rhodium, are also components of catalysts and studies have shown significant elevated concentrations of PGEs (and cerium) in road dust, depending on the traffic volume and driving behaviour [19]; another environmental study analysed the platinum content in airborne samples, which was usually high when cerium content was also high, confirming that traffic is the source of these pollutants, especially in populated urban areas [20, 21]. Gomez et al. [22] found lower platinum (rhodium) content in airborne particles in Munich as compared to Madrid. Therefore, the higher cerium content in serum of the Spanish mothers can be explained by these differences in environmental cerium concentrations in the different traffic areas and by a higher incorporation of cerium through inhalation. Interestingly, the cerium values of the Spanish serum samples showed different cerium levels depending on the date of sampling. The subjects, from whom blood samples were collected in March 2009, had higher cerium concentrations in serum than those who had given their blood in mid-May 2009 (see Fig. 3), whereas all cerium values of breast milk were similar and independent on the sampling time. Fig. 4 shows that no correlation between the cerium concentrations of the Spanish serum and milk samples was found. In Fig. 4, also the cerium concentrations of the two German plasma samples (which were above LOQ) and of the corresponding milk samples were included. The varying cerium levels of the Spanish serum samples could be traced to different concentrations of particulate matter due to changes in climate and heating situation as well as traffic volume in March and in May in Madrid, which was also reported by the study of Artinano et al. [23].

In the recent literature [17] blood plasma values of cerium from “not traceable” to 30 ng/L were found for adult males and females living in northern Germany. Considering these low plasma cerium concentrations (mean <8 ng/L) and our own measurements in the plasma samples from Munich (mean <10 ng/L), together with the analysed cerium concentrations in all breast milk samples (mean 15 ng/L), no significant enrichment of cerium from plasma/serum to milk could be assumed during lactation, since both cerium concentrations were in the same range. Even with higher cerium values in the Spanish serum samples (mean 38.8 ng/L) an increased transport of cerium from the serum to the breast milk to reach similar values was not indicated. In the former study from Saxonia [5] where higher cerium values in human milk (mean 120 ng/L) were found, an increased transfer of cerium from plasma to milk cannot be affirmed since no plasma samples were analysed in that study. Cerium transport and cerium content in human milk seem to be regulated and independent on the cerium content in blood. In a similar manner, studies on the transfer of the trace elements copper, selenium and cobalt have shown that transport of these metals from blood to milk (colostrum) was reduced and their concentrations in colostrum were lower than in the maternal serum [24] indicating an regulatory effect of the mammary gland on these trace element transfer.

Thus, concerning a possible radiation exposure from radioactive cerium isotopes to newborns and infants through breast milk feeding, the results of our study could not confirm an increased level of cerium in human breast milk compared to plasma values.

Conclusions

The present study substantially contributed to define reference values of cerium in human breast milk collected from German and Spanish breast-feeding mothers at various stages of lactation from 5 days to 51 weeks post partum. The arithmetic mean values of the cerium

concentrations in human milk varied between the quantification limit of 5 ng/L up to 65 ng/L; and the median value amounted to about 13 ng/L. The data were about a factor of eight lower than the values found in another study.

Cerium concentrations in the German plasma samples were mostly under the detection limit, whereas the Spanish samples showed cerium values between 21.6 to 70.3 ng/L. These higher data could be explained by an enhanced intake of cerium in humans in Madrid, compared to Munich, possibly caused by increased cerium concentrations in particulate matter due to a higher traffic volume.

The results in the present study showed that depending on the environmental condition varying cerium values in plasma/serum could be found and an increased level of cerium in human breast milk compared to plasma/serum values could not be confirmed. Cerium transport and cerium amount in human milk seem to be regulated and independent on the cerium content in plasma. Cerium content in plasma could be an indicator for environmental cerium which is not valid for breast milk.

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Table 1a. Data of participating breast-feeding mothers from Munich (Germany). Some mothers collected breast milk on several days

Subject codes	Age (y)	Height (cm)	Weight (kg)	Lactation period (weeks)	Additional children (n)
Study 2007					
CeMu0	34			18	0
CeMu1	39			0.7	1
CeMu2	31			1	1
CeMu3	-			1	0
CeMu4	33			0.8	0
CeMu5	40			0.7	0
CeMu6	29			4	0
CeMu7	37			20	0
CeMu8	37			51	0
CeMu9	36			6+19	1
Study 2008/9					
CeMu10	34	167	55	16+38	0
CeMu11	31	167	85	16+20	1
CeMu12	28	165	63	44	0
CeMu13	30	170	72	34+42	1
CeMu14	31	170	80	54+16	0
CeMu15	22	167	65	6+8+12	0
CeMu16	41	177	75	3+6+8+14+20	0
CeMu17	33	170	65	48	0
CeMu18	33	165	55	16+24	2
CeMu19	32			24	0
CeMu20	32	179	70	22	0
CeMu21	26	172	61	28	0
CeMu22	36	172	65	4+8+12	1
CeMu23	29	158	75	6	0
CeMu24	38	183	70	6	3
CeMu25	36	155	58	12	0
CeMu26	28	168	63	18	0
CeMu27	34	167	61	12+16	0

Table 1a. continued

CeMu28	32	170	70	34	0
CeMu29	33	176	69	11	0
CeMu30	29	175		2+4+6+9+13	1
CeMu31	34	162	52	12	0
CeMu32	33			14	0
CeMu33	29	180	76	6+16	0
CeMu35	36	158	62	12	0
CeMu34	32	165	72	26	0
CeMu36	35	163	63	16	1
CeMu37	39	155	51	2+4+6+8+12	0
CeMu38	30			6	0
CeMu39	28	164	67	6	0
CeMu40	29	173	77	6	0
CeMu41	32	168	72	6	1

Table 1b. Data of participating breast-feeding mothers from Madrid (Spain)

Subject codes	Age (y)	Height (cm)	Weight (kg)	Lactation period (weeks)	Additional children (n)
Study 2009					
CeMa42	30	165	73	4	0
CeMa43	44	153	47	4	0
CeMa44	32	160	65.5	4	0
CeMa45	32	157	67	4	0
CeMa46	40	180	68	4	1
CeMa47	29	173	59	4	0
CeMa48	36	168	57	4	0
CeMa49	41	162	59	4	1
CeMa50	35	160	47	4	2
CeMa51	32	176	65	4	1
CeMa52	37	175	69	4	0
CeMa53	26	158	64	4	1
CeMa54	33	164	61	4	1
CeMa55	41	153	66	4	0
CeMa56	34	167	68	4	0
CeMa57	34	163	83	4	0
CeMa58	36	158	53	4	0
CeMa59	38	169	67	4	0
CeMa60	35	159	56	4	2
CeMa61	33	170	75	4	0
CeMa62	32	158	62	4	0
CeMa63	32	169	70	4	0
CeMa64	29	161	50	4	0
CeMa65	31	160	58	4	0
CeMa66	32	170	61	4	0
CeMa67	32	160	57	4	0

Table 2. Instrumental parameters of ICP-MS

Instrument: ELAN DRC II, Perkin Elmer (Sciex, Toronto, Canada)
RF power: 1250 W
Plasma gas: 15 L Ar/min (Air Liquide, Gröbenzell)
Nebulizer: Meinhard (Glass Expansion)
Spray chamber: Cyclon (Perkin Elmer)
Nebulizer gas: 0.85 L Ar/min
Isotope: ^{140}Ce
Internal standard: ^{193}Ir, at 1 µg/L
Dwell time: 200 ms
Replicates: 3; 6 sweeps per reading
Sample introduction: Perimax peristaltic pump with "Antipulse-Head", Spetec, Erding.
Sample flow rate: 1.2 mL/min
Calibration: 8 point calibration (0-1000 ng/L): $r^2 = 0.99993$
These parameters were the optimal conditions for this instrument.

Table 3. Concentrations of cerium in breast milk and blood plasma/serum of German and Spanish lactating mothers

Milk	Numbers of samples (n)	Range (ng/L)	Mean (SD) (ng/L)	Median (ng/L)	n < LOQ*
German mothers					
2007	11	11-25.8	15.4 (5.9)	13	4
2008/9	51	5.04-65.8	15.7 (5.8)	12.6	3
Spanish mothers					
2009	26	5.03-45.7	13.9 (9.2)	10.6	3
Plasma/serum	Numbers of samples (n)	Range (ng/L)	Mean (SD) (ng/L)	Median (ng/L)	n < LOQ**
German mothers					
2007	not determined	--	--	--	--
2008/9	31	<10-12.7	<10	<10	29
Spanish mothers					
2009	26	21.6-70.3	38.8 (11.4)	40.4	0

*LOQ of milk 2007: 10 ng/L, 2008/9: 5 ng/L;

**LOQ of blood plasma/serum 2008/9: 10 ng/L

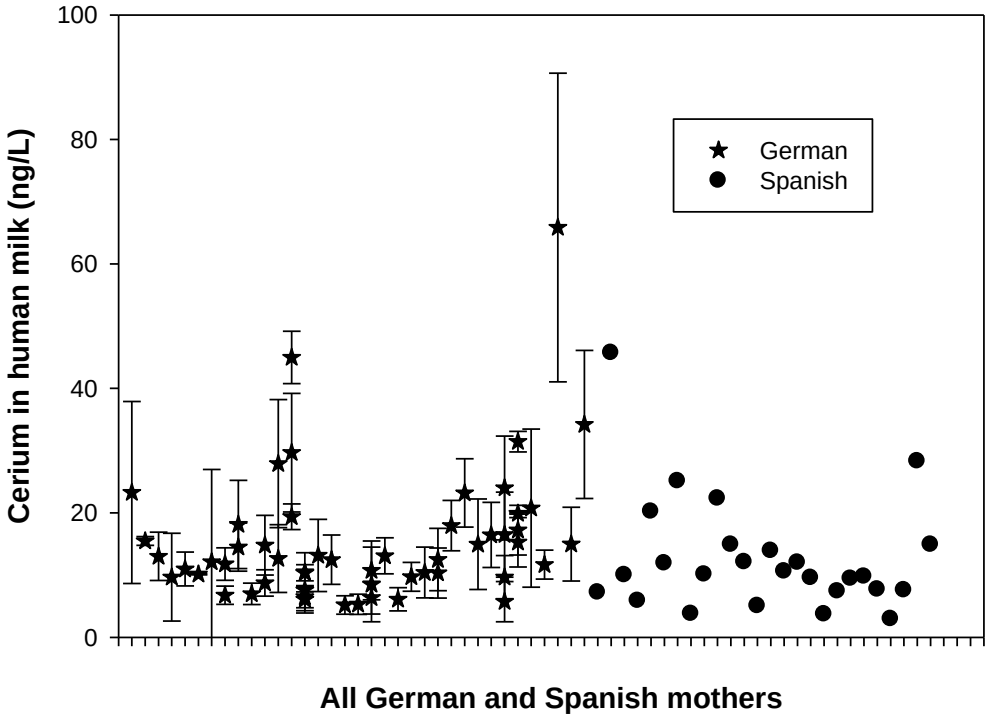

Fig.1. Cerium concentrations of milk samples collected from participating German and Spanish mothers including all data of the mothers who had donated milk on several occasions. The values for each German mother were obtained from two or more specimens prepared from the same sample and are expressed as arithmetic mean. The uncertainty corresponds to the standard error of the mean (SEM). The data from Madrid are from a single preparation measured in triplicate. The measurement uncertainty corresponds to <3.5%.

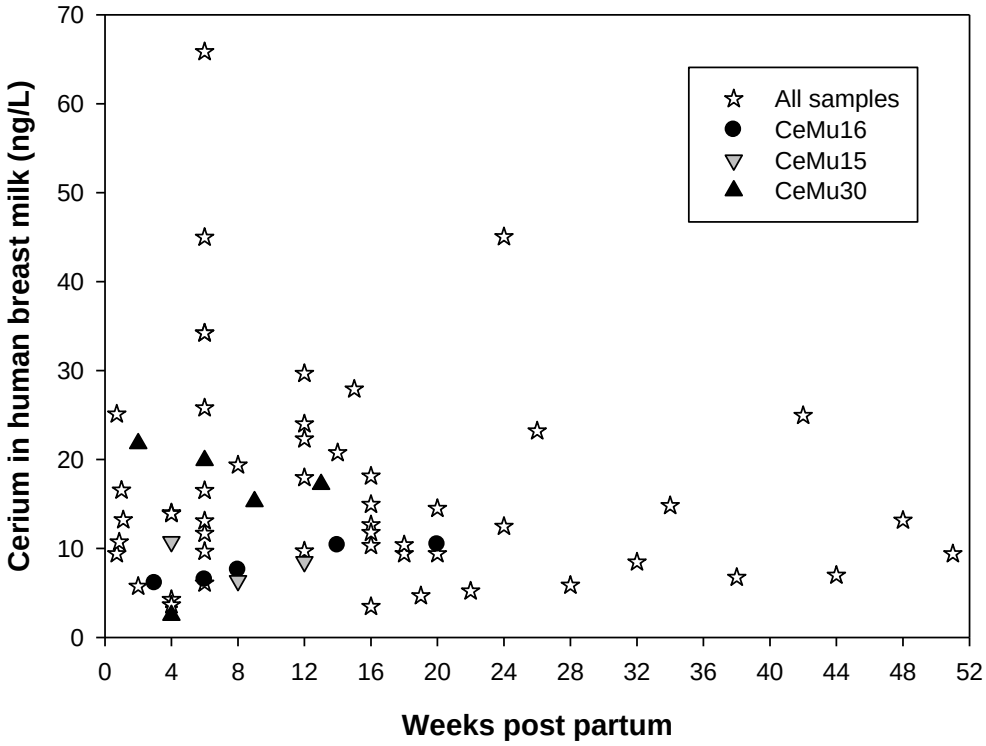

Fig. 2. Mean cerium concentrations in human breast milk of German lactating mothers as a function of time after delivery (weeks post partum). In particular, the time course of cerium concentrations in breast milk of three mothers (CeMu15, CeMu16, CeMu30) who had donated for several times is shown.

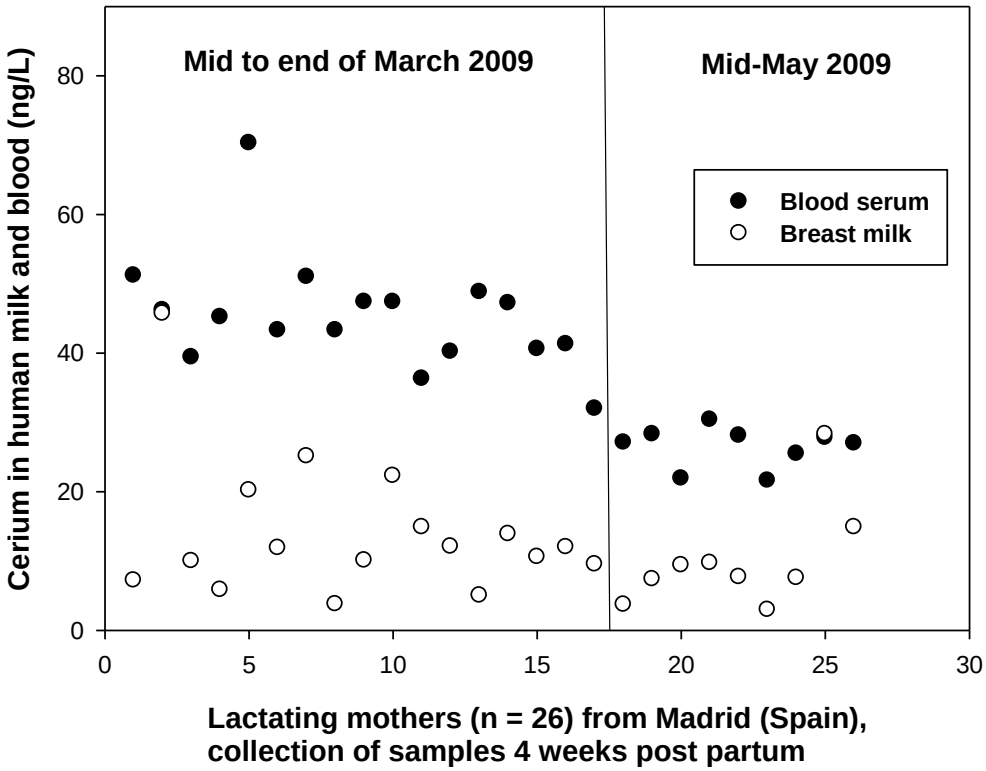

Fig. 3. Cerium concentrations in human breast milk and serum of 26 Spanish lactating mothers depending on the season of sample collection.

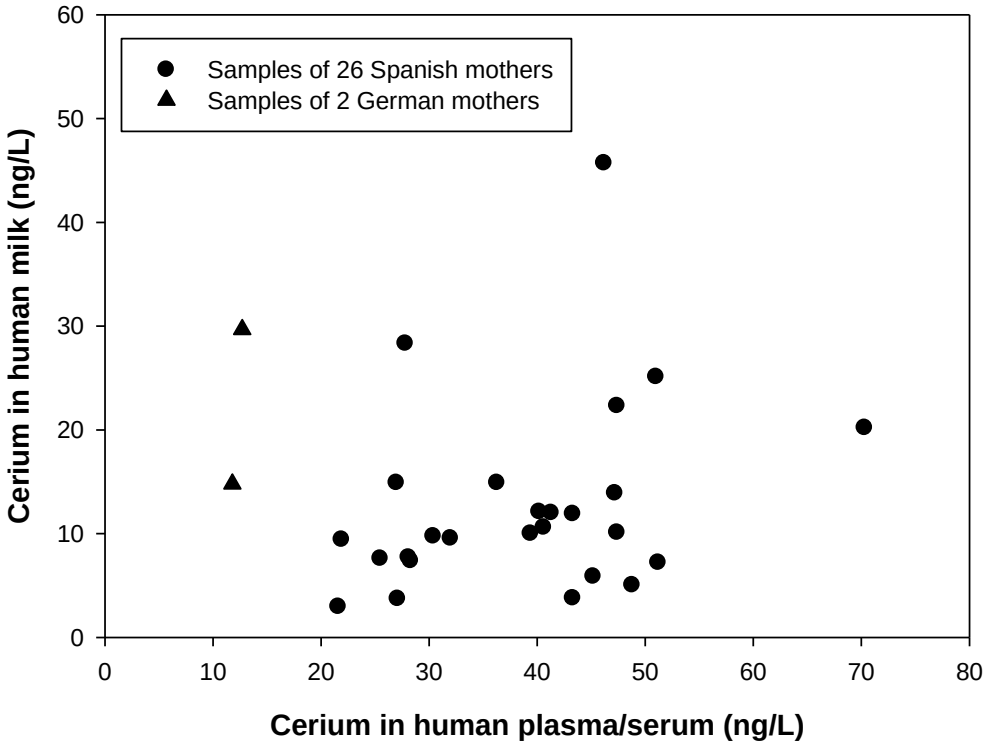

Fig. 4. Relation between the cerium concentrations in human plasma/serum and breast milk of Spanish and German lactating mothers.