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Identification of the origin of infantile saturnism based on lead isotopic and elemental ratios

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Abstract

Background: In France, the prevalence of childhood lead poisoning is becoming rare since

the avoidance of lead in paints in 1949 and the gradual replacement of water pipes. Chronic

lead toxicity is well known and is well correlated to blood lead concentration.

Aim: Here we report a case of severe lead poisoning occurring in a young female child with a

pica behavior. Methods: A blood sample and four environmental samples were analyzed

using inductively coupled plasma- mass spectrometry (ICP-MS). Lead concentration, lead

isotopes and elemental ratios were compared.

Results: The determination of ²⁰⁸Pb/²⁰⁶Pb, ²⁰⁶Pb/²⁰⁷Pb and Cd/Pb has allowed us to identify

the origin of lead poisoning.

Discussion-conclusion: The source of contamination was eliminated and the child benefited

from a psychological and medical follow up. Her outcome was positive. This case illustrated

the potential interest of the use of elemental and isotopic ratios for clinical practice as the

ICP-MS measurement has allowed a quick response and a rapid eviction of the

contamination's source.

Running title: Total lead concentration, lead isotopic and elements ratios for determination of

the origin of saturnism

Keywords: lead poisoning, isotopic, ICP-MS, elements ratio

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Introduction

Lead (Pb) is well known to be at the origin of severe toxicities, some are referred to the term of saturnism. Lead toxicity is mainly developing after oral or pulmonary exposure [1]. Apart from occupational exposure, the main source of lead is water (due to the old water pipes) and the paints containing lead even if it was avoided since 1949 in France. Moreover the presence of lead in gasoline has been banned since January 2000 in France [2]. The lead blood concentration in the French population has considerably decreased since the 50ies and is nowadays ranging from 25 to 75 μ g/L in adults (95th percentile of 73 μ g/L) [3]. The median concentration and the 95th percentile were in 2013 of 13 μ g/L and 32.8 μ g/L respectively, in a cohort of 484 children aged from 6 months to 6 years [4].

The clinical symptoms for chronic lead intoxication are well correlated with blood lead concentration. For blood lead concentration less than $100 \,\mu\text{g/L}$, there is a risk of developing cognitive effects, decrease of IQ, hearing and growth, behavioral and attention problems for children. At higher level, biological signs (decrease of hemoglobin synthesis...), neurological signs, nephropathy can occur [5]-[6]. For children, encephalopathy can appear for concentration higher than $1000 \,\mu\text{g/L}$ [1].

Lead is a heavy metal having four isotopes: 204 (1.4%), 206 (24.1%), 207 (22.1%) and 208 (52.4%). The gold standard used to determine the environmental source of contamination of lead is multi collector inductively coupled plasma mass spectrometry (MC-ICP-MS). But, this technology is quite expensive therefore only few centers are equipped with it, the delay to obtain the results can be long (few months) and more steps are necessary to prepare the samples for the measurements involving clean room facility.

Here we present the case of a female child presenting chronic lead intoxication. To determine the source of this intoxication, an environmental investigation was conducted. Blood and environmental samples were analyzed to determine lead (total concentration and isotopic ratios) and elements ratios were studied to complete the analysis and identify the main source of Pb contamination of the child.

Case history

In February 2018, an 8-year-old child was referred to pediatric emergency department by her general practitioner following the discovery of anemia. She complained of abdominal pain

and constipation which had been progressing for several months. She also had food vomiting and asthenia for several weeks. The clinical examination found diffuse abdominal pain, constipation and chronic pain in the lower limbs. There was no clinical impact of the anemia and the neurological examination was normal. The diagnosis of infant lead poisoning was established based on the blood lead concentration at 946 µg/L. Biological analyses confirmed iron-deficiency microcytic anemia (hemoglobinemia at 8.0 g/dL), blood ionogram and renal function were normal. Radiological images of the abdomen showed radiopaque ponctiform images compatible with chronic lead impregnation. An X-ray of a lower limb showed banded metaphyseal opacity of the long bones visible at the tibial, fibular and femoral metaphyses. The electroencephalogram was normal. The child was hospitalized in pediatric emergency department for chelation by succimer (DMSA) 300 mg three times a day and calcium EDTA 1000 mg/m²/day by a continuous infusion for 5 days. The blood lead concentration was verified seven days after the end of chelation and was at 667µg/L. A new chelation was performed at home by succimer alone (300 mg three times a day during 5 days then twice a day) for 14 days and iron supplementation was prescribed. Medical monitoring based on clinical course and controls of blood lead concentrations were implemented. A regular child psychological follow-up was initiated for the management of pica which evolved since the age of 2 years. The evolution of blood lead levels was illustrated in Fig.1. An initial decrease of the lead blood concentration of the child was observed due to chelation therapy and discontinuation of pica behavior with control of blood lead level at 388 µg/L in July 2018. A first environmental survey did not reveal lead in the child's home or in her current school. Lead was found in her former nursery school, attended 2 years ago, which could explain a previous exposure of the child. However, an increase of the blood lead level from November 2018 has motivated to inquire further environmental investigation in order to find a current source of intoxication.

Material and Methods

The home-school journey was reproduced with the child and samples were taken from the scraping areas for analysis. The determination of blood lead concentration was performed in all members of the family. At home, some degradations of paint above the bed were observed but low lead concentration was measured.

Blood sample of the child (sampled in 2018 November) and environmental samples from different sources (paint of walls in school, paint of two gates) were sent to the toxicology laboratory of Angers hospital for analysis. The sample 1 was paint from the front door of the school, the sample 2 is white paint sampled from a wall of the refectory, the sample 3 is creamy white paint from the door of a hall and the sample 4 from the wall (white paint near the windows) of a class room.

All samples were analyzed in duplicate.

For the blood sample of the child, the samples were half pre-diluted using Triton 0.2% (Sigma, Saint- Quentin Fallavier, France). The total lead calibration (Pb, first ionization energy:7.415 eV) was performed using a matrix mimicking blood composed of Milli Q water ((Millipore Reference A+, Merck, Molsheim, France), glycerol 1% (VWR, Leuven, Belgium) and cysteine 0.1% (Sigma, Saint Quentin Fallavier, France) and ranged from 12.5 to 400 µg/L. The method was validated and accredited by Cofrac organism (ISO 15189 norm). Internal quality controls used were Clincheck blood trace elements (Recipe, Munich, Germany). After 1/20 dilution using nitric acid 0.5% solution containing the internal standard (Re, first ionization energy:7.88 eV) at concentration of 10 µg/L, the analysis was performed using ICP-MS (Elan DRC-e, Perkin Elmer, Villebon sur Yvette, France). The main instrumental parameters were as follows: plasma power of 1300 W, plasmagen flow (Ar): 15L/min, nickel sampler and skimmer, quartz cyclonic nebulization chamber, micromist meinhard nebulizer (flow rate: 0.96 ml/min), analysis in three replicates.

For environmental samples, 100 mg were weighted and they were first mineralized using 1 ml of nitric acid (Suprapur, Merck, Darmstadt, Germany), at 70°C during 2 hours. To allow a comparison, blood sample of the child was also mineralized and treated in the same way. The lead calibration (10-400 μ g/L) and cadmium calibration (2.5-400 μ g/L) were performed in 1% nitric acid. Each sample was 1/20 diluted using nitric acid 0.5% solution containing the internal standard (Re) at a concentration of 10 μ g/L.

For the isotopic lead ratios determination, the matrix used for this analysis was 1% nitric acid. The method of bracketing was used by inserting NIST 981control between each sample for mass bias correction [7]. The method used is a linear correction. The certified reference values (%) of lead isotopes in NIST981 control were: 1,4255 ±0.0012 for ²⁰⁴Pb, 24.1442 ±0.0057 for ²⁰⁶Pb, 22.0833± 0.0027 for ²⁰⁷Pb and 52.3470±0.0086 for ²⁰⁸Pb.The instrumental

parameters of ICP-MS were modified as following: sweeps number: 10, replicates: 12, running time: 10 min to evaluate isotopic lead ratios.

Results

The controls of the blood lead concentration in the siblings and the mother were into the range of general population. The lead concentration measured in the blood sample of the child (sampling in November 2018 or approximately 10 months after her first admission to hospital) was of $506.7\mu g/L$.

The first analysis of lead in environmental samples realized by an environmental technician was insufficient to clearly rule out some sources. The total lead concentrations measured in environmental samples ranged from 50.9 (± 9.38) mg/g in sample 3 to 189.7 (± 20.38) mg/g in sample 1 (table 1). The lead concentration (0.47 ± 0.04 µg/g) has also been again determined in the blood sample after mineralization (versus 506.7 µg/L without mineralization). All those samples contain an amount of lead which is largely high enough to contaminate the child with a low absorption rate.

For the blood sampled in the child, the isotopic lead ratios were of 2.112 (± 0.003) for $^{208}\text{Pb}/^{206}\text{Pb}$ and 1.158 (± 0.004) for $^{206}\text{Pb}/^{207}\text{Pb}$. The comparison of $^{206}\text{Pb}/^{207}\text{Pb}$ ratios determined in environmental samples has led to the eviction of the sample 3 (1.180 ± 0.003); moreover the $^{208}\text{Pb}/^{206}\text{Pb}$ ratio for this sample was of 2.074 (± 0.014). The comparison of Cd/Pb ratios with this of blood sample (0.00125 ± 0.00003) was selective and allowed to eliminate the sample 2 (0.02234 ± 0.00218).

Discussion

The cases of severe saturnism in occidental countries occur rarely. The child presented a high blood lead concentration (946 μ g/L) at her first admission to hospital, but it was still moderately well tolerated (time 0, Fig. 1). No serious neurological (i.e. encephalopathy) was developed. After chelation therapy, the blood lead concentration decreased to 506.7 μ g/L in November. The percentage of lead ingested and absorbed by children was estimated at 50% after a meal and up to 60-80% on an empty stomach [8]. Lead is mainly stored in bones; indeed, lead in teeth and bones of a child represent about 73% of the total lead burden [8]. Dignam *et al.* assessed that more than one year (382 days) is needed for children to decrease blood lead concentration higher than 100 μ g/L to less than 100 μ g/L [9].

The signs observed in children with lead poisoning (lead blood concentration less than 450 μ g/L) can be unspecific even absent. A lead intoxication should be investigated when a child complains of persistent gastrointestinal troubles (constipation, abdominal pain, vomiting...) and/or unexplained behavioral modifications, neurological signs, anemia... in a context excluding other causes of poisoning (opioid ingestion, carbon monoxide poisoning...), iron deficiency, Wilson's disease, encephalitis, other causes of encephalopathy [10]. The earlier the lead poisoning is diagnosed, the better the outcome will be, all the more because a chelation therapy is available.

In addition to the medical care, the second important point was the eviction of the source of contamination, which required an environmental study. To determine isotopic ratios, the most important parameters which should be optimized were dwell time, number of sweeps per replicate and number of replicates for each analysis [11]. The lead isotopic solution NIST 981 has been intercalated between each sample to correct isotopic ratio by linear extrapolation. Lead has a highly variable isotopic composition and ²⁰⁴Pb, ²⁰⁶Pb, ²⁰⁷Pb and ²⁰⁸Pb are treated as stable isotopes [12]. The 206, 207 and 208 isotopes result of radioactive decay of ²³⁸U, ²³⁵U and ²³²Th respectively, whereas ²⁰⁴Pb is a stable isotope whose abundance is constant in space and time [12]. The isotopic composition of lead is not affected by industrial activity such as smelting, refining...[13]. The isotopic ratio of Pb can be used to determine sources of pollution with Pb [13]-[14].

The isotopic lead ratios $^{206}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{206}\text{Pb}$ are used to study environmental sources of pollution for numerous of natural and biological media [15]. The $^{206}\text{Pb}/^{207}\text{Pb}$ ratio was the most studied as the isotopic ratio varies in a relation with the radioactive decay of U and Th [12]. In Europe, the $^{208}\text{Pb}/^{206}\text{Pb}$ ratio was between 2.08 and 2.02 [12]. The $^{206}\text{Pb}/^{207}\text{Pb}$ ratio in European coals ranges between 1.16 and 1.21 [13];[17]-[19]. In France, Cloquet *et al.* (2015) studied the evolution of metals concentrations in lichens between 2001 and 2009. The median isotopic lead ratios for lichens collected in urban area were: 1.15 (\pm 0.0146) for $^{206}\text{Pb}/^{207}\text{Pb}$, 2.11 (\pm 0.0153) for $^{208}\text{Pb}/^{206}\text{Pb}$ [16].

The ratio ²⁰⁶Pb/²⁰⁷Pb measured by Monna *et al.* in fly ash sampled from an incinerator in south of France, varied from 1.14 to 1.16 [14]. Carignan *et al.* analysed fly ash and flue gas from solid waste combustors in France and obtained ²⁰⁶Pb/²⁰⁷Pb values ranging from 1.15 to 1.16 [20]. A recent investigation of Pb isotopic composition of urban waste incineration flue gases from north-eastern France showed ²⁰⁶Pb/²⁰⁷Pb values close to 1.155 [21].

In our case, the ²⁰⁶Pb/²⁰⁷Pb ratios measured in blood sample were of 1.15 (±0.004) and ranged from 1.15 to 1.18 (sample 3) in environmental samples, and were about 2.10 for ²⁰⁸Pb/²⁰⁶Pb except for sample 3 (2.07±0.014). The ²⁰⁸Pb/²⁰⁶Pb ratios measured in our environmental samples were similar to those published which were between 1.98 and 2.12 [22]-[23]. Thus, the environmental sample 3 can be excluded to be at the origin of the contamination. As the isotopic lead ratios have allowed us to exclude one environmental sample among the four, one and/or other exclusion criteria should be tested.

Oulhote *et al.* studied the potential sources of lead contamination in children. The main identified sources were dust, soil and water [24]. As paint was the most evident origin of contamination, other elemental ratios were also studied to be more selective.

The main components of paint are binders (resin, oils or inorganic compounds used to get the dry paint film when the solvent evaporates), pigments such as cadmium derivatives (CdS (yellow), Cd₂SSe (orange), CdSe (red)...), titanium (TiO₂ (grey), chromium (Cr₂O₃ (green), cobalt (Co₃(PO₄)₂ (violet), Co₂SnO₄ (blue...), extenders/fillers (gypsum, ground silica...), thinners (turpentile oil...), driers (red lead, lead acetate, manganese dioxide...), additives (plasticizer, biocide, corrosion inhibitor (sodium molybdate, zinc molybdate...), glow in the dark properties (SrAl₂O₄)...). Based on these data and the toxicokinetics of cadmium, the elemental ratio Cd/Pb seemed to bring a potential added-value. The gastrointestinal absorption of cadmium has been evaluated to represent 5% of the ingested quantity and is influenced by age and nutritional status. A deficiency of bivalent ions concentrations such as iron, zinc can induce an increase of absorption of cadmium in the gastrointestinal tract. In blood, cadmium is mainly stored in red blood cells [1]. Cadmium is largely distributed in the organism. Its half-life in blood is estimated to be between 80 and 100 days. According to modelling data, cadmium could have two phases of elimination in blood: a first step with a half-life of 60 days and a second with a half-life of 120 days [1]. Cadmium is binding with albumin, red blood cells, metallothonein before its storage in tissues and organs. It accumulated mainly in kidneys (30%), liver and in a smaller way in bones, muscles and skin. Based on its long half-lives (4-19 years in liver and 6-38 years in kidneys), the body load of cadmium increases with age. Moreover, as it seems to be few released from tissues and organs, blood cadmium concentration decreases slowly after the end of exposure. Thus the comparison of Cd/Pb ratios seemed to be relevant.

The determination of Cd/Pb ratio has allowed us to exclude samples 2 and 4 (ratios at 0.02234 (± 0.00218) and 0.00209 (± 0.00063) respectively versus 0.00125 (± 0.00003) for blood sample

and $0.00100 (\pm 0.00019)$ for environmental sample 1). The measurement of the isotopic lead ratios (208Pb/206Pb and 206Pb/207Pb) and elemental ratios (Cd/Pb) has allowed identifying one sample among the four (table 2). The Cd/Pb determined in blood sample was of 0.00125 (± 0.00003) . The profile of the environmental sample which was the most similar to the blood sample was the number 1; indeed the ratios were 2.111 (± 0.0175) for 208 Pb/ 206 Pb and $1.154(\pm 0.0064)$ for 206 Pb/ 207 Pb and 0.00100 (± 0.00019) for Cd/Pb. This sample corresponded to paint of a gate of the school.

The element ratios coupling with Pb isotope ratios has allowed us to identify the environmental sample possibly at the origin of the intoxication which was due to a previous and old layer of paint recovering a gate.

Conclusion

The occurrence of saturnism has become rare in France but it should be early diagnosed in order to avoid clinical impairment. The combination of the determination of lead isotopes (²⁰⁸Pb/²⁰⁶Pb and ²⁰⁶Pb/²⁰⁷Pb) with the determination of Cd/Pb has allowed us to identify within a short time the source of contamination. A rapid response was required as the blood lead concentration of the child increased again. The family has moved to a new home. Restoration work of the gates was begun. The medical care of the child based on a psychological and medical monitoring and the eviction of the source of contamination were associated with a better outcome of the child. Blood samples of the child are required to monitor the decrease of lead concentration. Besides, a control of blood lead concentration was performed one month later (december) and it was at 481 µg/L.

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Conflict of interest

The authors declare they have no conflict of interest.

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FIGURES AND TABLES LEGENDS

- Fig.1. Evolution of blood lead concentration of the child
- Table 1. Mean total lead concentration in blood sample and environmental samples
- Table 2. Lead isotopic ratios in blood sample and environmental samples

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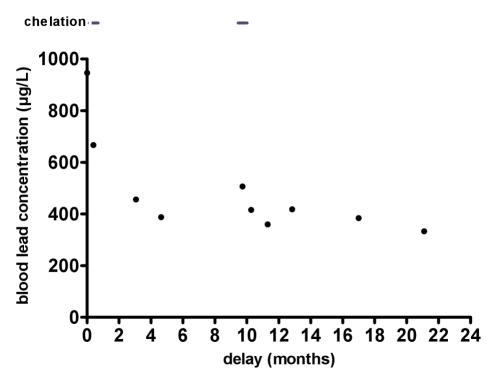


Table 1.

Sample	Mean total lead concentration (± standard deviation)		
Child blood sample	0.47 (±0.04) μg/g		
1	189.7 (±20.38) mg/g		
2	19.7 (±1.36) mg/g		
3	50.9 (±9.38) mg/g		
4	60.0 (±9.05) mg/g		

Table2. Results of isotopic lead ratios and Cd/Pb ratios

	mean ratios (corrected values for lead were used)						
Elements ratios/ sample (2 s)	²⁰⁶ Pb/ ²⁰⁴ Pb	²⁰⁷ Pb/ ²⁰⁴ Pb	²⁰⁸ Pb/ ²⁰⁴ Pb	²⁰⁸ Pb/ ²⁰⁶ Pb	²⁰⁶ Pb/ ²⁰⁷ Pb	Cd/Pb	
Blood sample	17,8686	15,4370	37,7465	2,1124	1,1575	0,00125	
(patient)	(0.2308)	(0.2475)	(0.5444)	(0.0031)	(0.0036)	(0.00003)	
	17,8826	15,4492	37,8118	2,1114	1,1540	0,00100	
1	(0.0285)	(0.1103)	(0.2540)	(0.0175)	(0.0064)	(0.00019)	
	17,9882	15,5879	37,9798	2,1068	1,1513	0,02234	
2	(0.3477)	(0.5257)	(0.9801)	(0.0138)	(0.0165)	(0.00218)	
	17,9033	15,5083	37,6611	2,0739	1,1800	0,00023	
3	(0.2668)	(0.1829)	(0.5264)	(0.014)	(0.0033)	(0.00002)	
	17,9314	15,5754	37,7787	2,1050	1,1592	0,00209	
4	(0.374)	(0.0659)	(0.6940)	(0.0051)	(0.0192)	(0.00063)	