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Blood lead levels and risk factors for lead exposure among pregnant women in western French Guiana: the role of manioc consumption

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ABSTRACT

Concerns about lead (Pb) poisoning in French Guiana first arose in 2011, after the discovery of excessively high levels of the metal among children in a small neighborhood without any clear source of Pb. Since 2012, blood lead levels (BLL) measurement has been proposed to all pregnant women in western French Guiana. The aim of this study was to determine BLL in pregnant women in this region and identify factors associated with elevated BLL. An observational study of a consecutive sample of women who delivered in the maternity ward of the hospital was conducted. Risk factors were investigated using a questionnaire administered post-delivery by midwives (N = 531). Approximately 25 and 5% of women displayed BLL of \geq 50 µg/L and \geq 100 µg/L, respectively. The geometric mean was 32.6 µg/L. Factors that were significantly associated with an elevated BLL after modeling (multivariate linear regression) included: place of residence along the Maroni river, low level of education, daily consumption of manioc derivatives, weekly and daily consumption or personal preparation of manioc flour during pregnancy and weekly consumption of wild game. This study provides insight into the regional and social disparities in BLL in French Guiana and on potential sources of exposure. Evidence indicates that foods that are primarily produced and consumed in the Guiana Shield significantly affect BLL. Taken together with existing data, our results demonstrate that specific actions in terms of prevention, screening and care are required to be adapted and put into place in order to reduce exposure.

Keywords: Lead poisoning; Epidemiological Study; French Guiana; Manioc

Introduction

Lead (Pb) is a non-essential metal that is highly toxic to several physiological systems in humans, including the nervous, renal, cardiovascular, reproductive, immune, and hematologic systems (Counter et al., 2015; García-Lestón et al., 2012; Pollack et al., 2015). The most vulnerable populations are children and pregnant women (Buchanan et al., 2011; Lamadrid-Figueroa et al., 2006), and there are no apparent established thresholds for levels at which a number of critical health effects occur (NTP, 2012). Low intelligence quotients (IQ) were reported in children with blood lead levels (BLL) of less than 100 μg/L (Bellinger, 2008; Lanphear et al., 2005); however it should be noted that these findings are contradictory. BLL vary over the course of pregnancy, following a U-shaped curve with the lowest point between 12 and 20 weeks after the last menstrual period. In the second trimester of pregnancy, low BLL can been explained by hemodilution, whereas in the third trimester, higher BLL may occur due to the mobilization of calcium and Pb from maternal bone to mineralize the fetal skeleton (Miranda et al., 2010; Rothenberg et al., 1994). Lead readily crosses the placental barrier (Lamadrid-Figueroa et al., 2006; Osman et al., 2000; Pollack et al., 2015), and its primary effects on pregnancy progression include: the risk of gestational hypertension and/or pre-eclampsia (Kennedy et al., 2012), an increased risk of spontaneous abortion (Borja-Aburto et al., 1999), danger of premature labor, prematurity, and low birth weight and height (Taylor et al., 2015; Zhu et al., 2010). Finally, Pb was found to exert a detrimental effect on fetal development, and especially neurocognitive function, in utero (Jedrychowski et al., 2008; Hu et al., 2006; Schnaas et al., 2006), making pregnant women a high-risk group.

Lead is responsible for a significant disease burden in developed countries (Bellinger, 2012; Valent et al., 2004) but the largest disease burden is seen in developing countries, including Latin America, where this metal continues to pose a significant public health risk and entails high costs (Attina and Trasande, 2013). Legislative measures have been gradually introduced to reduce exposure by removing Pb from paint, food cans, water pipes

and petrol (Tagne-Fotso et al., 2016), and population-wide BLL have fallen quickly in the USA (Tsoi et al., 2016) and Europe (WHO, 2009) in response to these measures.

French Guiana is a French overseas department situated in northeastern South America, between Brazil to the east and south and Suriname to the west. It is a large country of approximately 84,000 km², and with a population of 246,500 in 2013. St-Laurent-du-Maroni is the second largest city, separated from Suriname by the natural border created by the Maroni River. The population of the city is growing rapidly (+4.3% per year), and characterized by its youth (52.5% of inhabitants were younger than 20 in 2013) and melting-pot nature (due to multiple waves of immigration). Its economy is characterized by weak development, a high level of unemployment (47.8% in 2013) and persistence of traditional ways of life such as hunting, fishing, and slash-and-burn farming. The Western French Guiana Hospital Center (Centre Hospitalier de l'Ouest Guyanais, CHOG) is the sole secondary healthcare provider for a large area and treats patients from a region that spans both sides of the border (Figure 1), which was estimated to be home to approximately 100,000 individuals in 2013.

The problem with Pb poisoning in west French Guiana arose in 2011, after discovery of excessive metal poisoning in a 3-year-old girl (BLL = 1724 µg/L) (Rorive et al., 2015). Up until that point, no apparent studies had been performed in French Guiana, as Pb poisoning did not seem to be a public health concern. The results from an investigation conducted by CIRE Antilles-Guyane after this case came to light showed that 93% (13/14) of children under 7 year of age living in the vicinity exhibited a BLL > 100 µg/L. Environmental investigations suggested multifactorial exposure that includes a nutritional element, primarily from derivatives of bitter manioc (Barbosa Jr. et al., 2009; Carneiro et al., 2013; Freire et al., 2014). Given the lack of knowledge regarding Pb exposure in French Guiana, systematic assessment of BLL in pregnant women was implemented in 2012 in western French Guiana. BLL assessment is performed by health professionals ideally during the prenatal visit in the 4th month of pregnancy and is repeated during the 3rd trimester if the first BLL value is > 50 µg/l. BLL is determined at delivery if not assessed previously. Thus, the objectives of this

study were to (1) determine Pb levels in pregnant women in western French Guiana (population-wide BLL) and (2) identify factors associated with elevated BLL.

Methods and Materials

Study population

A descriptive, cross-sectional, single-center anonymous study was conducted over a period of 12 consecutive weeks from September to November 2013. The study population included women who delivered in the CHOG maternity ward, whose BLL was measured at least once during the course of their pregnancy, and who agreed to participate or had parental consent (for mothers under the age of 18). Patient information, patient consent and data were collected by midwives on the maternity service who were trained for the study.

Data collection

The following information was collected using a questionnaire translated into local languages: sociodemographic characteristics (age, place of birth, native language, primary place of residence), socioeconomic characteristics (level of education, professional status, legal status, perceived financial situation), nutrition throughout pregnancy (frequency with which certain foods identified as potential risks were consumed, such as bitter manioc derivatives: couac (manioc flour), cassava (manioc pancakes), crabio (detoxified cooking liquid used as a sauce), tapioca (bitter manioc starch), wassaï (fruit of the wassaï palm tree), pemba (local clay), wild game (mammals such as Capybaras, Cuniculus paca, and javelinas, and local birds such as pigeons, curassows, etc.)), lifestyle factors' (tobacco use, and alcohol consumption), the source of drinking water and preparing couac during pregnancy. The maternal BLL were recorded from medical records after all other information was collected, to avoid biasing the collection of patient information. We also recorded the gestational age (as calculated from the date of last menses or corrected by the first trimester ultrasound) at which the BLL was assessed.

Whole blood collection and determination of Pb levels

Blood samples were collected by a health professional using standard procedures. Blood was collected and stored in purple-top vacutainers (Becton-Dickinson) with EDTA. Vacutainers were frozen and stored at -20°C in the CHOG until shipping. Pb analyses were carried out in a central laboratory (Cerba, Paris) and measured by atomic absorption spectrometry. The limit of detection (LOD) and the limit of quantification (LOQ) were 10 μ g/L. All specimens with a Pb concentration greater than 200 μ g/L were reanalyzed for confirmation. In cases where the BLL was below the LOQ, values were rounded up to LOQ/ $\sqrt{2}$ (Hornung, 1990), given that only a small percentage of the data was censored (<5%) and BLL were distributed normally when this imputation was applied.

Statistical analyses

The rate of participation was calculated based on total number of deliveries that took place during the study period. When the BLL was determined at multiple points during the pregnancy, the highest recorded level was employed. BLL ≥ 50 µg/L and ≥ 100 µg/L were evaluated with a 95% confidence interval (CI 95%). The Pb variable was log-transformed in order to obtain a variable with a normal distribution (skewness-kurtosis test > 0.05). The log of BLL were compared according to the women's characteristics using one-way analysis of variance (ANOVA) tests. Multivariate analyses (generalized linear model) were then performed. The explanatory variable was the log of the maternal BLL during pregnancy. First, all explanatory variables were tested using binary models (model 1). Secondly, socioeconomic and sociodemographic variables that were significant using a threshold value of 20% were introduced into a multivariate model and progressively eliminated to obtain an "adjusted model" that contained only significant variables (using a threshold value of 5%). Age was used as the primary adjustment variable. Thirdly, nutritional and occupational factors were tested one by one using the adjusted model. Finally, nutrition and occupational factors (that were significant using a threshold value of 20%) were introduced into a

multivariate model and progressively eliminated according to a descending procedure to obtain a "final model" containing only the significant variables (using a threshold value of 5%). We calculated the adjusted R² and tested the validity of the final model (normality of residuals). The analyses were performed using STATA® v13.1 software.

The study was declared to the CNIL and was approved by the Institutional Review Board (IRB00003888) of the French National Institute of Health and Medical Research (INSERM).

Results

During the study period, 613 women delivered, 554 were eligible for inclusion (59 did not have BLL assessed during pregnancy), and 531 were included (5 declined to participate, 8 left the maternity ward before inclusion, and 10 did not participate for other reasons). The overall participation rate was 86.6%. The population is described in Table 1. The average age was 27 years. Approximately 40% of women were born in French Guiana and 51.7% in Suriname, 81.2% of the women were of bushi-nenge origin, 62.9% lived in St. Laurent du Maroni, 17.2% resided along the Maroni River and 7% reported Suriname as their primary residence. Approximately 25% of the population did not attend school. The geometric mean (GM) of BLL was 32.6 μ g/L (max: 259 μ g/L) where 25.8% (CI 95 % = [22.1-29.5]) and 5.1% (CI 95 % = [3.2-7.0]) of the women had BLL \geq 50 μ g/L and \geq 100 μ g/L, respectively. Only 4.1% of BLL were under the LOQ.

The comparison between GM of BLL according to sociodemographic and socioeconomic characteristics is presented in Table 1. BLL were significantly higher in: women born in Suriname whose first language was nengue tongo, who resided along the Maroni River (Apatou or Grand Santi), with a low level of education (an inverse correlation between BLL and level of education was observed), had irregular employment ("jobbing") or undocumented status, and did not have enough money to live on. The highest BLL were noted for women living in Apatou and Grand Santi (49.4 µg/L and 59.8 µg/L, respectively). Women who had no schooling displayed BLL of 45.1 µg/L. BLL were mostly recorded from

week 20 to delivery (62%), then from week 12 to week 20 (32%). BLL collected from week 12 to week 20 were significantly lower.

The comparison of BLL according to nutritional habits during pregnancy is depicted in Table 2. BLL were significantly higher in women who regularly (daily or weekly) consumed manioc, all manioc derivatives combined (specifically couac, domi and cassava), wild game and fish. A positive correlation was found between BLL and frequency with which manioc derivatives were consumed. Notably, 73.9% of the population regularly consumed manioc derivatives, and 61.4% regularly consumed couac. The BLL was higher when couac was prepared personally (56 μ g/L) and when woman helped prepare couac during her pregnancy (49.6 μ g/L) or indicated that rainwater was consumed (40.5 μ g/L) (Table 3). Drinking bottled water was associated with lower BLL (21.6 μ g/L). No correlation was observed with alcohol and tobacco consumption during pregnancy.

The results from the multivariate analysis are presented in Table 4. Among the socioeconomic and sociodemographic variables, only native language, place of residence, level of education and gestational age at the time BLL was assessed, remained significantly associated with an elevated BLL in the multivariate model after the descending procedure (data not shown). These four variables, as well as age, were included in the adjusted model. In the final model (model 3), the variables associated with a risk of elevated BLL were: speaking nengue tongo as a first language, place of residence (Grand Santi), a low level of education (primary school only or no schooling), consumption of couac (weekly or daily), participating in couac preparation during pregnancy and weekly consumption of wild game. Drinking rainwater no longer appeared to be a risk factor for elevated BLL in this final model. The adjusted R² of the final model was 0.31, and the graphic distribution of residuals was normal. The "consumption of manioc derivatives" variable was not included in the final model to avoid the risk of overadjustment. However, when it was substituted for the "couac consumption" variable in the final model, daily consumption of manioc derivatives was significantly associated with an elevated BLL. Finally, 90% of women who participated in this study had at least one of the risk factors identified in the final model.

Discussion

In our study, the GM of BLL in women was 32.6 μ g/L, while 25.8% had a BLL above the threshold for intervention in minors in France (50 μ g/L since 2015) (HCSP, 2014). The average BLL observed is approximately 4-fold higher than that found in metropolitan France in 2011 (study of 1968 women delivering in 211 maternity wards: BLL = 8.30 μ g/L) (Guldner et al. 2014), and 5-fold higher than that found in USA between 2003 and 2008 (NHANES Study, BLL = 6.4 μ g/L) (Jones et al., 2010). However, the BLL observed in this study did not reach the levels noted in Brazil, where average BLL between 57 and 127 μ g/L were found in pregnant women or women of reproductive age (Barbosa Jr. et al., 2009; Carneiro et al., 2013; Zentner and Rondó, 2004).

The first set of risk factors identified in our study was associated with belonging to a disadvantaged socioeconomic class. These risk factors were identified in many studies (Barbosa Jr. et al., 2009; Carneiro et al., 2013; Hertz-Picciotto et al., 2000; Jones et al., 2010; McKelvey et al., 2007). There are many potential explanations for the link between a precarious socioeconomic status and Pb poisoning: nutritional deficiencies such as iron and calcium promote absorption of metal, or environmental sources (Tagne-Fotso et al., 2016). Pregnant women in western French Guiana are particularly likely to have anemia (70%) and iron deficiency (Louison-Ferté et al., 2014). In addition, the soil in French Guiana is particularly poor in iron and calcium, which might promote the persistence of deficiencies in populations whose food intake is primarily based on farming (ANSES, 2015).

Independent of socioeconomic characteristics, elevated BLL were identified in women who consumed manioc derivatives in a dose-dependent manner. Analyses performed by the French authorities in 2011 found high Pb content in some manioc and couac samples. An analytical study of Pb in manioc and its derivatives was conducted by the French-Guiana Regional Agency of Health in 2012-2013 by sampling communities, then farmers (ANSES, 2015). In total, 86 manioc samples and 50 couac samples were collected from homemade or

familial sources of manioc treatment or preparation (industrial production does not exist in French Guiana). The average metal content based on "fresh weight" was 0.06 mg/kg in manioc roots and 0.19 mg/kg in couac. According to this investigation, 24% of couac samples and 14% of root samples exceeded the limit permitted for sale (established by the European Regulation CE 1881/2006 as 0.1 mg/kg of fresh manioc). These data are a cause for concern, because couac is a staple food in French Guiana, as it is in Brazil (mainly in the northern and northeastern regions) and Africa (Adayemi et al., 2016). In our study, 21.5% of women consumed daily couac, and 46% consumed couac at least twice a week. Several different hypotheses may account for this contamination, including initial contamination of the manioc roots from soil or Pb transfer during manioc processing: shredding, grinding and cooking on hotplates. There is no ongoing or historic industrial or mining activity (outside of gold mining) that might explain contamination of soil or waterways. An expert assessment conducted in French Guiana (by the Bureau de Recherches Géologiques et Minières de Guyane in 2013) provided little information. This study did not measure concentrations in soil, only in waterway sediments. The observed Pb levels were relatively comparable to those that occur naturally in large rivers (Horowitz, 1999), although elevated levels were observed in certain areas of French Guiana. No data are available for the Grand-Santi and Apatou regions. Finally, these data do not exclude the possibility of soil contamination in French Guiana, and more analyses are needed (ANSES, 2015). Traditional agricultural practices based on slashing and burning may be at the origin of zones with the highest concentrations of metal. Contamination of manioc flour by Pb transfer from hotplates during cooking was proposed by Barbosa Jr. et al. (2009), who found significantly higher levels of metal in manioc flour compared to manioc root (flour: 0.19 ± 0.1 mg/kg, manioc: 0.017 ± 0.016 mg/kg). This finding was also reported in a similar analysis performed later in another village (Barbosa Jr. et al., 2009). In French Guiana, a significant degree of metal enrichment was observed during shredding and grinding of manioc roots due to the use of homemade tools, but not during the cooking stage (ANSES, 2015). This observation, which was only conducted at two sites, does not allow us to draw any formal conclusions. In addition, in our

study, women who participated in preparing couac during their pregnancies were found to exhibit higher Pb levels. Data suggest that Pb poisoning might also occur by inhaling metal particles while preparing couac.

The other risk factors identified in our investigation were weekly consumption of wild game, which is well-known (Tagne-Fotso et al., 2016), however, this was not apparent in this study. We did not find any link between geophagy (consuming pemba, local clay) and BLL, even though this has been identified as a source of lead poisoning in other contexts (Bakhireva et al., 2013; Thihalolipavan et al., 2013). In contrast, Lambert et al. (2010) noted elevated Pb in pemba itself in French Guiana. It is possible that this item was underreported due to reluctance to admit to consuming pemba to a healthcare provider. It is also possible that pemba sequesters Pb (due to its chelating ability), thereby rendering it less bioavailable (ANSES, 2015). Drinking rainwater appeared to be linked to metal exposure, even though the association was not significant. In French Guiana, house roofs are typically composed of sheets of steel, and thus it is possible that rainwater becomes contaminated due to degradation of the metal. Environmental investigations would be required to confirm this link. Cooking utensils, particularly those made of ceramic, tin and crystal, are also known as sources of Pb exposure (Lynch et al., 2008). However, these utensils were not found in environmental field studies. Lead poisoning due to the use of cooking utensils of unregulated quality remains a possibility in French Guiana, as Pb may be released during cooking, especially of acidic foods (ANSES, 2015). This information was not collected during our study, nor was information collected regarding traditional remedies or cosmetics. Waste treatment is another issue in French Guiana, particularly in western French Guiana, where all waste is discarded in landfills without being recycled or incinerated. In addition, there are no means of recycling hazardous waste such as car batteries, and illegal waste recovery may occur via Suriname. This could be the origin of localized soil contamination. Sucking on a piece of metal containing a high concentration of Pb is another possible source of contamination, particularly in children (Meyer et al., 2008). This source of contamination could explain the Pb poisoning of the 3-year-old girl in Mana, where car frames containing

batteries were accessible to children (ANSES, 2015). However, this possibility is essentially limited to the coastal areas, as there are still few cars in the Maroni river villages, which can only be accessed from the river.

There are a number of strengths of our study: a high level of participation (86.6%), a relatively large number of women included in the study (N = 531) and the fact that data collection was performed in local languages. There are several limitations that need to be noted. First, this was an observational study, and BLL were measured at different points during pregnancy. We did observe a U-shaped curve, with the lowest point between 12 and 20 weeks of pregnancy, as reported in the literature (Hertz-Picciotto et al., 2000; Miranda et al., 2010). However, this variable did not modify the link we observed between nutritional factors and BLL. Secondly, 59 women (9.6% of the deliveries that occurred during the study period) were not included because their BLL were not collected during pregnancy. We did not collect any information about these women, but it is likely that they received little or no follow-up, and that the BLL collection was omitted at delivery. Many women arrive at the CHOG in labor without any previous follow-up. According to the regional delivery register, this was true for 9.2% of the woman who delivered at the CHOG in 2013, versus only 0.6% in our sample. This may have led to a recruitment bias, as these women often live in precarious situations. Thirdly, is the choice of using maximal BLL, which may have led to a slight overestimation of metal levels. However, this overestimate does not appear to have exerted a substantial effect, as the GM of the first BLL was 32 µg/L. The fourth limitation is linked to the fact that BLL during pregnancy is not solely due to contemporary exposure, but is also associated with remobilization of metal that has accumulated in the bones in the past (Miranda et al., 2010).

Since 2012, systematic screening has been provided for pregnant women in western French Guiana. Based on the results from our study, this screening needs to be continued as 90% of women displayed at least one risk factor and 25% of women exhibited a BLL above the threshold for intervention. Consequently women may benefit from health and dietary

advice, as well as iron and calcium supplementation. The impact of this metal exposure on cognitive development in children is problematic, even more so when combined with other heavy metal poisoning. Indeed, mercury (Hg) poisoning has been recognized in French Guiana for about 20 years, and associated with consumption of river fish and panning for gold (Fréry et al., 2001). Mercury poisoning primarily affects the residents of Haut-Maroni.

Conclusion

This is the first study conducted in pregnant women in western French Guiana to provide evidence to raise public health problem. This study has shed light on regional and social disparities in BLL in western French Guiana and potential sources of contamination. Our study identified foods that are widely prepared and consumed by the local population as possible metal sources. Given the existing data, recommendations and specific actions in terms of prevention, screening and care for pregnant women and women of reproductive age need to be adapted and put into place, as well as cooperation with bordering countries that are facing similar health issues.

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Figure 1: Area served by the St-Laurent-du-Maroni Hospital



Table 1: Participants' characteristics and BLL (μg/L).

			%	GM*	Divolue
Age	<18	<u>n</u> 37	7.0	32.7	P-value 0.42
Age	18-24	198	37.3	31.2	0.42
	25-35	219	41.2	32.7	
	>35	77	14.5	36.3	
		• •		00.0	
Place of	French Guiana	214	40.4	32.2	p<10 ⁻³
birth	Surinam	274	51.7	35.9	•
	Brazil	17	3.2	25.6	
	Other	25	4.7	15.1	
N 1 (*)		00		47.0	4.0-3
Native	French	33	6.3	17.2	p<10 ⁻³
language	Nenge tongo	427	81.2	37.3	
	Amerindian language	7	1.3	19.4	
	Portuguese	20	3.8	23.8	
	Other	39	7.4	18.1	
Primary	St Laurent	333	62.9	30.0	p<10 ⁻³
place	Mana/Awala	54	10.2	25.6	ρ .σ
of residence	Apatou	49	9.3	48.7	
Ol recidence	Grand Santi	42	7.9	59.8	
	Surinam	37	7.0	28.9	
	Other	14	2.7	28.0	
				_0.0	
Level of	No schooling	124	23.6	45.1	p<10 ⁻³
education	Primary	60	11.4	41.8	·
	Secondary	146	27.8	31.1	
	High school	160	30.4	27.5	
	University	36	6.8	18.1	
					4.0-3
Employment	Student	102	19.4	30.1	p<10 ⁻³
status before	Employed	85	16.1	23.9	
pregnancy	Unemployed	21	4.0	31.4	
	Irregular employment	49	9.3	40.1	
	Homemaker	270	51.2	35.7	
Legal status	French citizen or foreign citizen with	238	48.6	28.9	0.0007
g	10-year French territory residence card				
	Temporary documented migrant (1-	77	15.7	31.3	
	year stay document)				
	Undocumented migrant	175	35.7	37.0	
_			04.0	40.0	
Financial	Not enough	114	21.6	40.9	p<10 ⁻³
means	Just enough	269	50.9	33.0	
	Adequate	145	27.5	27.0	
Gestational age	<12 weeks	34	6.4	29.3	0.006
at BLL collection	12-20 weeks	169	31.8	26.7	3.000
at DEL CONSCION	>20 weeks	328	61.8	36.6	
* CM: ========		020	01.0	55.0	

^{*} GM: geometric mean of BLL (µg/L)

Table 2: BLL (µg/L) according to nutritional habits and lifestyle factors during pregnancy.

		•			-
		n	%	GM**	р
Manioc	Never	171	32.6	30.6	p<10 ⁻³
	Monthly	140	26.7	29.0	
	Weekly	157	29.9	35.1	
	Daily	57	10.8	44.2	
All	Never	33	6.2	19.2	p<10 ⁻³
derivatives	Monthly	81	15.3	24.8	
	Weekly	250	47.4	33.3	
	Daily	164	31.1	40.1	
Couac*	Never	93	17.6	26.1	p<10 ⁻³
	Monthly	111	21.0	26.3	μ
	Weekly	210	39.8	35.8	
	Daily	114	21.6	40.3	
Domi*	Never	127	24.4	25.3	p<10 ⁻³
Domi	Monthly	133	25.5	30.1	pilo
	Weekly	185	35.5	37.6	
	Daily	76	14.6	42.5	
Cassava*	Never	210	40.1	29.7	0.005
Cassava	Monthly	160	30.5	29.7 31.2	0.005
	Weekly	118	22.5	36.7	
	Daily	36	6.9	41.7	
0 1: #		070	74.0	00.4	0.00
Crabio*	Never	376	71.9	33.4	0.23
	Monthly Weekly	126 11	24.1 2.1	30.2 42.8	
	Daily	10	1.9	28.8	
	Daily	10	1.5	20.0	
Tapioca*	Never	359	68.9	33.6	0.03
	Monthly	127	24.4	29.6	
	Weekly	30	5.8	30.9	
	Daily	5	0.9	65.8	
Wild game	Never	210	40.4	28.9	p<10 ⁻³
	Monthly	205	39.4	32.7	
	Weekly	85	16.4	43.9	
	Daily	20	3.8	37.9	
Fish	Never	25	4.8	30.0	0.001
	Monthly	86	16.3	27.2	
	Weekly	291	55.2	32.1	
	Daily	125	23.7	38.9	
Wassaï*	Never	103	19.6	27.2	0.02
	Monthly	130	24.7	33.9	
	Weekly	205	39.0	33.6	
	Daily	88	16.7	35.5	
Pemba*	Never	286	54.8	31.9	0.27
1 Ciliba	Monthly	112	21.5	33.5	U.Z.I
	Weekly	64	12.2	31.8	
	Daily	60	11.5	38.3	
Tobacca	Vos	10	2.4	25.0	0.14
Tobacco	Yes No	18 513	3.4 96.6	25.9 32.9	U. 14
		0.10	00.0	02.0	
Alcohol	Yes	180	33.9	34.1	0.27
*Cours /	No	351	66.1	31.9	Vendia (det
Couac (man	ioc flour), Cas	sava (ma	anioc par	ıcakes), (Crabio (det

*Couac (manioc flour), Cassava (manioc pancakes), Crabio (detoxified cooking liquid used as a sauce), Tapioca (bitter manioc starch), Wassaï (fruit of the wassaï palm tree), Pemba (local clay) ** GM: geometric mean of BLL (μg/L)

Table 3: BLL (µg/L) according to couac preparation and water consumption during pregnancy.

			n	%	GM**	p-value
Source of the couac		Personal	69	16.6	55.7	p<10 ⁻³
		Other*	346	83.4	31.7	
Personally prepared couac		No	313	60.8	27.2	p<10 ⁻³
r crochany proparoa c		Yes	202	39.2	44.8	μ .σ
Helped prepare couac		No	415	81.4	30.3	p<10 ⁻³
		Yes	95	18.6	49.3	
187 (Dublic comb	No	225	44.2	25.4	0.02
Water consumption	Public supply	No Yes	235 296	44.3 55.7	35.1 30.8	0.03
	Dellied etc.	NI.	400	07.0	04.0	40-3
	Bottled water	No	463	87.2	34.9	p<10 ⁻³
		Yes	68	12.8	20.7	
	Rain water	No	386	72.7	30.1	p<10 ⁻³
		Yes	145	27.3	40.4	
	Well water	No	498	93.8	32.4	0.33
		Yes	33	6.2	36.4	
	River water	No	498	93.8	32.3	0.15
		Yes	33	6.2	38.5	

^{*} market, grocery store, family/friends, from the producer.

^{**} GM: geometric mean of BLL (µg/L)

Table 4: Linear regression model of the logarithm of the BLL according to socioeconomic characteristics and nutritional factors (regression β coefficients and CI 95%).

		Model 1 (binary)	Model 2: Model 1 + Adjusted variables*	Model 3: Final model
Age	<25 years [25-35 years] >35 years	0 0.04 [-0.08 ; 0.16] 0.14 [-0.03 ; 0.32]		0 0.02 [-0.10 ; 0.13] 0.09 [-0.06 ; 0.25]
Native language	French and other Nenge tongo	0 0.68 [0.54 ; 0.81]		0 0.40 [0.25 ; 0.55]
Place of residence	SLM Mana/Awala Apatou Grand-Santi Other	0 -0.16 [-0.34; 0.02] 0.48 [0.29; 0.67] 0.69 [0.49; 0.89] -0.05 [-0.23; 0.14]		0 0.02 [-0.15; 0.20] 0.16 [-0.02; 0.34] 0.28 [0.07; 0.48] -0.10 [-0.27; 0.08]
Education level	High School University Secondary Primary None	0 -0.42 [-0.65 ; -0.19] 0.12 [-0.02 ; 0.26] 0.42 [0.23 ; 0.60] 0.49 [0.35 ; 0.64]		0 -0.25 [-0.47; -0.03 -0.04 [-0.17; 0.10] 0.19 [0.01; 0.37] 0.20 [0.05; 0.35]
Gestational age	12-20 weeks <12 weeks >20 weeks	0 0.09 [-0.15 ; 0.33] 0.32 [0.19 ; 0.44]		0 0.21 [-0.002 ; 0.42] 0.28 [0.17 ; 0.39]
Consumption of Manioc	Never or monthly Weekly Daily	0 0.16 [0.03 ; 0.28] 0.37 [0.19 ; 0.55]	0 0.06 [-0.06 ; 0.17] 0.14 [-0.03 ; 0.31]	
Consumption of manioc derivatives	Never or monthly Weekly Daily	0 0.34 [0.21 ; 0.48] 0.52 [0.38 ; 0.67]	0 0.17 [0.04 ; 0.30] 0.24 [0.10 ; 0.39]	
Consumption of Couac	Never or monthly Weekly Daily	0 0.29 [0.17 ; 0.41] 0.40 [0.26 ; 0.55]	0 0.15 [0.04 ; 0.26] 0.19 [0.05 ; 0.33]	0 0.15 [0.03 ; 0.26] 0.17 [0.02 ; 0.31]
Participation in the Preparation of Couac	No Yes	0 0.48 [0.34 ; 0.62]	0 0.24 [0.10 ; 0.38]	0 0.20 [0.05 ; 0.34]
Consumption of Fish	Never or monthly Weekly Daily	0 0.14 [-0.003 ; 0.28] 0.32 [0.16 ; 0.49]	0 0.10 [-0.03 ; 0.22] 0.15 [-0.005 ; 0.30]	
Consumption of Wassaï	Never or monthly Weekly Daily	0 0.07 [-0.05 ; 0.19] 0.12 -0.04 ; 0.28]	0 -0.04 [-0.15 ; 0.07] -0.01 [-0.16 ; 0.14]	
Consumption of Game	Never or monthly Weekly Daily	0 0.35 [0.20 ; 0.50] 0.19 [-0.09 ; 0.48]	0 0.19 [0.05 ; 0.33] 0.01 [-0.26 ; 0.27]	0 0.17 [0.03 ; 0.30] 0.00 [-0.26 ; 0.26]
Consumption of Pemba	Never or monthly Weekly Daily	0 -0.04 [-0.20 ; 0.13] 0.15 [-0.02 ; 0.33}	0 -0.05 [-0.21 ; 0.10] 0.05 [-0.11 ; 0.21]	
Rain water	No Yes	0 0.28 [0.15 ; 0.40]	0 0.10 [-0.01 ; 0.22]	

 $^{^{\}star}$ The adjusted variables used are age, level of education, native language, place of residence and gestational age Significant β coefficients are indicated in bold