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Metal exposure and reproductive disorders in indigenous communities living along the Pilcomayo River, Bolivia

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ABSTRACT

Background: The Pilcomayo River is polluted by tailings and effluents from upstream mining activities, which contain high levels of metals. The Weenhayek live along this river and are likely to have elevated exposure.
Objectives: To assess whether the Weenhayek have increased risk of reproductive and developmental disorders related to elevated metal exposure in comparison with a reference population.
Methods: We assessed reproductive and developmental outcomes, i.e. fertility, fetal loss, congenital anomalies, and walking onset by means of structured interviews. We sampled hair, water and fish to assess the relative exposure of the Weenhayek. Samples were analyzed for Pb and Cd with ICP-MS techniques.
Results: The Weenhayek communities studied had a higher prevalence of small families (OR 2.7, 95% CI 1.3–6.0) and delayed walking onset (OR 2.7, 95% CI 1.4–5.1) than the reference population. Median Pb levels in Weenhayek hair were 2–5 times higher than in the reference population, while Cd levels were not elevated. In water and fish, both Pb and Cd levels were increased in the Weenhayek area.
Conclusions: We found indications for increased risks of small families and delayed walking onset among the Weenhayek living along the Pilcomayo River. Lactants form a high risk group for lead exposure.

1. Introduction

Acid mining drainage and erosion of waste rock and tailing dump piles are mining-related processes that can lead to discharge of metals into river basins (Salomons, 1995; Luoma and Rainbow, 2008). Humans can be exposed to these metals by consuming polluted fish and other aquatic organisms. Another potential exposure pathway is the consumption of polluted river water (ATSDR, 2007a). Indigenous riverside populations may form a specific risk group since they are more likely to consume relatively large quantities of fish (e.g. Fréry et al., 2001) and may depend upon the river as a water source.

Bolivia has traditionally been a mining country. In 2007, 5% of its Gross Domestic Product (GDP) came from nonfuel minerals. One of the rivers which is affected by mining activities is the Pilcomayo river (Fig. 1). This river originates in the Bolivian Andes (Cordillera Oriental, ±5200 m above sea level) and cuts down for 560 km until it reaches the Gran Chaco plains near the town of Villamontes. Subsequently, the river flows southward where it forms the natural border between Argentina and Paraguay.

Based upon the lead isotopic composition of the channel bed and the decrease of lead concentrations downstream, Miller et al. (2002, 2007) concluded that the predominant source of lead in the upper Pilcomayo (for at least up to 200 km downstream from Potosí) is related to mining and milling ores of the Cerro Rico. Mining and milling related effluents of the Cerro Rico are drained by the Tarapaya (Fig. 1). Tailings and flotation effluents are likely to contribute most significantly, since the elevated metal levels in sediment are also associated with pyrite, sulfur and sulfide (Hudson-Edwards et al., 2001; Smolders et al., 2003).

Three studies investigated tailing dam failures in tributaries of the Pilaya (Macklin et al., 1996, 2006; Hudson-Edwards et al., 2001; Villarroel et al., 2006) and showed that although metal contamination due to these failures had a significant short-term impact on the water quality and sediment of the lower reaches of the Pilcomayo downstream of the confluence with the Pilaya (Fig. 1), metal concentrations in the upper Pilcomayo were higher and were more likely to have a long-term impact. The lower reaches of the Pilcomayo consistently show low, near background, metal concentrations in sediments (Hudson-

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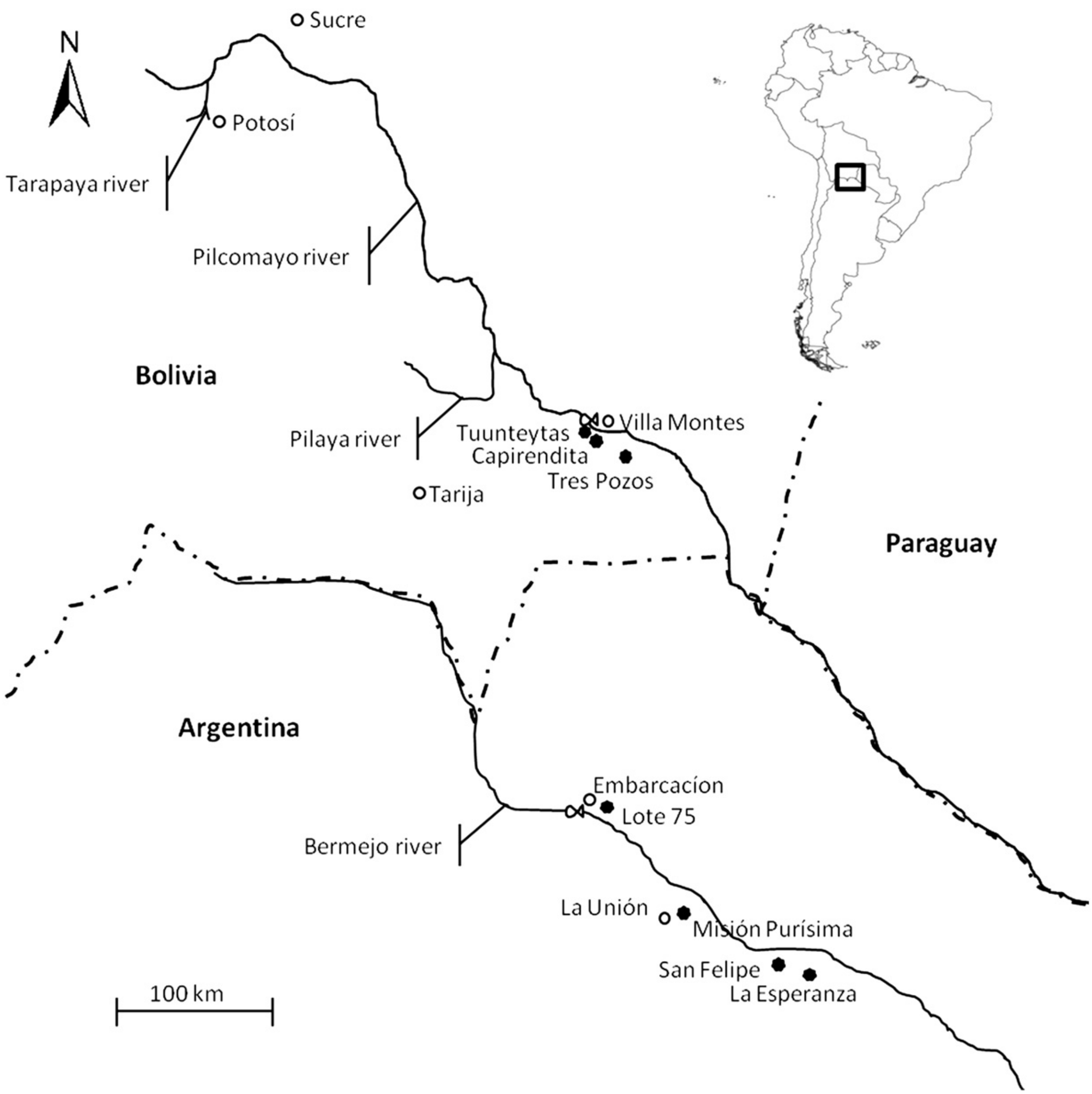

Fig. 1. Location map showing sample areas and study populations.

Edwards et al., 2001; Smolders et al., 2003; Macklin et al., 2006) as a result of storage within the channel bed and dilution through the input of large amounts of relatively clean sediment (Hudson-Edwards et al., 2001; Smolders et al., 2002, 2003). Nevertheless, Smolders et al. (2003) found a three to fivefold increase of metal concentrations in sediments deposited in the Gran Chaco area in the late 1990s compared to sediments deposited before 1985, although the concentrations in the upper sediment layer were still low.

The Weenhayek live along the lower reaches of the Pilcomayo from the area of Villamontes downstream to the Argentinean border. The Weenhayek are an indigenous tribe traditionally living from hunting, gathering and fishing (Alvarsson, 2001). Since the seventies, fishing (mainly for the *Sábalo*) forms their principal economical income. These communities consume an important part of their fish production (FAO, 2004). Therefore, concentrations of metals in the scalp hair of a small number of Weenhayek people were determined in 2003 (Smolders et al., 2006). The concentrations reported were relatively high, also compared to nearby habitants from Villamontes, and raised concerns about potential adverse health effects, especially reproductive and developmental disorders that may be caused by elevated metal exposure. Particularly, Pb and Cd have known reproductive and/or developmental effects described in the literature (ATSDR, 1990, 1992a, 1992b, 2004, 2005, 2007a, 2008). Elevated lead exposure has been related to several reproductive effects in women and men (ATSDR, 2007a, 2007b), while for cadmium only reproductive effects in men are known (ATSDR, 2008). Neurologic and developmental effects of lead in children are well-known in the literature (ATSDR, 2007a, 2007b), but developmental effects of cadmium are unknown (ATSDR, 2008).

Therefore, an in-depth study was performed in 2006 that focused on metal exposure and potential adverse health effects, i.e. reproductive and developmental disorders. The main goal of this study was to assess whether the Weenhayek have an increased risk of reproductive and developmental disorders associated with elevated lead and cadmium exposure in comparison with a reference population living in a relatively unpolluted area. The secondary goal was to estimate whether the total daily lead and cadmium intake of the Weenhayek exceeds the Tolerable Daily Intake (TDI). The Methodology and detailed Results and Discussion related to this secondary goal are described in the Appendix.

2. Methods

2.1. Reference area

The Bermejo river basin in Salta Province, Argentina was selected as a relatively unpolluted reference area with characteristics comparable to the Pilcomayo. Lead and cadmium levels in unfiltered water and sediment of the Bermejo were significantly lower compared to the Pilcomayo river near Villamontes (Smolders, 2006). The Pilcomayo and Bermejo rivers are both tributaries of the Paraguay–Paraná rivers and part of the La Plata basin (Fig. 1). The sample areas (Pilcomayo and Bermejo) and study populations (Weenhayek and Wichí) are located in the same geological region (Chaco plain).

Both rivers have similar hydrogeochemical characteristics: (1) marine and continental sedimentary rocks and Precambrian metamorphic rocks in the headwaters and Quaternary volcanic rocks in the Chaco plain (Iriondo, 1993; Depetris and Pasquini, 2007), (2) similar annual water discharge fluctuations with maximum discharges of $\pm 1000\ m^3\ s^{-1}$ (Bermejo) and $\pm 600\ m^3\ s^{-1}$ (Pilcomayo) in February–March (Depetris, 2007), (3) high sediment loads, i.e. in the Pilcomayo $10.6\ g\ L^{-1}$ (Smolders et al., 2002) and in the Bermejo $7–8\ g\ L^{-1}$ (Depetris and Pasquini, 2007), (4) classification as iron mudstones (Depetris and Pasquini, 2007), (5) similar composition of Total Suspended Solids (TSS), e.g. for SiO_2 and Fe_3O_2 62.96 μg/g and 4.98 μg/g in the Bermejo and 52.43 μg/g and 5.09 μg/g in the Pilcomayo, respectively, and (6) relatively comparable concentrations of Rare Earth Elements (REE) dissolved in water (Depetris and Pasquini, 2007).

2.2. Water and fish sampling

Between May (onset of the fishing season) and October (end of the fishing season) 2006, water samples were obtained from the Pilcomayo river ($n = 44$). The fishing season corresponds with the dry season. On several days in October 2006, water samples were taken from the Bermejo river in Embarcación ($n = 4$). Samples were collected along the banks of the rivers at the lower channel's inner curve at a single location per river. Unfiltered water was sampled because Weenhayek and Wichí reported not to decant water. The pH was recorded with a field instrument (Schott handylab pH 11; precision = 0.005). The water samples (20 ml) were conserved by directly adding 1% nitric acid (Merck, 65%), preventing complex formation and sorption.

The *Bagre* (*Pimelodus clarias*) fish was caught with hook and line in the Pilcomayo river (Villamontes) between May and September 2006 ($n = 28$) and in the Bermejo River (just south of Embarcación) in October 2006 ($n = 5$). Muscle tissue was collected from behind the dorsal fin. All samples were stored in pre-washed (with diluted HNO_3) polyethylene containers at −20 °C until pre-analysis.

2.3. Study populations

The total Weenhayek population fluctuates between 2000 and 3000 people, living in 21 communities. In this study, we included three villages along the Pilcomayo River, namely Capirendita, Tres Pozos and Tuunteytas. The total numbers of people living in these villages were 426, 229 and 170, respectively (INE, 2001). Capirendita was chosen because it is the largest Weenhayek village. Tres Pozos and Tuunteytas were selected to compare our results with the previous study in the same villages (Smolders et al., 2006).

As reference population, we chose indigenous Wichí who live along the Bermejo River. Weenhayek and Wichí are likely to have great genetic similarities, since their languages are closely related and they share the same cultural heritage (Alvarsson, 2001; Lewis, 2009). The populations have a comparable way of living, which is relatively self-sufficient and primitive, organized around fishing, hunting, gathering, handicrafts and small-scale stock. From the reference population, we included the relatively large village of Lote 75, near Embarcación, and three small villages in the vicinity of La Unión: Misión Purísima, San Felipe and La Esperanza. The total numbers of people living in these villages were approximately 280, 100, 90 and 100, respectively.

Since we worked with more or less isolated indigenous communities we needed to adopt certain principles of cross-cultural research to achieve collaboration and consent (Piquemal, 2000). We worked with local NGOs that enjoyed a high level of confidence to introduce us to the community (leaders), since the introduction of unfamiliar persons by familiar persons is common in these communities. After consent of the leaders, we organized informal meetings with inhabitants of the selected communities. Since the vast majority is illiterate, we used poster presentations to introduce our study. General information was given about the background of the study, the methods used (interviews and hair sampling) and the communication of the results after completion of the study. The presentations were held in Spanish and in the indigenous language. Translation was especially necessary for older women who understood and spoke Spanish insufficiently. To increase participation, a small gift (food products) was given after participation.

2.4. Interviews

The interview questions were translated from Spanish into the indigenous language. The interviews were pilot tested to ensure no information got lost and interpretation remained unchanged after translation (Nieuwenhuijsen, 2003). The interviews were conducted by two instructed female Weenhayek interviewers and two instructed female Wichí interviewers, in September and October 2006 in Bolivia and in October 2006 in Argentina. The two separate sets of interviewers received identical instructions. Pilot interviews were done by the instructed interviewers with persons acting as participants to ensure that the interpretation remained as intended by the researchers. Furthermore, the researchers were present at the location of the interviews and could be consulted in case the answers were unclear for the interviewers. The interviews were held with women only, since they are likely to provide the most accurate information regarding the reproductive and developmental disorders included in this study. Random groups of women were asked to participate in each community.

We assessed two reproductive outcomes, i.e. family size as a proxy for fertility and fetal loss (including stillbirths, miscarriages and absence of menstruation for at least two months, ending with blood loss and pain). Regarding family size, a question about the first names of the children was included to ensure that this variable was recorded adequately. We also assessed two developmental outcomes related to lead (ATSDR, 2007a, 2007b), i.e. selected congenital anomalies (Needleman et al., 1984) and onset of independent walking (Schwartz and Otto, 1987). We focused on two types of congenital anomalies which are relatively easy to identify visually, i.e., hemangiomas and lymphangiomas on the skin. Pictures and a verbal description accompanied the questions about these congenital anomalies. Walking onset was expressed in months after birth. Fetal loss was registered only if it occurred during the last two pregnancies and anomalies only when related to the last two births. Outcomes were registered only for the last two events, since earlier events are subject to less accurate memories. Other questions concerned potential confounding factors, i.e., age, year of birth of the children, settling history, smoking habits, alcohol use, use of contraceptives, and potential external reasons for fetal loss and delayed walking onset. We also asked whether the youngest child was still breastfeeding.

2.5. Hair sampling

At the time of the interview, hair samples were taken from the fine hairs at the nape of the neck (Gunter and Miller, 1986) from all women who were willing to cooperate and their youngest child ($n = 293$). The samples were stored and transported in polyethylene bags and kept in a dark place at room temperature (±25 °C).

2.6. Sample analysis

2.6.1. Pre-analysis

Pre-analysis of water samples consisted of settling of the suspended matter. Hair samples were washed three times, both with acetone and deionized water, in order to diminish external contamination. Fish and hair samples were dried in a ventilated oven at 40 °C until no further weight loss was observed. The samples were weighted before (wet weight) and after (dry weight) the drying process. However, determination of the water content of the fish samples was deemed unreliable because it was too low relative to the detection limit of the balance (0.1 g) for the majority of samples. Therefore, average water contents of muscle tissue of *Bagre* (79% ± 7%) were obtained from the literature (Filho et al., 2010) and were used to convert dry weights to fresh weights.

Dry samples of fish and hair were digested in sealed Teflon vessels in a microwave oven (Ethos D Microwave Lab station from Milestone) after adding ultra-pure HNO_3 (Merck) and an excess of ultra-pure H_2O_2 (Merck) (JECFA, 2011). The digestion procedure used for fish is a preferred method in metal analysis of food (JECFA, 2011).

2.6.2. Metal analysis

Diluted destruates and water were analyzed for lead and cadmium, using Inductive Coupled Plasma Mass Spectrometry (ICP-MS). For all samples, Sc, Ga and Te were used as internal standards and blanks were included as well. For water and fish, the accuracy was estimated by the use of an external reference solution (QC; Merck multi-element standard) and the precision by repeated analyses. For hair, the accuracy was estimated by the use of certified reference material of human hair (Yoshinaga et al., 1997) and the precision by the use of duplicates. Cadmium concentrations in some water and fish filet samples were below the detection limit, i.e. 0.0025 μg/L Cd in water and 0.00005 μg/g Cd in fish filet. The concentration of these samples was assumed to equal the detection limit.

2.7. Statistical analysis

All statistical analyses were performed using SPSS version 17.0. Non-parametric methods were chosen because the vast majority of the data were not normally distributed, even after log transformation. For the environmental parameters, medians with 25th and 75th percentiles were calculated with the non-parametric method implemented in SPSS, while for hair the larger number of samples permitted calculation of 10th and 90th percentiles. Mann–Whitney U tests were used to test for differences between groups and Kruskal–Wallis tests for differences between multiple age categories. The reproductive and developmental outcomes were evaluated by means of logistic regression analyses, in which the Weenhayek were considered exposed to heavy metals and the Wichí as low or non-exposed. This resulted in ORs with 95% confidence intervals (95%CI) as effect measures. Dichotomous outcomes were defined as follows: small family as 0–2 children, fetal loss as at least one loss in the last two pregnancies, congenital anomalies as presence of hemangiomas or lymphangiomas in any of the last two live-born children, and delayed onset of walking as 18 months or older in one or both of the last two children. Because all children lactated until after they started walking, duration of lactation was not included as an explanatory variable for delayed walking onset. Only maternal age was used as a potential confounder, while restriction was applied for some other covariables due to small numbers.

In the analysis of family size, only those women were included who did not use anti-conception for more than 1 year during their life and who had lived in the village since their 16th birthday (140 women). For fetal loss, only women were included who had had two pregnancies after 1985 and reported clearly about the fetal loss (134 women). No fetal loss was recorded if an obvious external reason existed for the absence of menstruation (e.g. inflammation) or the woman was highly unlikely to be pregnant (use of anti-conception). For congenital anomalies, births after 1985 with a complete report on the anomalies were included (298 births). In the analysis of walking onset, children born after 1985 who were older than 18 months or younger and already walking were included if the mother remembered the timing of onset, did not smoke and lived in the village during pregnancy, and there was no obvious external reason for delayed walking (e.g. spasm) (192 children). The cut-off criterion of cases after 1985 was chosen to reduce recall problems and because post 1985 milling operations may have more impact on downstream areas.

3. Results

3.1. Metal levels in river water and fish

Blanks for water and fish filet revealed an average potential external contamination of 0.0% for both lead and cadmium, except for lead in fish filet (17.9%). The estimated accuracies of the water analyses were an average overestimation of 4% for lead and 6% for cadmium and of the fish analyses an average underestimation of 2% for lead and 1% for cadmium. The precision of the water analyses was an average relative standard deviation of 24% for cadmium and 7% for lead. For fish the relative standard deviation was 19% for cadmium and 25% for lead.

The lead and cadmium levels in Pilcomayo river water and *Bagre* muscle were statistically significantly higher than in Bermejo river water and *Bagre* muscle (Table 1), but did not exceed WHO drinking water guidelines (Pb: 10 μg/L; Cd: 3 μg/L; WHO, 2008), nor EU fish standards (Pb: 0.20 mg/kg fresh weight; Cd: 0.05 mg/kg fresh weight; European Commission regulation No. 78/2005). The average measured pH was similar in both rivers, i.e. 8.4 (range: 7.8–8.6) in the Pilcomayo and 8.3 (range: 8.0–8.7) in the Bermejo.

3.2. Estimated intake of lead and cadmium

We estimated the intake of lead and cadmium (for details and assumptions see the Appendix) among Weenhayek only, because lead and cadmium levels in Bermejo river water and fish were much lower than in the Pilcomayo and Wichí adults consume less river water and fish than Weenhayek adults. The Wichí consume on average 0.8 glasses river water/week (max. 3 glasses/week) and on average 1 fish filet/week (max. 3 filets/week). The Weenhayek consume

Table 1
Median (25th and 75th percentiles in parentheses) Pb and Cd levels in water and fish tissues from the Pilcomayo and Bermejo rivers.

	Pilcomayo		Bermejo	
	μg/L	μg/g wet weight	μg/L	μg/g wet weight
Metal	Unfiltered water (n = 44)	Bagre muscle (n = 28)	Unfiltered water (n = 4)	Bagre muscle (n = 5)
Pb	1.65[a] (0.72–2.74)	0.05[c] (0.04–0.07)	0.43[a] (0.11–1.07)	0.03[c] (0.02–0.03)
Cd	0.030[b] (0.010–0.063)	0.002[d] (0.001–0.004)	0.003[b] (0.003–0.003)	0.000[d] (0.000–0.000)

p-values (two-tailed) comparison between:
(1) Pilcomayo and Bermejo river water: a = 0.048; b = 0.005;
(2) *Bagre* muscle from the Pilcomayo and Bermejo rivers: c = 0.004; d = 0.001.

Table 2
Median Pb and Cd levels in hair (μg/g), 10th and 90th percentiles in parentheses.

Metal	Weenhayek			Wichí	
	Adult women	Non lactants (18–144 months)	Lactants (0–72 months)	Adult women	Children (0–144 months)
	(n = 113)	(n = 35)	(n = 61)	(n = 56)	(n = 27)
Pb	13.3[a] (3.3–47.1)	19.0[c,e] (5.4–102.3)	47.6[c,g] (14.1–149.8)	3.0[a] (1.3–7.0)	9.4[e,g] (4.1–23.8)
Cd	0.04[b] (0.02–0.10)	0.07[d,f] (0.02–0.13)	0.12[d,h] (0.05–0.24)	0.04[b] (0.02–0.12)	0.13[f,h] (0.04–0.74)

p-values (two-tailed) for a, b, c, d, e, f, g and h were: 0.000; 0.867; 0.000; 0.000; 0.007; 0.002; 0.000 and 0.505, respectively.

on average 21 glasses river water/week, 10 *Sábalo* filets/week, 13 *Bagre* filets/week, 6 *Sábalo* bone portions/week and 3 *Bagre* bone portions/week.

The estimated lead and cadmium intake of the Weenhayek due to exposure to riverine contact media, i.e. river water, fish and soil/dust, was compared with the TDI (Table S.2, Appendix). Relative intake was consistently higher for children than for adults. In the fishing season, the estimated daily intake of lead and cadmium was generally below the TDI for the average consumption scenario, except for children exposed to the 75th percentile of lead in soil/dust, water and fish. Children with a worst case consumption pattern exceeded the TDI for all quartiles of lead concentration. In the non-fishing season, the estimated daily intake of lead and cadmium was below the TDI for both scenarios, except for children with a worst case consumption pattern exposed to the 75th percentile of lead concentrations in *Sábalo* filet and soil/dust.

Based upon the lead concentrations measured in 1997 (Table S.3, Appendix), the estimated daily intake of lead by adults exceeded the TDI for the average case scenario and the worst case scenario, when the upper percentiles of lead concentration were assumed (Table S.4, Appendix). Children exceeded the TDI in 1997 for both scenarios and for all percentiles of lead concentration estimated. The estimated daily intake of cadmium in 1997 only exceeded the TDI for the worst case scenario among adults when upper percentiles of cadmium were assumed. For children, the cadmium intake exceeded the TDI both for the average and the worst case scenario, except when lower percentiles of cadmium were assumed.

3.3. Hair metal concentrations

The blanks revealed an average potential external contamination of 4.3% for lead and 0.0% for cadmium. Duplicates showed average differences of 21% (Pb) and 17% (Cd), which are measures for the precision of the analyses. The analysis of certified reference material, a measure for the accuracy of the analyses, resulted in an average recovery of 91% for lead and 80% for cadmium.

Table 3
Descriptive information of the study populations. Range in parentheses if applicable.

	Weenhayek	Wichí
Number of women interviewed	130	82
Number of women included in the analyses	114	64
Average age of the women at time of study	30 (16–60)	33 (16–59)
Average maternal age at childbirth	26 (13–58)	26 (15–49)
Average maternal age of women with fetal loss	27 (14–43)	24 (20–28)

Metal concentrations in children's hair were analyzed by age class, i.e. 0–9, 10–17, 18–72 and 73–144 months, and by lactant/non-lactant. Children older than 144 months were excluded. Because of low numbers, classes were pooled into three groups, i.e. Wichí children, Weenhayek lactants (0–72 months) and Weenhayek non-lactants (18–144 months). One Weenhayek non-lactant was younger than 18 months and was excluded. Due to outlier correction, in which we set the maximum hair metal concentration on the median plus three times the standard deviation, 7 values were adjusted, i.e. 5 hair lead levels (4 Weenhayek mothers and 1 Wichí child) and 2 hair cadmium levels (1 Weenhayek non-lactant and 1 Wichí child).

The results of the analysis on hair are presented in Table 2. Adult Weenhayek women had statistically significantly higher hair lead levels than Wichí women, while the cadmium levels did not differ. Both Weenhayek lactants and non-lactants had higher hair lead levels than Wichí children, but this seemed to be reversed for cadmium. Among the Weenhayek children, lactants had higher Pb and Cd levels in hair than non-lactants.

3.4. Reproductive and developmental outcomes

In total, 212 women participated in the interview study (Table 3). None of these women reported to drink more than 4 glasses of alcohol per week and all were above the age of 16. Of the 212 interviews performed, 178 were included in the analyses, according to the following inclusion criteria: maternal age below 65 years, most recent child born after 1985, mother lived in the same community during the last pregnancy and no report of smoking more than 6 cigarettes per day. Of the Weenhayek women included, 69 were from Capirendita, 27 from Tres Pozos and 18 from Tuunteytas. Of the reference population, 38 came from Lote 75, 12 from San Felipe, 9 from La Esperanza and 5 from Misión Purísima. The average age of the women was 30 among the Weenhayek and 33 among the Wichí, but the average age at childbirth was 26 years for both groups. The results of the analyses on the reproductive and developmental outcomes are presented in Table 4. Three Weenhayek women and one Wichí woman had no children, of which one Weenhayek woman had a fetal loss. Among the Weenhayeks, small families with only one or two children were much more common than among the Wichí (OR 2.7, 95% CI 1.3–6.0), but no difference was found in the occurrence of reported fetal loss. However, there seemed to be a higher prevalence of the congenital anomalies studied (OR 2.6, 95% CI 0.7–9.2) among the Weenhayek. Their children also had an increased risk of delayed walking onset (OR 2.7, 95% CI 1.4–5.1).

Table 4
Number of cases and odd ratios for the reproductive outcome measures.

Reproductive disorder	Number of cases Weenhayek[a]	Number of cases Wichí[a]	Odds ratio with 95% CI unadjusted	Odds ratio with 95% CI adjusted for maternal age
Small families	37 (n = 84)	12 (n = 56)	2.9 (1.3–6.2)	2.7 (1.3–6.0)
Fetal loss	15 (n = 87)	6 (n = 47)	1.4 (0.5–4.0)	0.7 (0.2–2.8)
Congenital anomalies	15 (n = 191)	3 (n = 107)	3.0 (0.8–10.5)	2.6 (0.7–9.2)
Delayed walking onset	48 (n = 106)	20 (n = 86)	2.7 (1.5–5.1)	2.7 (1.4–5.1)

[a] n = total number of women or children in the specific group.

4. Discussion

We investigated whether the Weenhayek had an increased risk of reproductive and developmental disorders and elevated lead and cadmium exposure compared to a reference population (Wichí). Median lead levels in Weenhayek hair were 2–5 times higher than in our reference population, especially among Weenhayek lactants. Our results also showed associations between small families, selected congenital malformations and delayed walking onset and living in the Weenhayek area. Before further discussing the implications of our results, we first address some methodological issues.

4.1. Metal analyses

There was some experimental uncertainty in the metal analyses in all media, which was estimated by quality controls. Over-or underestimation may have occurred, with a maximum underestimation of 47.7% and a maximum overestimation 59.8%. However, the uncertainty for each medium and metal was too small to influence our conclusions.

4.2. Hair metal concentrations

There are controversial opinions about the use of metal in hair as a biomarker of exposure, but despite its limited value to quantify the extent of exposure, scientific consensus exists that metal in hair can be a useful qualitative tool for exposure assessment (ATSDR, 2001). Several studies have shown that metal levels in hair can be used to identify situations with elevated metal exposure, provided that comparisons are made with a reference group within the same study (e.g. Pereira et al., 2004; Sanna et al., 1990; Strumylaite et al., 2004).

Comparison of metals in hair between different studies is complicated by differences in, for example, hair color and smoking behavior. Therefore, we selected three studies among relatively unexposed populations with comparable circumstances, i.e. dark hair and nonsmokers for comparison (Table 5; Mortada et al., 2002; Goullé et al., 2005; Park et al., 2007). The median cadmium levels in hair of the Wichí (0.04–0.13 μg/g) were in the same range as those in the reference studies (0.01–0.31 μg/g). The same was true for the lead levels (3.0–9.4 μg/g versus 0.4–5.4 μg/g), although the hair lead levels in Wichí children were somewhat elevated (9.4 μg/g). The agreement of our results with reference values from the literature supports the validity of our hair sampling methods.

The lead levels in hair of Weenhayek adults (13.3 μg/g) were elevated compared to Wichí adults, reference values and inhabitants of Villamontes, but lower than in 2003 (Table 5; Smolders et al., 2006). Remarkably, the lead levels in hair of inhabitants of the nearby Villamontes (Fig. 1; Smolders et al., 2006) were in the same range as the reference values (Table 5), possibly because they consumed less river water and fish compared to the Weenhayek. In 2006, the cadmium levels in Weenhayek adults (0.04 μg/g) were not elevated compared to Wichí adults, reference values and adult inhabitants of Villamontes (Table 5). In 2003, however, cadmium levels in Weenhayek adults were highly elevated compared to 2006.

Table 5
Lead and cadmium levels in hair (μg/g): reference values and Weenhayek and Villamontes population (Smolders et al., 2006), in parentheses 5th and 95th percentiles.

	Pb	Cd
Reference values		
Mortada et al., 2002	5.37	0.310
Goullé et al., 2005	0.41	0.011
Park et al., 2007	1.68	0.080
Study area		
Weenhayek adults, 2003 n = 41	34.4 (7.7–68.8)	2.565 (0.470–4.655)
Villamontes adult inhabitants, 2004 n = 18	2.2 (1.1–3.7)	0.054 (0.001–0.160)

4.3. Reproductive and developmental outcomes

We performed interviews to quantify the prevalence of reproductive and developmental disorders. The usage of clinical data is generally more accurate but was unfeasible, since the Weenhayek rarely make use of hospitals. Advantages of interviews are a larger study size and greater statistical power than would be achievable with other techniques (Nieuwenhuijsen, 2003). We presumed that the participants were representative for the communities studied, since the women were randomly invited to participate, few refused and a relatively high percentage of the total adult female population in the communities actually participated, i.e. 45% on average.

We have no information on non-response, but since participation was voluntary, selection may have occurred leading to a lower or higher participation of families with disorders, resulting in under- or overestimation of the prevalence in both populations. However, the interviewers and participants were not aware of the outcomes of interest and their definitions, e.g. small families as 0–2 children and delayed walking onset as ≥18 months. Furthermore, we have no reason to assume that the Weenhayek responded differently to our questions than the Wichí. Therefore, we consider it unlikely that our study has a large amount of selection bias or recall bias, implying that our odds ratios were not substantially affected by systematic over- or underreporting. This is corroborated by the fact that we did not find a difference in the prevalence of fetal loss. However, random misclassification may have led to underestimation of the odds ratios and to over- or underestimation of the prevalences of especially fetal loss and congenital anomalies. These are relatively difficult to identify by indigenous people who generally lack formal education and have limited access to detailed health information. This should be kept in mind when comparing our data with prevalence data reported in the literature.

The reported numbers of small families among the Weenhayek and Wichí could not be compared with the literature because other studies mostly use time-to-pregnancy as an indicator for fertility. The prevalences of fetal loss were 17% and 13% among the Weenhayeks and Wichí, respectively, which is roughly within the normal range (10–15%; Rothman et al., 2008). The joined prevalence of hemangiomas and lymphangiomas was 3% for the Wichí which is close to the normal range (1.1–2.7%; Enjolras, 2006; Williams, 2008), while the Weenhayeks showed an increased prevalence (8%). The walking onset of Weenhayek children was delayed compared to what is considered normal (8–18 months; ADAM, 2009), i.e. 45% of the Weenhayek children started walking at the age of 18 months or older. For the Wichí children this was 23%. The fact that the prevalence data for our reference population are in reasonable agreement with literature data supports the validity of our interview methods.

4.4. Interpretation of the results

The increased risk of small families might be related to chronic lead exposure of Weenhayek men, which results in decreased sperm quality and quantity, as well as in alterations of male endocrine function, which can occur at blood lead levels between 40 and 70 μg/dL (Goldman and Hu, 2007). Decreased sperm quality might also be related to cadmium exposure, but the studies that showed an association (Telisman et al., 2000; Akinloye et al., 2006) did not adjust for smoking which is an important route of cadmium exposure (ATSDR, 2008). Hemangiomas and lymphangiomas are possibly caused by the interaction of lead with other teratogenic risk factors (Needleman et al., 1984). Increased risks (1.9–2.7) of these anomalies may occur at relatively low blood lead levels (6.3–24 μg/dL; Needleman et al., 1984). Delayed walking onset has been linked with postural instability (Assaiante, 1998) which can be triggered by lead exposure at blood lead levels of 21 μg/dL

(Bhattacharya et al., 1990), but may also be explained by lower scores on upper-limb speed, dexterity and fine-motor functioning (Dietrich et al., 1993) or by disturbances in other neuromotor functions, such as reaction time and sway oscillations due to lead exposure (Després et al., 2005). Some of these effects have been observed at blood lead levels of <10 μg/dL (Després et al., 2005). It has not been clearly demonstrated that cadmium may cause similar developmental effects (ATSDR, 2008).

We did not find an increased risk of fetal loss, although stillbirths and miscarriages occur at blood lead levels in the same range as sperm alterations (>25 μg/dL; Gardella, 2001). Possible explanations may be lower exposure among women and difficulty recalling these painful events. The Weenhayek could also have been exposed to elevated levels of mercury and arsenic due to mobilization of upstream sediments (Hudson-Edwards et al., 2001). Elevated arsenic exposure is known to have an impact on stillbirths (ATSDR, 2007b) and mercury may have developmental effects (ATSDR, 1999). However, these metals were not evaluated in this study as the laboratory was not adequately equipped to measure inorganic arsenic and methylmercury.

Prenatal care was very rare among both the Wichí and Weenhayek. Although Wichís more often went to hospital to give birth, this could not have influenced fertility or the risk found for delayed walking onset. Differences in nutritional status could lead to different prevalences of certain outcomes, such as motor functioning (Walker et al., 2007). Undernutrition often leads to anemia which in turn could lead to fetal loss. However, since we did not find a difference concerning fetal loss between the Weenhayek and Wichí, we have no reason to assume large differences in the prevalence of undernutrition.

Other causes, not included in this study, that could explain the associations found between small families, congenital malformations and delayed walking onset and being Weenhayek are exposure to pesticides, chemicals in waste water (e.g. nonylphenol), genetic differences between the study populations, and, in the case of fertility, alcohol consumption and lead exposure among Weenhayek men (Muthusami and Chinnaswamy, 2005; ATSDR, 2007a, 2007b), as well as national regulations for family planning. Although we have no reason to assume that these factors are different between the populations studied, we cannot exclude it either. Therefore, we recommend further research into these issues.

4.5. Potential explanations related to elevated exposure

Based upon the estimations of the intake of lead and cadmium, we conclude that it is unlikely that current lead and cadmium exposure levels caused the observed health outcomes. Only children in the worst case scenario, i.e. soil-pica behavior and high consumption levels of fish, exceeded the TDI for lead substantially (i.e., by a factor 1.7). However, lead intake in the past, as estimated for 1997, exceeded the TDI for adults in the average case scenario and the worst case scenario, depending on the lead percentiles assumed, while the TDI for children was generally exceeded in both scenarios. The lead intake in 1997 was most likely even underestimated, because we assumed the same lead levels for soil/dust as in 2006, since we lacked these data for 1997.

The increased prevalences of small families, congenital malformations, and delayed walking onset are possibly related to this historically higher lead exposure, because the health outcomes in our study were collected retrospectively over the period 1985–2006, on average going back 5 years. We hypothesize that the lead levels in hair at the moment of sampling (in 2006), could be elevated through remobilization of lead stored in maternal bone during pregnancy and especially breastfeeding (e.g. Gulson et al., 1998; Ettinger et al., 2004; ATSDR, 2007a, 2007b; JECFA, 2011), which could be a significant lead source for women and their infants. This hypothesis is supported by our findings of elevated hair lead levels in Weenhayek lactants, that were a factor 2.5 higher than in Weenhayek non-lactants and a factor 5.1 higher than in Wichí children.

There is strong consensus that polluted sediment from the mining areas is strongly diluted by "clean" sediment, which results in concentrations that do not substantially deviate from background levels in river sediments downstream of the confluence with the Pilaya river (Hudson-Edwards et al., 2001; Smolders et al., 2003; Macklin et al., 2006). Nevertheless, suspended solids collected during the dry season in Villamontes in 1999 contained very high metal concentrations (Table 6). Although the sediment load was very low (<0.03 g/L), the content of Zn, Cu, Pb and Cd was very high and comparable to levels measured in suspended solids of the highly polluted Tarapaya River (Smolders et al., 2003). This can most likely be explained by long distance transport of disposed tailings in the Potosí area. These very small sized particles (0.3–0.05 mm) are typical products of the flotation method that has been used since 1985 to concentrate the metal-bearing minerals. Interestingly, unfiltered water samples and suspended solids collected in the dry season in 2006 and 2010 revealed lower lead concentrations than samples taken between 1997 and 1999 (Table 6). These notable differences in lead concentrations coincide with the construction of tailing impoundments near the city of Potosí which started to function since 2004 with the aim to collect tailings and effluents from the numerous milling operations along tributaries of the Pilcomayo near Potosí (Halcrow and Serman Asociados, 2006). Miller et al. (2007) suggested that sediment-borne trace metal dispersal may decline in response to lower precipitation. However, precipitation in the upper Pilcomayo was similar in 2003 and 2006 (Miller et al., 2007; SENAMHI, 2012). Therefore, we suggest that the decrease in lead levels in the lower reaches of the Pilcomayo River is probably mainly related to the construction of the tailing impoundments. This decrease also coincides with lower hair lead concentrations in 2006 compared to 2003 and may explain the differences in the estimated lead intake between 1997 en 2006.

5. Conclusions and recommendations

We conclude that we found indications for increased risks of small families, hemangiomas and lymphangiomas on the skin, and delayed walking onset among the Weenhayek communities studied compared to our reference population. Furthermore, these communities showed elevated lead exposure while cadmium levels in hair were not elevated.

Table 6
Mean lead levels in water and suspended solids, in parentheses the range, modified from Smolders and Lanza de Smolders (1998), Smolders et al. (1999, 2003), Smolders (2006) and unpublished data.

Year	Unfiltered water	Filtered water	Suspended solids
	μg/L		μg/g
Before construction tailing impoundments			
1997 (n = 4)	27.0 (21.5–32.5)	1.5 (0.2–2.9)	No data
1998 (n = 5)	35.6 (21.0–62.0)	1.4 (0.2–4.2)	No data
1999 (n = 6)	19.8 (9.3–30.1)	0.7 (0.3–1.2)	1495 (352–3345)
After construction tailing impoundments			
2006 (n = 39–44)	1.7 (0.7–2.7)[a]	0.4 (0.2-1.4)[a]	47 (39–63)[a]
2010 (n = 9)	1.2 (0.6–3.1)	0.9 (0.2–2.3)	36 (20–36)

[a] Median (25th and 75th percentiles in parentheses).

We hypothesize that lead intake via river water, fish consumption and soil/dust in the past could explain these increased risks, which we studied retrospectively. Important exposure pathways may be the mobilization of lead stored in bones for women and the fetus, and lead in breast milk for children, since the lead levels in hair of Weenhayek lactants were the most elevated.

Preliminary health recommendations include calcium supplements for pregnant women, which may reduce absorption and toxic effects of lead (Gulson et al., 1998; Hernandez-Avila et al., 2003) and a shorter period of lactation (currently 2–3 years). It is highly advisable to breastfeed the child the first six months to protect against infectious diseases, especially gastrointestinal infections, which largely contribute to child morbidity and mortality in developing countries (Kramer and Kakuma, 2002; Sguassero, 2008) and to improve motor and cognitive function (Walker et al., 2007). For non-breastfed children of age 6–24 months, the guidelines of the WHO (2005) should be taken into account for adequate nutrition. Further research is desirable to determine whether maternal bone is currently a primary lead source for women and the fetus, whether this exposure can cause the observed disorders and whether a shorter lactation period is associated with an earlier walking onset.

Monitoring of the Pilcomayo and the health situation remains important in the future, since other studies suggest potential impact downstream due to remobilization of stored metals in historic deposits in the upper Pilcomayo and main tributaries, e.g. the Pilaya, which have been studied insufficiently (Hudson-Edwards et al., 2001; Macklin et al., 2006). Apparently, the tailing impoundments functioning since 2004 coincide with lower lead concentrations in unfiltered water samples and scalp hair and lower estimated lead intake by the Weenhayek. Nevertheless, these impoundments also imply important risks, because accidents may result in the release of huge amount of tailings (Macklin et al., 1996, 2006; Hudson-Edwards et al., 2001; Villarroel et al., 2006), which may suddenly deteriorate the health of riverine populations downstream.

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Appendix A. Supplementary data

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