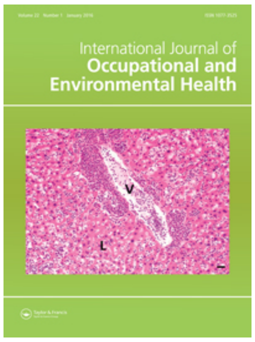

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Mercury and neuromotor function among children in a rural town in Chile

Johan Ohlander[1], Stella Maria Huber[1], Michael Schomaker[2], Christian Heumann[3], Rudolf Schierl[1], Bernhard Michalke[4], Oskar G. Jenni[5], Jon Caflisch[5], Daniel Moraga Muñoz[6,7], Ondine S. von Ehrenstein[8], Katja Radon[1]

[1]Center for International Health Ludwig-Maximilians-University (LMU) at the Institute and Outpatient Clinic for Occupational, Social and Environmental Medicine, University Hospital Munich (LMU), Munich, Germany, [2]Centre for Infectious Disease Epidemiology and Research, University of Cape Town, Cape Town, South Africa, [3]Department for Statistics, Ludwig-Maximilians-University Munich, Munich, Germany, [4]Research Unit Analytical BioGeoChemistry, Helmholtz Center Munich – German Research Center for Environmental Health GmbH, Neuherberg, Germany, [5]Department of Pediatrics, Child Development Center, University Children's Hospital Zürich, Zürich, Switzerland, [6]Facultad de Medicina, Universidad Diego Portales, Santiago, Chile, [7]Past staff of Facultad de Medicina, Latin American Center of the Center for International Health Ludwig-Maximilians-University, Universidad Católica del Norte, Coquimbo, Chile, [8]Department of Community Health Sciences, University of California Los Angeles, Los Angeles, CA, USA

Background: Mercury (Hg) exposure from artisanal gold mining has adverse effects on the neuromotor function in adults. However, few studies have examined this relationship in children.

Objectives: To investigate the impact of Hg exposure on children's neuromotor function.

Methods: Cross-sectional data on Hg risk factors and demographics were collected from n = 288 children (response = 68.9%). Based on complete cases (CCs) (n = 130) and multiple imputations (n = 288), associations between fingernail Hg and four different neuromotor function components were calculated using multiple logistic regression adjusted for confounders.

Results: Of the children, 11.1, 14.9, 63.9, and 10.4% had pathologic pure motor skills, adaptive fine motor skills, adaptive gross motor skills, and static balance, respectively. No significant association between fingernail Hg and any neuromotor component was found. However, Hg burning in the household was significantly associated with children's pathologic pure motor skills (OR 3.07 95% CI 1.03–9.18).

Conclusion: Elemental Hg exposure in the household might have adverse long-term effects on children's pure motor skills.

Keywords: Gold mining, Mercury, Neurotoxicity syndromes, Postural balance, Motor skills, Child development

Introduction

In Chile, small-scale miners often practice artisanal gold mining. Mined ore-bearing stone is ground and mixed with liquid mercury (Hg), heated over a Bunsen burner, producing pure gold and Hg vapors (elemental Hg/Hg0).[1] This process is often performed inside the homes or backyards of miners, exposing the miner, and nearby individuals to Hg vapors. Hg vapors are absorbed into the blood stream and can pass the blood–brain barrier to accumulate in the cerebellum, affecting protein synthesis, destroying membranes, and leading to denaturation of intracellular and cytoskeleton proteins and enzymes.[2]

Elemental Hg is toxic for the nervous system, and may be especially harmful for developing children.[3,4] Apart from a few studies that failed to find any effect of Hg on neuromotor function (e.g. Wastensson et al. 2008) research on adult populations has shown both acute and long-term toxic effects among artisanal gold miners.[5,6] Furthermore, synthesized evidence on the long-term effects of occupational Hg exposure demonstrated adverse neurobehavioral effects on attention, memory, and motor performance.[7–9] Additionally, elevated Hg levels and neurological abnormalities, such as frontal impairment, tremor, or parkinsonism, have been reported in gold miners.[3,10]

Correspondence to: Johan Ohlander, Center for International Health Ludwig-Maximilians-University (LMU) at the Institute and Outpatient Clinic for Occupational, Social and Environmental Medicine, University Hospital Munich (LMU), Munich, Germany. Email: johan.ohlander@med.uni-muenchen.de

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Table 1 Number of measurements. N = 288

Tests completed*	*N* (%)
0 of 3	0 (0)†
1 of 3	66 (22.9)
2 of 3	55 (19.1)
3 of 3	167 (58.0)

*Tests performed: questionnaire survey, fingernail Hg-sampling, and the Zurich neuromotor assessment.
†The study population constituted the 288 children who filled in the questionnaire.

Less is currently known about the potentially adverse effects of Hg on children's neuromotor function and development. Studies involving children have mainly focused on Hg exposure from fish consumption, prenatal Hg exposure, or exposure of pre-school children.[11,12] For example, mothers exposed to MeHg through ingested contaminated marine life had Hg-poisoned fetuses and their children presented psychomotor retardation.[11] In contrast, prenatal Hg exposure was not associated with neuromotor development among Inuit children examined at preschool age, although Hg concentrations at preschool age were associated with Tremor amplitude.[12]

Among the few studies on children's exposure to elemental Hg resulting from Hg burning, an impaired neuromotor function in children aged 9–17 years has been reported.[1] Additionally, Counter *et al*. found negative neurological effects among children in Nambija and Ecuador exposed to elemental Hg.[13] Given the potential risk of neuromotor deficiencies due to Hg vapor exposure, more research on children is needed to quantify effects and to develop corresponding preventive measures. Our previous research reported increased odds of elevated Hg levels among Chilean school children whose parents practice artisanal gold mining.[14] We therefore reinvestigated this child population on potential neuromotor dysfunctions as a result from elemental Hg exposure.

Methods

Design and participants

This study is based on the cross-sectional analyses of data from a child population living in rural Chile, collected in 2008 and 2009. We previously analyzed these data to identify risk factors for elevated Hg values.[14] The target population (n = 432) was recruited from two public schools (covering 83.5% of children in grades 1–6) in a town with approximately 10,000 inhabitants in central Chile. Data were collected using a pre-validated questionnaire assessing potential risk factors and exposure pathways of Hg, a priori confounders, and demographics, administered by teachers during school handed to a total of 418 children (age range 6.0–14.9 years, 14 children had moved or did not attend school regularly). Of the 418 children, 288 (68.9%) completed the questionnaire, 184 (44%) provided fingernail samples, and 205 (49%) completed a standardized test of the neuromotor function. Trained experts, using identical methods, performed both tests in the morning at two schools. Data collection resulted in 130 CCs. Using multiple imputations, 288 cases were used for data analysis. Of the 288 children, 58.0% provided all three types of measurement (questionnaire, ZNA test, fingernail Hg) (Table 1).

This study was approved by the Ethics Committee of the University Hospital Munich (LMU) (Project-No. 399-08) and by the Ethics Committee of the University Católica del Norte in Coquimbo, Chile (Project-No. 04/08). Written informed consent was obtained from the legal guardians of the children and the children provided assent. The principles of the Declaration of Helsinki with its amendment of Somerset West (1996) were considered in designing the study.

Fingernail Hg assessment

Long-term Hg exposure was measured by examining fingernails of the children. The nail sampling was made using stainless steel nail clippers. Samples (n = 184) were weighed into quartz vessels and subjected to digestion with 1 ml HNO3. The vessels then underwent high-pressure digestion for 10 hours at 170 °C. After adding ultrapure water (18.5 MOhm) up to the 10 ml mark, total Hg was analyzed by inductivity coupled plasma sector field mass spectrometry (ICP-sf-MS). For quality control, every tenth measurement, three matrix blank determinations and a control determination of a standard for Hg were included and analyzed with the samples. Results were calculated using a computerized lab data management system that related the measurements to calibration curves, blanks, control standards, and the sample weights. Parents were informed of their children's Hg concentrations and recommendations to limit the Hg exposure were provided to families and local authorities.

The limit of detection (LoD) for Hg determination was calculated as three times the standard deviation (SD) of a blank (3 σ criterion) in measurement solution and resulted in 0.001 μg/L. For relating this value to mass of fingernails, the exact weighed mass per sample was considered for calculation, resulting in a LoD of 0.002 μg Hg/g fingernails.

Neuromotor function assessment

At the two schools, children's neuromotor functions were evaluated using the Zurich Neuromotor Assessment (ZNA).[15,16] The ZNA is a standardized validated test to evaluate neuromotor functions of children aged 5–18 years based on age- and sex-specific norm values. The ZNA test evaluates performance (time) and movement quality (frequency and degree of associated movements). We analyzed the components Z-1, Z-2, Z-3, and Z-4, which correspond to summary measures of different types of timed performance skills: Z-1 measures pure motor skills (repetitive, alternative, and sequential movements), Z-2 adaptive fine motor skills (ability to put pegs in correct place on pegboard using each hand at a time), Z-3 adaptive gross motor skills (dynamic balance, ability to jump over obstacle sideways and forward), and Z-4 static balance (ability to stand on one leg with hands above head). Each respective summary measure for Z1–Z4 was recalculated to a z-score and compared to that of an age- and sex-standardized population. Child performance was expressed in terms of number of SDs from the mean of the standardized population for each component. Further, for each of Z1–Z4, performance was considered "pathologically poor" for *z*-scores that deviated more than two SDs from the mean of the standard population (+2SD). Thus, *z*-scores > +2SD for each ZNA component indicate underdeveloped neuromotor skills compared with the standard population.

Statistical analysis

Due to a relatively high item non-response, a bootstrap-based MI approach implemented with the R package Amelia II (version 1.7–2)[17] was applied, yielding seven imputed datasets with no missing values. The full imputation procedure has been described in detail elsewhere.[14] Amelia II assumes the data to be multivariate normal and missing at random (MAR). Thus, sufficient normality transformations were made prior to imputation (e.g. Hg values were Box–Cox transformed). All statistical analyses were performed with both the incomplete and the imputed data, in the former case with a CC analysis. Descriptive statistics for the imputed results were calculated using Rubin's rules.[18] The ZNA components were, according to the ZNA test-specific pathological cut-off, dichotomized into a pathological (*z*-score > +2SD) and a normal (*z*-score ≤ +2SD) category.

Using the R package Zelig (version 4.2–1), which allows for combined analyses of various imputed datasets, bivariate and adjusted multiple logistic regression models were calculated to model associations between fingernail Hg and the odds of having pathologic neuromotor component *z*-scores, with respect to all four neuromotor component outcomes.[19] A priori confounder adjustments in all four multiple models included: age (years), gender (female vs. male), fish consumption (>4 times per week, 1–4 times per week vs. <1 time per week), mother's contact with Hg during pregnancy (yes vs. no), whether somebody was burning Hg in the household (yes [inside the house or patio] vs. no), and where the child played most frequently (outdoors vs. indoors). As no child had Hg values above 1 μg/g, the exposure variable Hg was multiplied by 100 to enable modeling of the linear outcome response corresponding to a one-unit increase of the Hg variable. Finally, as the children visited two different schools, potential clustering effects on the association between fingernail Hg and neuromotor function were investigated by adding random-effect terms to each of the four multiple regression models.

Results

Pre-imputation, 54% of the total study sample (n_{tot} = 288) was male and the mean age was 9.6 years (SD = 1.9 years) (Table 2). The median Hg level in fingernails was 0.11 μg/g, ranging from 0.02 to 2.36 μg/g. Nineteen percent of children had a father working in traditional gold mining, while more than one in three children were exposed to Hg burning in the home environment. The ZNA test showed that 9.8, 12.2, 63.7, and 6.9% of the children had pathologic (> +2SD) pure motor skills, adaptive fine motor skills, adaptive gross motor skills, and static balance, respectively. Moreover, children whose mother had been in contact with Hg during pregnancy had higher Hg values than children whose mothers were unexposed during pregnancy (Table 3).

The descriptive statistics using imputed data were similar to the descriptive statistics without imputed data (Table 2). The imputed data yielded generally increases in the pathologic values of the neuromotor components: 9.8% vs. 11.3% (pure motor skills), 12.2% vs. 15.0% (adaptive fine motor skills), 63.7% vs. 63.9% (adaptive gross motor skills), and 6.9% vs. 10.4% (static balance). Further changes comprised a minor increase in the percentage of children whose parents worked in an industrial copper mine (17.9% vs. 19.1%), and fewer children whose parents worked outside mining (59.4% vs. 55.3%).

Tables 4 and 5 show the results of the multivariate modeling of the impact of fingernail Hg on all four neuromotor components. The logistic regression showed no statistically significant associations between fingernail Hg and pathologic neuromotor skills with regard to all four ZNA components, respectively. These results were consistent both before and after imputation.

Before imputation, the multiple regression showed a statistically significant association between having somebody burning Hg in the household and pathologic pure motor skills, both in the bivariate (OR 3.05 95% CI 1.10–8.48) and the multiple regression (OR 5.63 95% CI 1.23–25.8) analyses. Having somebody burn Hg in the household was statistically significantly associated with increased odds of having pathologic adaptive gross motor skills in the adjusted model (OR 2.81 95 CI 1.13–7.03). Finally, increasing age was significantly associated with pathologic adaptive gross motor skills, both in the bivariate (OR 1.34 95% CI 1.13–1.56) and the multivariate model (OR 1.45 95% CI 1.14–1.84).

Post-imputation analyses showed a statistically significant association between having somebody burning Hg in the household and increased odds of having pathologic pure motor skills (OR 3.07 95% CI 1.03–9.18); the

Table 2 Pre- and post-imputation descriptives of all study variables. M = mean. SD = standard deviation. Med = median. R = range

	Pre-imputation		Post-imputation (N = 288)*		NA† (%)
Demographics					
Age (years) *M* (SD)	9.59 (1.92)		9.59 (1.92)		0
Gender: *N* (%)					0
Male		156 (54.2)		156 (54.2)	
Female		132 (45.8)		132 (45.8)	
Mother employed: *N* (%)					8.3
No		191 (72.4)		208 (72.2)	
Yes		73 (27.6)		80 (27.8)	
Father employed: *N* (%)					13.5
No		23 (9.2)		28 (9.7)	
Yes		226 (90.8)		260 (90.3)	
Somebody smoking in household: *N* (%)					10.1
No		188 (72.6)		208 (72.2)	
Yes		71 (27.4)		80 (27.8)	
Number of siblings: *N* (%)					5.9
0		26 (9.6)		30 (10.4)	
1–2		170 (62.7)		177 (60.7)	
>2		75 (27.7)		81 (28.9)	
Exposure and exposure pathways:					
Hg (μg/g): Med (*R*):	0.11 (0.02–2.36)		0.10 (0.02–2.38)		36.1
Somebody burning Hg in household: *N* (%)					17.7
No	163 (68.5)		196 (67.9)		
Yes	75 (31.5)		92 (32.1)		
Child playing most frequently: *N* (%)					17.7
Indoors		125 (45.0)		129 (44.6)	
Outdoors		153 (55.0)		159 (55.4)	
Fish consumption: *N* (%)					4.2
<1 times/week		85 (30.8)		90 (31.3)	
1–4 times/week		131 (47.5)		135 (46.9)	
>4 times/week		60 (21.7)		63 (21.8)	
Mother in contact with Hg during pregnancy: *N* (%)					12.5
No		176 (69.8)		196 (68.1)	
Yes		76 (30.2)		92 (31.9)	
Father's occupation: *N* (%)					18.8
Industrial gold mine		9 (3.8)		17 (5.8)	
Industrial copper mine		42 (17.9)		55 (19.1)	
Traditional gold mining		44 (18.8)		57 (19.8)	
Outside mining		139 (59.4)		159 (55.3)	
Time spent indoors: *N* (%)					22.2
<3 h/day		23 (10.3)		34 (11.7)	
3–6 h/day		49 (21.9)		64 (22.2)	
>6 h/day		152 (67.8)		191 (66.2)	
Outcome variables					
Pure motor skills: Med (*R*)	0.44 (−2.84–3.86)		0.49 (−2.88–3.86)		28.8
Fine motor skills: Med (*R*)	0.69 (−1.48–4.31)		0.75 (−1.80–4.31)		28.8
Adaptive gross motor skills: Med (*R*)	2.57 (−1.50–15.2)		2.84 (−2.03–15.2)		28.8
Static balance: Med (*R*)	0.18 (−2.33–3.26)		0.21 (−2.41–3.26)		29.9
Prevalence pathologic values: *N* (%)					
Pure motor skills		20 (9.8)		32 (11.3)	28.8
Fine motor skills		25 (12.2)		43 (15.0)	28.8
Adaptive gross motor skills		130 (63.7)		184 (63.9)	29.2
Static balance		14 (6.9)		30 (10.4)	29.2

*Descriptives for variables post-imputation were calculated using Rubin's rules.
†NA = missing value. Column displays percentage of missing values in variable.

effect was borderline significant in the bivariate analysis (OR 2.60 95% CI 0.98–6.90) (Table 4). Contrary to the pre-imputation model, we found no significant association between Hg burning inside the household and pathologic adaptive gross motor skills (Table 5). Increasing age remained a significant risk factor for pathologic adaptive gross motor skills after imputation, both in the bivariate (OR 1.29 95% CI 1.10–1.51) and the multivariate (OR 1.33 95% CI 1.14–1.57) models.

The mixed-effects model showed a negligible intra-class correlation coefficient, indicating a between-school variance close to zero. Consequently, the results of the mixed-effects model did not differ substantially from those of the logistic regression model (data not shown).

Discussion

To our knowledge, this is one of few studies to examine the effects of elementary Hg exposure due to Hg burning on school children's neuromotor function. We found in our multiple models no statistically significant association between fingernail Hg levels and children's neuromotor function. However, our results suggest increased odds of

Table 3 Mercury levels by selected variables pre-imputation. *N* = 184

	N	%	Hg (μg/g)	*P*-value	%NA*
Age (years)					0
5–7	19	10.3	0.13		
7–9	60	32.6	0.24		
9–11	58	31.5	0.16		
11–13	41	22.4	0.17		
13–15	6	3.2	0.13	0.515	
Gender: *N* (%)					0
Male	92	50.0	0.17		
Female	92	50.0	0.20	0.584	
Fish consumption					3.3
<1 times/week	49	27.5	0.19		
1–4 times/week	92	51.7	0.20		
>4 times/week	37	20.8	0.16	0.46	
Mother in contact with Hg during pregnancy					8.2
No	117	69.2	0.15		
Yes	52	30.8	0.30	0.037	
Somebody burning Hg in household					16.3
No	103	66.9	0.15		
Yes	51	33.1	0.29	0.055	
Child playing most frequently					2.2
Indoors	79	43.9	0.20		
Outdoors	101	56.1	0.18	0.751	

*Column displays percentage of missing values per variable.

having pathologic pure motor skills among children who had somebody burning Hg in the home environment (OR 3.07 95% CI 1.03–9.18). The pure motor skills comprise a child's most fundamental motoric abilities. These are principally controlled by the motor cortex, a cerebral structure in which Hg particularly accumulates. Moreover, in contrast to the adaptive components, the motor skill development is less affected by experience and practice. Thus, our results might be an indication that children exposed to Hg burning in the home environment have pathologic pure motor skills due to an impaired neural substrate.

Nevertheless, as fine motor skills are dependent on the pure motor skills, we also expected a negative effect of elementary Hg exposure on children's fine motor skills, in line with previous findings of Bose-O'Reilly *et al*.[1] In one of few other studies investigating the effect of elementary Hg exposure on the neuromotor function of school children, 166 children aged 9–17 years living in Zimbabwe and Indonesia were examined using the so-called Matchbox test, a measure of fine motor skills.[1, 20] The results found significantly worse fine motor skills among children living in Hg-exposed areas and among children who worked with Hg, when compared to the non-exposed control group. Our findings however showed no significant associations between fingernail Hg and pathologic fine motor skills (OR 1.00 95% CI 0.99–1.02). The disparity in these findings might be partly explained by the different neuromotor tests (ZNA vs. Matchbox test) and Hg-sampling methods used (fingernail vs. blood, urine, and hair samples).

The variable 'Having somebody burning Hg in the household' was used as a proxy for elementary Hg exposure. Compared with fingernail Hg, this proxy variable showed a stronger and statistically significant association with children's neuromotor function. One possible reason might be that the proxy better captures long-term Hg exposure, as Hg burning in affected children's homes might occur continuously over a long period of time. As the pure motor skills are present at a very early period in childhood (prenatal phase – approximately three years), our reported negative impact of Hg exposure on children's pure motor skills might indicate that Hg exposure in early life has a higher impact on a child's neuromotoric development, compared with exposure during later phases when a child's brain is more developed.

Fingernail sampling, a point measure, does not capture Hg exposure during the prenatal phase. Despite its reliability as a long-term marker of Hg, it is limited to the growth cycle of children's nails.[21] Despite this natural limitation of fingernail sampling, a correlation of 0.52 was found between our fingernail Hg measurements and urinary Hg measurements, the latter made in a sub-sample of n = 23 children who participated in this study and a subsequent study of children (N = 174) in 2010 at the same two schools (authors' unpublished data). This increases the validity of our fingernail Hg measures and lowers the risk of exposure misclassification. It is thus possible that the absence of any effect between Hg levels and neuromotor function is a result of a low study power to detect small differences, despite the existence of an association. Nevertheless, blood sampling remains the gold standard of Hg biomonitoring and should thus be applied when possible. Due to logistic reasons, this method was not feasible in this study population. We therefore decided to perform the more feasible and less invasive fingernail sampling.

Although unrelated to fingernail Hg or any other Hg proxy, we found that 63.2% of children showed pathologic adaptive gross motor skills. The adaptive gross motor skills include adaptive dynamic balance, which is influenced by physical activity. Thus, the high prevalence of children with pathologic adaptive gross motor skills may

Table 4 Associations between mercury exposure and pathologic Z-1 (pure motor skills) and Z-2 (fine motor skills) values, respectively. Unadjusted and adjusted (pre- and post-imputation) odds ratios (OR) with 95% confidence intervals (CI). N=288

	Pure motor skills	Pre-imputation		Post-imputation*		Fine motor skills	Pre-imputation		Post-imputation*	
	N (%) > Z-1 pathologic cut-off (N=32)†	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	N (%) > Z-2 pathologic cut-off (N=43)†	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Age (years)	.	1.19 (0.94-1.50)	1.36 (0.92-2.02)	1.17 (0.94-1.46)	1.18 (0.94-1.50)		1.20 (0.97-1.49)	1.01 (0.72-1.42)	1.15 (0.95-1.39)	1.17 (0.96-1.43)
Gender:										
Male	21 (65.6)	1	1	1	1	30 (69.8)	1	1	1	1
Female	11 (34.4)	0.48 (0.18-1.31)	0.58 (0.12-2.83)	0.61 (0.19-1.93)	0.64 (0.19-2.15)	13 (30.2)	0.43 (0.17-1.07)	0.37 (0.10-6.88)	0.48 (0.23-1.01)	0.46 (0.21-1.03)
Hg (100*µg/g)‡	.	0.97 (0.91-1.04)	0.95 (0.85-1.06)	0.99 (0.96-1.03)	1.00 (0.96-1.03)		1.00 (0.99-1.02)	1.01 (0.99-1.02)	1.00 (0.99-1.01)	1.00 (0.99-1.02)
Fish consumption:	13 (40.6)	1	1	1	1	13 (30.2)	1	1	1	1
<1 times/week										
1-4 times/week	13 (40.6)	0.41 (0.14-1.17)	0.28 (0.05-1.52)	0.75 (0.32-1.78)	0.74 (0.30-1.82)	23 (53.5)	1.02 (0.40-2.61)	1.62 (0.38-6.89)	0.78 (0.34-1.81)	0.80 (0.34-1.89)
>4 times/week	6 (18.8)	0.53 (0.15-1.86)	0.60 (0.09-4.18)	1.20 (0.55-2.64)	1.19 (0.54-2.64)	7 (16.3)	0.45 (0.11-1.80)	1.21 (0.18-8.13)	0.75 (0.37-1.54)	0.72 (0.34-1.52)
Mother in contact with Hg during pregnancy:										
No	21 (65.6)	1	1	1	1	31 (72.1)	1	1	1	1
Yes	11 (34.4)	0.92 (0.33-2.53)	0.31 (0.05-2.06)	1.00 (0.40-2.53)	0.69 (0.20-2.40)	12 (27.9)	0.86 (0.33-2.21)	0.94 (0.22-4.06)	0.75 (0.29-1.92)	0.87 (0.29-2.62)
Somebody burning Hg in household:										
No	15 (46.9)	1	1	1	1	33 (76.7)	1	1	1	1
Yes	17 (53.1)	3.05 (1.10-8.48)	5.63 (1.23-25.8)	2.60 (0.98-6.90)	3.07 (1.03-9.18)	10 (23.3)	0.80 (0.29-2.18)	0.70 (0.17-2.81)	0.62 (0.20-1.95)	0.58 (0.16-2.17)
Child playing most frequently:										
Indoors	10 (31.2)	1	1	1	1	17 (39.5)	1	1	1	1
Outdoors	22 (68.8)	2.25 (0.78-6.50)	2.12 (0.33-13.5)	1.81 (0.77-4.28)	1.44 (0.58-3.59)	26 (60.5)	1.58 (0.64-3.87)	0.74 (0.23-2.45)	1.21 (0.54-2.70)	1.36 (0.44-4.23)

*Post-imputation unadjusted and adjusted (for all variables in table) odds ratios based on all seven imputed datasets combined.
†Calculated post-imputation.
‡As no child had Hg values above 1 µg/g, the Hg variable was multiplied by 100 to enable modeling of the linear outcome response corresponding to a one-unit increase of the Hg variable.

Table 5 Associations between mercury exposure and pathologic Z-3 (adaptive gross motor skills) and Z-4 (static balance) values, respectively. Unadjusted and adjusted (pre- and post-imputation) odds ratios (OR) with 95% confidence intervals (CI). N=288

	Adaptive gross motor skills	Pre-imputation		Post-imputation*		Static balance	Pre-imputation		Post-imputation*	
	N (%) > Z-3 pathologic cut-off (N=184)†	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	N (%) > Z-4 pathologic cut-off (N=30)†	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Age (years)	.	1.34 (1.13-1.56)	1.45 (1.14-1.84)	1.29 (1.10-1.51)	1.33 (1.14-1.57)	.	0.99 (0.75-1.31)	0.84 (0.53-1.34)	0.96 (0.75-1.23)	1.00 (0.75-1.32)
Gender:										
Male	101 (54.9)	1	1	1	1	13 (43.3)	1	1	1	1
Female	83 (45.1)	1.07 (0.60-1.89)	0.93 (0.42-2.06)	0.93 (0.52-1.67)	0.86 (0.46-1.58)	17 (56.7)	0.87 (0.29-2.61)	0.47 (0.10-2.11)	1.00 (0.41-2.42)	0.81 (0.23-2.90)
Hg (100*µg/g)‡	.	1.00 (0.99-1.01)	1.00 (0.99-1.02)	1.00 (0.99-1.01)	1.00 (0.99-1.02)	.	0.98 (0.92-1.04)	0.95 (0.87-1.04)	0.97 (0.92-1.03)	0.97 (0.92-1.03)
Fish consumption:										
<1 times/week	53 (28.8)	1	1	1	1	7 (23.3)	1	1	1	1
1-4 times/week	90 (48.9)	1.44 (0.73-2.84)	1.40 (0.55-3.56)	1.20 (0.71-2.04)	1.32 (0.76-2.30)	15 (50.0)	2.06 (0.41-10.3)	2.80 (0.27-28.5)	1.65 (0.66-4.11)	1.83 (0.72-4.66)
>4 times/week	41 (22.3)	1.22 (0.54-2.75)	0.87 (0.28-2.69)	0.83 (0.49-1.39)	0.83 (0.48-1.42)	8 (26.7)	3.40 (0.63-18.4)	11.1 (0.99-124.2)	0.94 (0.45-1.95)	0.89 (0.44-1.80)
Mother in contact with Hg during pregnancy:										
No	125 (67.9)	1	1	1	1	22 (73.3)	1	1	1	1
Yes	59 (32.1)	1.13 (0.59-2.13)	0.87 (0.35-2.20)	1.03 (0.60-1.80)	0.82 (0.45-1.50)	8 (26.67)	0.86 (0.25-2.91)	1.08 (0.16-7.38)	0.68 (0.22-2.07)	1.11 (0.24-5.07)
Somebody burning Hg in the household:										
No	120 (65.2)	1	1	1	1	4 (13.3)	1	1	1	1
Yes	64 (34.8)	1.47 (0.76-2.85)	2.81 (1.13-7.03)	1.43 (0.68-3.00)	1.53 (0.71-3.28)	26 (86.7)	0.15 (0.02-1.15)	0.16 (0.02-1.75)	0.68 (0.22-2.07)	0.31 (0.07-1.35)
Child playing most frequently:										
Indoors	83 (45.1)	1	1	1	1	16 (53.3)	1	1	1	1
Outdoors	101 (54.9)	0.97 (0.54-1.73)	0.93 (0.42-2.07)	0.90 (0.53-1.50)	0.94 (0.53-1.66)	14 (46.7)	0.55 (0.18-1.66)	0.32 (0.07-1.44)	0.65 (0.09-4.84)	0.66 (0.25-1.71)

*Post-imputation unadjusted and adjusted (for all variables in table) odds ratios based on all seven imputed datasets combined.
†Calculated post-imputation.
‡As no child had Hg values above 1 µg/g, the Hg variable was multiplied by 100 to enable modeling of the linear outcome response corresponding to a one-unit increase of the Hg variable.

partly reflect an underdeveloped dynamic balance due to low physical activity; affected children might be obliged to help their parents in their occupational activities, thus reducing their opportunities for physical activities related to school and leisure time. The high prevalence of children with pathologic gross motor skills might also be explained by a comparatively high BMI in these children, and corresponding reduced balance, potentially driven by mentioned physical inactivity. A follow-up study of our children showed a significant association between pathologic gross motor skills and high BMI (authors' unpublished data). As we lacked information on children's physical activity and BMI, we could not investigate this hypothesis further.

Study strengths include the ability to examine children in public schools highly representative of the study area (covering 83.5% of children in grades 1–6). This provided easy access to a representative and heterogeneous study group including children whose families carry out artisanal mining. Moreover, the study response of 68.9% can partly be traced back to the strong support of stakeholders in the community involved, such as the town mayor, community groups, principals, teachers, and staff of the schools. Finally, children's neuromotor skills were assessed using a standardized test, and elementary Hg was obtained through biomotoring.

Being a cross-sectional study, we cannot demonstrate causality. Furthermore, as Hg use was based on parental self-report, it is possible that the use of Hg in the home environment was occasionally misreported. This might have led to a misclassification of exposure and likely underestimated the detrimental effect of Hg on children's neuromotor development. Further, not all children were able to participate in the Hg-sampling and the neuromotor test, respectively. The relatively low response regarding fingernail sampling (44%) and the neuromotor test (49%) partly results from a lack of parental concern and understanding of long-term benefits of the study, potentially resulting from a relatively low degree of schooling. Moreover, children with severe neurologic sequalae probably did not attend school and were therefore excluded from the study. As children with severe neurologic sequalae potentially have comparatively high exposures of Hg, we might have underestimated presented associations between Hg and the ZNA components. Moreover, non-responders of the Hg sampling tended to be more frequently female (50.0% vs. 38.5%, $\chi^2 = 0.04$), and non-responders of the ZNA test had slightly higher Hg levels ($M = 0.19$ ug/g vs. 0.12 ug/g, P Anova = 0.37) (not reported in tables). However, regarding the participation of the Hg fingernail sampling and the ZNA test, we found no statistically significant difference between participating and non-participating children regarding the different risk factors of the ZNA components included in the multivariate model. Hence, non-participation should not have biased our reported associations. Due to logistic reasons, we were unable to measure Hg concentrations in indoor air. Nevertheless, a follow-up study on a sub-sample of our children using urinary Hg, showed no association between Hg and neuromotor functions. Finally, the influence of socioeconomic status (SES) on the ZNA test is unknown. As our reference population from Switzerland presumably has a higher SES than our studied children, which might be beneficial for their neuromotoric development, we may have slightly overestimated the prevalence of pathologic children in our study. Nevertheless, such potential bias should not have influenced our logistic regression results, as these are based on internal comparison of children with and without somebody burning Hg in the household.

Finally, we had no information on children's number of amalgam fillings. However, no significant impact between the number of amalgam fillings and Hg concentration in Ecuadorian gold miners was found, neither have any adverse neuropsychological or neurobehavioral effects due to dental amalgam fillings been found in clinical trials.[3,22,23]

Our analysis was based on MI, which today is a standard approach in epidemiology as the loss of power and the error introduced by excluding cases with missing values is considered a major bias.[24] Using MI, we increased our relatively small sample size, resulting in higher statistical power. Our pre- and post-imputation odds ratio estimates were not meaningfully different. The observed difference is explained by the difference in statistical power, and by the different assumptions the CC respective the MI analysis requires met. In fact, the more frequently used CC analysis requires more assumptions met to yield unbiased estimates than does the MI (MCAR respective MAR). Although the MAR assumption cannot be tested, it is the most common situation in epidemiological research.[25] Thus, the imputed estimates should be more reliable than those yielded by the CC analysis.

In this study, we show that elementary Hg exposure from Hg burning in the household might have long-term detrimental effects on children's pure motor skills. Thus, our results add to the existing body of evidence indicating that Hg amalgamation for extracting gold is a non-sustainable method, seen from an occupational health perspective. However, far less harmful methods for extracting gold exist today. The Borax method, for example, is a very promising nontoxic Hg-free alternative, and was found to be feasible and popular among small-scale miners in Zimbabwe.[26] To protect workers' and indirectly exposed children's health, we encourage regional authorities to investigate whether local conditions satisfy the implementation of Hg-free gold extraction such as the Borax method. Additionally, focus groups and teaching interventions could be developed by means of community based approaches directed toward protecting children in communities where Hg burning is common.

Conflict of interest

The authors declare that they have no conflict of interest.

Authorship specification

JO analyzed the data and wrote the manuscript. SMH, DMM, and KJ planned and executed the study. MS and CM provided expert knowledge in the statistical analysis. RS performed the Hg analysis of the fingernail samples. OGJ and JC performed the neuromotor function test and analysis. All authors have read and contributed to the writing of this manuscript.

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