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Exposure to Manganese: Health Effects on the General Population, a Pilot Study in Central Mexico

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To support a risk assessment of manganese exposure in two communities living within a manganese mining district a cross-sectional study was performed on a sample of the adult population of long-term residents. One community was exposed to a point source from an ore primary refining plant. Manganese is an essential mineral for human life. It is also the fourth in importance for industrial metal making. Data were collected on socioeconomic living conditions, emission sources, environmental media concentrations (air, water, soil, dust, food), respiratory symptomatology, and a neuropsychological examination (Mini-Mental Screening test, the Hooper Visual Organization test, the Ardila-Ostroski, and others). We examined 73 subjects (52 women), most of low socioeconomic status. Environmental air concentrations were 2 to 3 times higher than those in other urban concentrations. Manganese blood concentrations ranged from 7.5 to 88 μg/L, with a median concentration of 15, the upper quartile starting at 20 μg/L; the upper 10% was above 25 μg/L. Lead and manganese were highly correlated; there was an inverse relation to hemoglobin. Reduced levels of plasma lipid peroxidation were associated with blood manganese. Using multivariate logistic regression, we identified B-Mn as increasing the risk of deficient cognitive performance 12 times (Mini-Mental score of less than 17).

Key Words: **manganese; exposure assessment; cross-sectional; lipid peroxidation; neuropsychologic.**

BACKGROUND AND RATIONALE

Central Mexico has one of the largest manganese Mn^2 ore reserves in the Americas, with 32 million tons of proven reserves (1), mainly of manganese dioxide. Its commercial extraction was started in 1962 in a mining district with several agriculturally based communities and a very stable population representing a lifetime exposure to naturally and anthropogenically released manganese. Extraction is performed through both strip and underground mining, and primary refining is done on site. The local government commissioned a Manganese Exposure Risk Characterization project to address the community concerns about the ecological impact of mining activities and their health effects on the population.

Manganese (CAS-7439-96-5), found in its natural state in the earth's crust, is an essential mineral important to mitochondrial oxidative processes (2). It is a gray metal that blackens when oxidized, and its Mn^{2+} oxidation form is the most bioavailable. Greater absorption happens in acidic media and with oxidized manganese. Its bioconcentration is highest in the lower food chain. It is also used in nutritional supplements and multivitamin preparations. Its toxicological profile is being updated (2). World production has increased by 30-fold throughout this century due to industrial use. Its use is

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[2]Abbreviations used: ATSDR, Agency for Toxic Substances and Disease Registry, U.S.A.; B-Mn, blood manganese; B-Pb, blood lead; CAS, Chemical Abstract Series; EPA, Environmental Protection Agency, U.S.A.; LP, lipid peroxidation; MAO, monoamine oxidase; MMT, methylcyclopentadienyl manganese tricarbonyl; Mn, manganese; Mn^{2+}, manganese oxide; MRC, Medical Research Council; PM_{10}, particulate matter of less than 10 μm; Si, silica; SO_2, sulfur dioxide.

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widespread and it is the fourth most important mineral in the world for industrial metal making (3).

Scientific concern has arisen due to its widespread use in ceramics, the manufacture of matches, glass, soldering, metal alloys, batteries, aluminum cans, electronic components, and, for over 20 years, in gasoline additive compounds such as methylcyclopentadienyl manganese tricarbonyl (MMT). The reevaluation of Mn health risks by the EPA in 1994 led to the resolution to delay the decision on accepting Mn as a gasoline additive in unleaded gasoline, given the lack of better empirical data (4). Chronic human exposure and effects data, the absorption efficacy of the different exposure routes, and the shape of the dose–response—if any—are some of the key, biologically important questions. The discussions during a recent international symposium (5) highlighted the need for human exposure assessment and effect biomarkers studies.

For the present study we selected two biomarkers of the biochemical effect of manganese, on the basis of previous studies in manganese-exposed workers: platelet monoamine oxidase activity (MAO) (6) and plasma lipid peroxidation (7). There are also experimental reports in animals showing alterations in both brain and liver mitochondrial activity of MAO (8) and brain lipid peroxidation (9). Consistently, the most important health end point is the neuropsychologycal, including the cognitive, motor and sensory areas; for this pilot we selected a battery of tests adapted for Mexican rural conditions (10–17) that reliably and practicably could be done on the site.

We developed this study in collaboration with local health authorities. The purpose was to gather local exposure and effect information in order to complement the risk assessment. Furthermore, this study constituted a first approach to the understanding of local health conditions as related to the extraction and primary refining of manganese.

METHODS

We performed a cross-sectional study of two communities in the manganese-mining district. These communities were selected through a prioritization algorithm and consultation with a State Interinstitutional Commission. Community A was 2 km from the primary ore refining plant, residing in the uphill area surrounding the plant; the census data enumerated 1257 inhabitants. Community B was 25 km downhill and downstream from the point source; census data enumerated 692 inhabitants. The survey was accomplished throughout the months of the dry winter season. After dividing Community A into four sectors, we obtained a probabilistic stratified random sample without replacement of households and recruited one volunteer per household. Each volunteer was asked for an informed consent for his participation. Local medical services, including community-based health promoters, from the Ministry of Health and the Social Security provided mapping, morbidity and mortality information, and community access and were heavily involved in the project.

Several instruments were developed, including an informed consent form, a sociodemographic questionnaire (housing, food intake, occupation, risk perception), and a modified Chronic Bronchitis questionnaire that we adapted and previously tested (18–20) for Mexico from the British MRC questionnaire. Standardized and trained personnel applied the questionnaires to 46 subjects in Community A and 27 subjects in Community B, including a total of 21 men and 52 women.

Neuropsychological Examination

In order to address the neuropsychologic evaluation, a team of trained psychologists successfully applied to 44 and 27 subjects in Communities A (two subjects failed to perform the tests adequately) and B a test battery. We used the neuropsychological scheme (10). It consisted of timed tasks assessing different aspects of motor behavior. It contained 41 items that examine hand and bilateral motor speed and coordination, the ability to imitate motor movements, symbolic actions, conflictive reactions, and choice reactions. The items were scored, according to the manual, as follows: 0, within the normal range, 1, mild impairment, 2, severe impairment. This test was adapted to Spanish and the normal values were those described by Ardila and Ostroski (11). The Hooper visual organization test (HVOT) was also applied. The HVOT was developed to identify those patients with organic brain conditions in psychiatric hospitals. Thirty or more pictures of readily recognizable cut up objects make up the test. The subject's task is to name each object verbally when the test is individually administered; the HVOT proved to be very sensitive to dementia and disease duration in Parkinson's patients (12). The score is the total number of correct answers. Attention and concentration functions were monitored with the Trail Making Test (13), which assesses visual conceptual and visomotor tracking and it is administered in two parts. Trail Making requires the subjects to connect sequential numbers placed in circles randomly distributed on a plane. Trail making B, which includes

symbols set shifting, provides a measure of cognitive flexibility and requires the subjects to alternate from one number to one letter (1-A-2-B-3-C ...). The subject is urged to connect the circles "as fast as you can," without lifting the pencil from the paper. This is a test of complex visual scanning with a motor component. The digit span test, also applied, provided a measure of metal tracking and sustained attention (14). The subjects are asked to immediately recall a progressively increasing series of digits read by the examiner (minimum three, maximum nine). The digit span backward requires the subject to repeat the digits in reverse order (minimum two, maximum eight).

Verbal fluency was measured by the quantity of words produced in a restricted category (e.g., animals) or the words according to an initial letter (F, A, S) and usually within a time limit (1 min).

Cognitive function was assessed with the Mini-Mental State Examination. This formalized mental status examination is widely used as a brief screening instrument for dementia. Administration takes from 5 to 10 min. The standardized administration and scoring procedures are easily learned. Scores below 17 (adapted for the rural Mexican population) are considered abnormal (15–17). The time taken to administer the battery was 2.5 h. The same test was administered by the same person.

Biomarkers and Clinical Data

Blood samples of 40 ml were obtained by venous puncture in 46 and 27 subjects in Communities A and B and after previous skin cleansing following skin cleansing with a non-ionic detergent and rinsing with deionized water. The samples were then refrigerated before being transported, during transportation, and in the clinical laboratory. Blood was stored in Vacutainer tubes with EDTA as an anticoagulant until assayed (21). Besides blood manganese (B-Mn), blood lead (B-Pb), and hemoglobin (hb), we used plasma lipid peroxidiation (LP) and monoamine oxidase (MAO) as early effects biomarkers (7, 22, 23). All sampling materials were prewashed with 3% nitric acid. A subset of 20 and 12 volunteers from communities A and B were taken to a regional clinic for X-ray examination and classified with either fibrosis or nodulation or both by a certified medical pulmonary radiologist.

Environmental Samples

Environmental samples were obtained in the communities of each of the following media; wells at a depth of 1.5 to 2 m ($n = 14$); approximately 500 g. of soil samples dug from the bottom of a 0.18-cm^3 pothole ($n = 10$). Household PM_{10} was collected for 24 h from within the central room of 10 houses. A 5-day, 24-h PM_{10} outdoor air sample was collected with a PM_{10} High Volume sampler installed at the Community A church and on the Community B medical unit roof. River samples were obtained in Community B, since Community A had no river. Samples were obtained at five different points along the river at a depth less than 1 m in order not to disturb the sediment. Limited time and resources did not allow for individual subject personal sampling. Individual exposures were assigned by weighing each subject by its home distance to the closer monitoring site.

Lead and Manganese in Blood Analyses

Both lead and manganese in blood were analyzed by graphite furnace atomic absorption spectrophotometer (GFAAS), according to techniques previously reported (24, 25). A Perkin–Elmer 3110 atomic absorption spectrophotometer and an HGA-600 graphite furnace with AS-60 autosampler were used. Calibration curves for lead were constructed using an aqueous lead reference standard (NBS-3128, NBS Gaithesburg, MD). For manganese, curves were constructed with a Merck Titrisol standard solution. Quality control for lead analysis was assessed by our current participation in the lead Wisconsin State Laboratory of Hygiene proficiency program. Analyzing an Eastman-Kodak gelatin standard material (LabLEADER) consituted the internal quality control for manganese.

Analysis of Manganese in Food Samples

Ready-to-eat food samples were requested in a random subsample of 20 and 12 households from Communities A and B and refrigerated. Food samples were stored in polypropylene containers that were previously washed with 3% (v/v) HNO^3, rinsed with deionized water, and nitrogen gas-dried to avoid external contamination. The food was homogenized with a food processor with a stainless steel grinder. A sample of 1 g was carefully weighed and submitted to acid digestion using Suprapur (E. Merck) HNO_3 in a water bath at 60°C for 30 min. The clear solution was diluted with deionized water, and manganese was analyzed by GFAAS.

Platelet Monoamine Oxidase Activity

Platelets were isolated according to Mustard (21). The activity of monoamine oxidase was obtained by a technique described by Krajl (26), using a Perkin–Elmer LS100 luminescence spectrophotometer. Isolated platelets were resuspended in 780 μl of albumin-free Tirode and 200 μl of this resuspension was incubated for 1 h at 37°C, in a solution containing 250 μl of 0.5 M phosphate buffer (pH 7.4) + 500 μl of 0.6 M kynuramine + 500 μl of water. After incubation, 1 mL of 10% (w/v) trichloroacetic acid was supplemented and samples were centrifuged at 1500 rpm. Twenty-microliter aliquots from the supernatants were supplemented with 1 ml of 1 M Na OH. Florescence was determined at 315-nm excitation and 380-nm emission wavelengths. A calibration curve was constructed using 4-hydroxyquinoline as a standard. Results were reported as nmol of 4-hydroxyquinoline formation/h/mg of protein.

Plasma Lipid Peroxidation

Plasma was separated from blood and the monitoring of lipid peroxidation was performed by measuring lipid fluorescent product formation according to Lunec and Domardy (27), within the 6 h after sample withdrawal; 500 μl of the plasma samples was separated in a glass tube covered from light and supplemented with 4 ml of 2:1 (v/v) chloroform–methanol mixture. Tubes were capped and vortex-mixed for 2 min. The samples were centrifuged at 10,000 rpm for 10 min and the aqueous phase was discarded; 2.5 ml of the organic phase were transferred to clean glass tubes and vortexed with 1 ml of deionized water. Tubes were placed on ice for 15 min; 500 μl of the chloroformic phase was transferred into a quartz cuvette and mixed with 0.1 ml of methanol. Fluorescence was measured in a Perkin–Elmer LS50B Fluorescence Spectrophotometer at 370 nm of excitation and 430 nm of emission. Prior to the measurement of the samples, the sensitivity of the spectrophotometer was adjusted to 140 fluorescence units with a 0.1 mg/L quinine standard, prepared in 0.05 M sulfuric acid. Results were expressed as fluorescence units per milliliter of plasma. All samples were run in duplicate.

Data Management and Analysis

Data formats and questionnaires were checked on site and coded according to the coding manual identification. Data were entered into a database and their management procedures were documented, ready for auditing. Data were analyzed using Stata 5.0 software (28), storing all analysis procedure logs. We developed exploratory data analysis in order to check data, as well as to transform continuous variables into a near Gaussian distribution to assure nonviolation of assumptions of parametric analysis. When necessary, we used distribution- and reference-independent categorization, dichotomizing either at the median or with different centiles. Several indices (from the sociodemographic questionnaire) were constructed in order to group covariates related to indoor and outdoor emissions, ventilation and socioeconomic level. The indoor emission index considered all dust-related sources, including wood smoke, a household with uncovered soil, indoor water, and area heaters; the outdoor emission index included proximity to the industrial plant, road traffic, trash, and industrial residues accumulation. Bivariate analyses were first done in tabular form, selecting candidate variables for the models; depending on the distribution and types of variables tabular data were tested with χ^2 or using t tests to compare group means with unequal variances. One-tailed tests were used, as the hypothesis was of excess risk from manganese exposure and therefore used 90% confidence interval estimation. Multivariate modeling was done through least squares linear regression (29) when the dependent variable was continuous. We calculated prevalence risk ratios based on the regression coefficients when they were required. We built dummy variables for those categorical ones and used robust regression when we observed distribution restrictions in the covariates. We used logistic regression for dichotomic dependent variables and multinomial regression for nominal dependent variables. When confronting serially correlated residuals, we used Hildreth–Lu regression (30).

RESULTS

As seen in Table 1, most of the study subjects were women, housewives within an agricultural community, and poorly educated. On average they were adults (ranging from 14 to 93 years of age, 90% of them within an age range of 16 to 80 years), long-term residents with poor housing and sanitary conditions. Many use lead-glazed pottery for cooking and consume local foods. Both communities are similar in their socioeconomic and housing conditions, with fewer years of schooling in Community B, where the residents have lived somewhat longer

TABLE 1
Descriptive Statistics of the Study Population Characteristics

Variable	Category	Total	Community A	Community B
Population size		73	46	27
Sex	Men	21	14	7
	Women	52	32	20
Occupation	Miner	0	0	0
	Student	1	1	0
	Agriculture	14	7	7
	House	48	29	19
	Laborer	3	3	0
	Commerce	1	1	0
	Other	1	1	0
Any schooling	Yes	377	23	14
	No	36	23	13
	Average years	2.15 (SD, 3.03)	2.37 (SD, 3.36)	1.81 (SD, 2.48)
Average age		43.35 (SD, 17.43), range 14 to 93	41.43 (SD, 18.36), range 15 to 93	46.46 (SD, 15.67), range 14 to 75
Average years of residency at the home		19.10 (SD, 16.60), range 1 to 88	17.67 (SD, 17.23), range 2 to 88	21.58 (SD, 15.46), range 1 to 75
Indices	Socioeconomic (2–5)	2.57 (SD, 0.80)	2.65 (SD, 0.86)	2.42 (SD, 0.70)
	Internal emissions (0–4)	1.87 (SD, 0.88)	1.57 (SD, 0.84)*	2.41 (SD, 0.69)
	External emissions (1–8)	3.48 (SD, 1.94)	3.63 (SD, 2.14)	3.22 (SD, 1.55)
	Ventilation (1–11)	7.24 (SD, 2.80)	6.74 (SD, 2.82)	7.79 (SD, 2.69)
Household water supply		16	13	3
Drainage		34	22	12
Household rooms	1	2	2	0
	2	25	14	11
	3	30	19	11
	4 +	16	11	5
Exposure covariates	Glazed pottery cooking	53	31	22
	Eat locally grown food	73	46	27
	Perceived health risk	38	25	13
	Perceived environmental risks	56	40*	16
	Use pesticides	33	19	14
	Frequent alcohol consumption	11	7	14

Note. SD, standard deviation.
*$P < 0.05$ by t test.

than those in Community A. However, the second community had lower external emission sources, a higher internal emission index, and, because of a warmer local temperature, a higher ventilation index. Table 2 shows that average blood hemoglobin was under clinically normal levels, 13.18 with a range of 9.84 to 16.5, and with significantly lower levels in women than in men; lead levels (B-Pb) were 11.00, ranging from the lowest detected of 2.5 to 31 μg per deciliter. B-Mn ranged from 7.5 to 88 μg /L, with a median concentration of 15, the upper quartile starting at 20 μg /L; the upper 10% was above 25 μg/L. In Community A, B-Mn, MAO, and Hb were higher and B-Pb and plasma lipid peroxidation were lower than those in Community B. Table 3 displays the results of the environmental monitoring. River manganese at Community B showed a trend up to downstream. In Community A compared to B, manganese levels in food, soil, and well water were lower; total suspended particles, outdoor air, and indoor dust manganese were 2.7, 1.1, 3, and 3.14 times higher. The distribution of B-Mn by sector is shown in Fig. 1, depicting a lower and less variable concentration in the farther sectors. B-Mn geometric means in Community A (sectors 1 to 4) showed a border significant trend (slope, -0.09, $P = 0.10$) with distance from the source (sector 1, the closest to the point source of emission, a mean of 16.8, sector

TABLE 2
Descriptive Statistics of Biomarkers by Community

	Units	*n*	Mean (SD)	Minimum	Maximum
Total					
Blood Mn	μg/L	73	17.71 (11.99)	7.50	88.00
Blood Pb	μg/dl	73	11.00 (5.86)	2.5	31.00
Plasma lipid peroxidation	F.U.[a]	73	8.73 (11.52)	n.d.	88.00
MAO	nmol[b]	73	64.96 (29.82)	13.92	182.05
Hemoglobin	mg/100 mL	72	13.18 (1.40)	9.84	16.5
Community A					
Blood Mn	μg/l	46	18.26 (13.92)	10.00	88.00
Blood Pb	μg/dl	46	9.54 (5.06)	2.5	21.00
Plasma lipid peroxidation	F.U.[a]	46	4.64 (4.90)	n.d.	26.49
MAO	nmol[b]	46	73.45 (30.93)	31.06	182.05
Hemoglobin	mg/100 ml	45	13.67 (1.23)	10.91	16.50
Community B					
Blood Mn	μg/L	27	16.76 (7.81)	7.50	45.00
Blood Pb	μg/dl	27	13.48 (6.38)	3.00	31.00
Plasma lipid peroxidation	F.U.[a]	27	15.70 (15.67)	2.29	88.00
MAO	nmol [b]	27	50.51 (21.50)	13.92	88.00
Hemoglobin	mg/100 ml	27	12.36 (1.29)	9.84	15.47

Note. SD, standard deviation; n.d., not detectable (given a value of 0.00).
[a] F.U., Fluorescence units.
[b] MAO refers to platelet monoamine oxidase activity and is expressed in nanomols of 4-HOQ formed per hour per protein milligram.

2 with 15.6, sector 3 with 14.2, and sector 4, the farthest, with 16.5). Six cases of tremor and/or numbness were identified in Community B. All of them were in the age range of 46 to 56 years (average 50.2), two of them being males. Their B-Mn levels were in the range of 10 to 45 μg/L; compared with the remaining of 67 subjects their B-Pb and plasma lipid peroxidation levels were higher (means, 16.1 versus 10.6, and 13.3 versus 8.3, respectively), and the MAO, schooling, and Mini-Mental performance scores were lower (means, 41.6 versus 68.3, 0.7 versus 2.3, and 18.0 versus 21.0).

Age was significantly and inversely related to B-Mn level (partial correlation coefficient of -0.26, $P = 0.04$), being significant for women and not for men (Fig. 2). On bivariate scatter plots we observed a direct relationship between manganese and lead, as well as with the socioeconomic index, and an inverse relationship between plasma lipid peroxidation with manganese and hemoglobin and between manganese and hemoglobin, the first four being statistically significant and not the last one. The Hb reduction started at 10 μg/L of B-Mn. MAO was inversely associated with lead and peroxide, with borderline significance. Table 4 shows the main predictive least square regression models of these biomarkers, confirming the bivariate observations and showing the inverse manganese–hemoglobin association. A statistically significant multinomial logistic regression analysis (adjusted by community and smoking) of the blood manganese–blood peroxide concentrations demonstrates an inverse U curve of the regression coefficients. This curve is presented in Fig. 1, where the distribution of B-Mn was divided into six centiles, and the regression coefficients were observed compared to the third centile. Here, a coefficient of -0.65 referred to a reduction of the lipid peroxidation for those at the fourth centile.

Adjusted risk ratios for the different neuropsychological tests are included in Table 5. The models with the highest explanation of the effects are those related to motor strength, coordination, and cognitive performance. The motor test employed was fingertip touching. The most relevant of these are the results of the Mini-Mental Examination. We used a cutpoint of 17, to culturally and socially adapt it to the rural Mexican population. This model was adjusted by hemoglobin, alcohol, age, sex, and schooling since the later one is significantly related to test performance. The logistic regression odds ratio adjusted for schooling yielded a 4.9 (1.4 to 17.4) estimate (shown in Table 6). The unadjusted and adjusted models for the Mini-Mental test are also shown in Fig. 2, the first graph describing a lack of trend for the Mini-Mental test with increasing manganese concentrations, while the second graph

TABLE 3
Descriptive Statistics of Environmental Data by Community

	Units	*n*	Mean (SD)	Minimum	Maximum
Total					
River Mn	mg/L	7 days, 5 sites	45.34 (0.002)	0.00	240.25
Total food Mn	mg/100 dry g	16	2.02 (1.19)	0.65	5.25
Soil Mn	mg/kg	10 sites	119.25 (85.78)	11.84	264.00
Total suspended particles	$\mu g/m^3$	5 days	27.03* (21.59)	6.44	67.03
Outdoor air Mn in PM_{10}	$\mu g/m^3$	5 days	0.07* (0.02)	0.03	0.10
Well water Mn	mg/L	14	39.95 (75.59)	n.d.	241.90
Indoor air PM_{10}	$\mu g/m^3$	10 homes	28.56* (4.95)	17.87	35.19
Indoor dust Mn	mg/cm^2	10 homes	0.62* (0.55)	0.07	1.98
Community A					
River Mn	mg/L	no river	n.a.	n.a.	n.a.
Total food Mn	mg/100 dry g	7 samples	1.87 (1.19)	0.72	2.65
Soil Mn	mg/kg	5 sites	95.75 (74.91)	11.84	194.15
Total suspended particles	$\mu g/m^3$	5 days	43.68[a] (12.08)	26.81	56.69
Outdoor air Mn in PM_{10}	$\mu g/m^3$	5 days	0.10[a] (0.02)	0.09	0.10
Well water Mn	mg/L	7	33.59 (66.87)	n.d.	183.56
Indoor air PM_{10}	$\mu g/m^3$	10 homes	28.56[a] (4.95)	17.87	35.19
Indoor dust Mn	mg/cm^2	5 homes	1.10[a] (0.52)	0.73	1.98
Community B					
River Mn	mg/L	7 days, 5 sites	45.34 (87.85)	0.00	240.25
Total food Mn	mg/100 dry g	9 samples	2.13 (1.48)	0.65	5.25
Soil Mn	mg/kg	5 sites	142.74 (100.42)	19.77	264.00
Total suspended particles	$\mu g/m^3$	5 days	16.72[a] (25.19)	6.44	67.03
Outdoor air Mn in PM_{10}	$\mu g/m^3$	5 days	0.03[a] (0.003)	0.03	0.03
Well water Mn	mg/L	7	46.30 (88.40)	n.d.	241.90
Indoor air PM_{10}	$\mu g/m^3$	n.a.	n.a.	n.a.	n.a.
Indoor dust Mn	mg/cm^2	5 homes	0.35[a] (0.30)	0.07	0.83

Note. SD, Standard deviation; n.a., not available; n.d., not detectable.
[a] Expressed as geometric mean.
[b] Median of two filters.
* $P < 0.05$ by t test.

shows the estimated risk ratios for each tertile of B-Mn for reduced Mini-Mental score, and other tests displaying a U-shaped dose–response curve. Only the adjusted model describes the relation adequately.

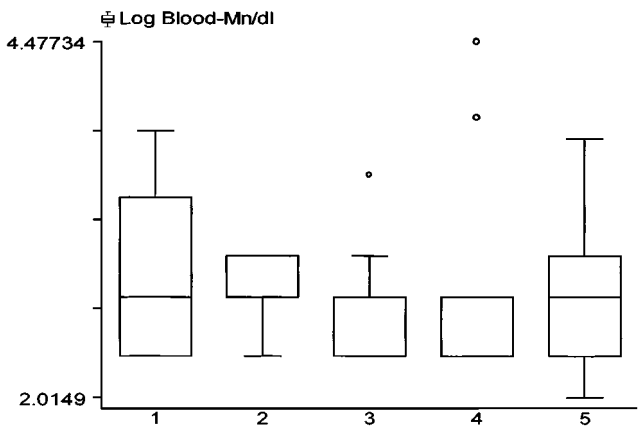

FIG. 1. Blood manganese by sampling sector (5, community B).

Seventeen of 73 subjects had classic chronic bronchitis (cough with phlegm during more than 3 months throughout 3 years), and another 2 had only a chronic cough. Twenty-eight of 32 subjects had positive dense nodulations and 20 had evident fibrosis. Multivariate logistic regression models showed no significant risk ratios related to community, B-Mn, or proximity to point source. Perception of health and environmental alterations was unrelated to manganese levels and to Mini-Mental Examination performance. As shown in Table 7, the perception of environmental alterations was directly related to schooling, the unawareness of the relevance of a source of pollution in Community B, and the frequent use of risk-bearing pesticides. Adjusted for the identified associated variables to blood manganese, hlu regression analysis identified the contribution of manganese in food, air, and home dust (Table 8); a model specific to Community B identified river manganese as another statistically significant factor.

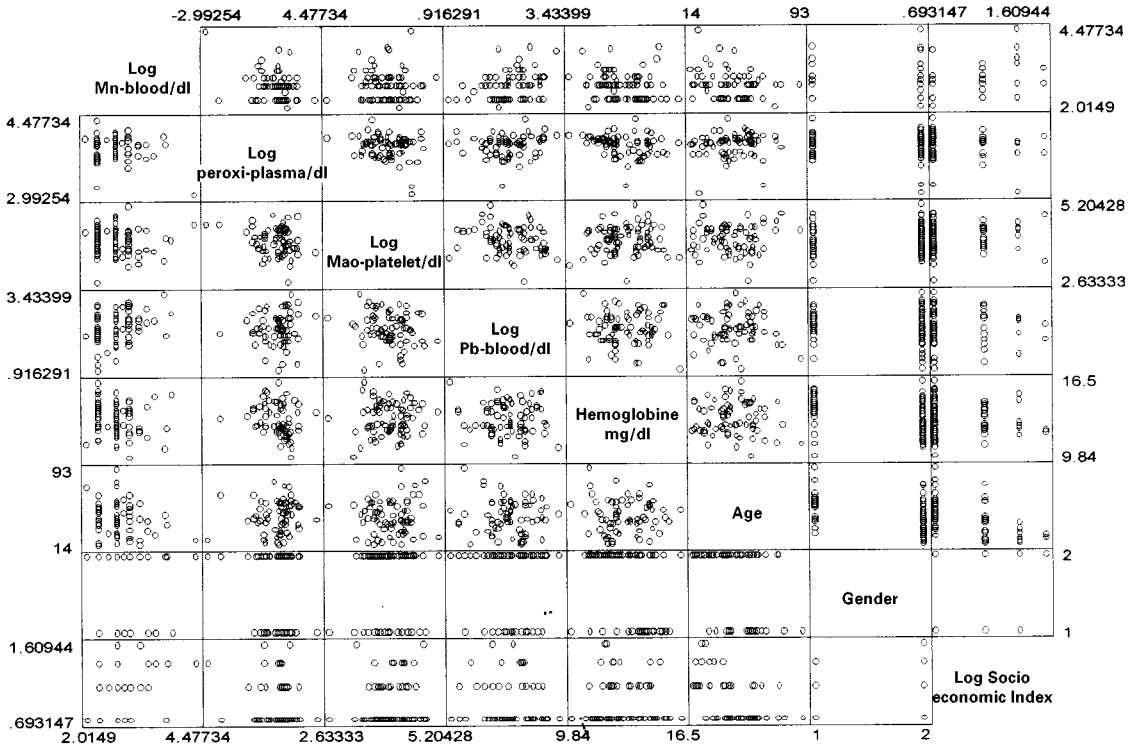

FIG. 2. Biomarkers by age, gender, and socioeconomic index.

DISCUSSION

Based on the results of this study, we identified plasma lipid peroxidation activity reduction, lower Mini-Mental performance, and poor motor function tests at above 15 μg/L of B-Mn. Obvious clinical tremor and numbness were identified at above 25 μg/L, and an inverse linear effect on hemoglobin was identified starting at 10 μg/L.

Biases in this study can occur due to its design, population selection, and instrument measurement among other factors. Although this is a cross-sectional study, inferences based on these findings are stronger given the chronicity of exposure and effects (31). However, the high prevalence might bias the risk estimators away from the null (32), and we suggest that the risk ratios be considered more as an index of effect rather than for obtaining its real magnitude. We have no information on high migration rate, but we can say that length of residency in the community and age are highly correlated, even when periods of time when subjects had lived outside the community are taken into account. This is relevant if we wish to consider especially susceptible subjects who may have left the mining district area; loss of susceptible subjects may bias results toward null. A definitive limitation of this design is that we are unable to identify changes over time, leaving open the question of the potential reversibility of effects resulting from lifetime exposures (33, 34), although acute clinical intoxication has been shown as reversible (35). A similar limitation is observed as for the analysis of potential progression of health effects (36). This limitation is enhanced, as we did not include subjects less than 14 years of age; this was a design limit requested locally. Therefore, our inferences relate only to teenagers and adults. The design does not allow us to examine subjects at very early ages or at the *in utero* stage, when the developing nervous system is most susceptible and when preferential absorption of manganese occurs.

For practical reasons and given the time limit for responding to governmental and community requirements, subjects were recruited at their homes once this was probabilistically selected. The potential bias of the worst case being self-selected is not supported by our risk perception survey. The population sample is certainly biased as regards age and sex structure toward those who are available during the working week, strengthening our inference that we are gathering information on those environmentally exposed at the community level.

The exposure biomarker B-Mn is a good body burden indicator (23, 37–41); this is the case when

TABLE 4
Least Squares Predictive Models of Biomarkers

Dependent variable	Independent variable	Coefficient	90% Confidence interval	Adjusted R^{2a}
Blood manganese (log)	Lead (log)	0.29	0.15–0.43	0.21
	Hemoglobin	−0.08	(−0.14–(−0.03)	
	Age	−0.004	(−0.009)–(−0.0002)	
	Sector distance[b]	−0.06	(−0.11)–(−0.009)	
	Constant	3.52	2.7–4.4	
Lead (log)	Manganese (log)	0.48	0.25–0.71	0.21
	Hemoglobin	0.07	(−0.00) −0.15	
	Community[c]	0.43	0.21–0.64	
	Constant	−0.60	(−2.01)–(0.80)	
Plasma lipid peroxidation (log)	Manganese (log)	−0.61	(−1.06)–(−0.17)	0.32
	Community	1.31	0.90–1.72	
	Constant	1.57	0.23–2.92	
Plasma lipid peroxidation (log)	Manganese (log)	−1.20	(−1.74)–(−0.67)	0.61
	Community	1.70	1.17–2.24	
	Smoker	−1.40	(−0.67)–0.40	
	Constant	2.77	0.87–4.67	
Monoamine oxidase (log)	Alcohol	0.39	0.17–0.60	0.24
	Community	−0.38	(−0.54)–(0.22)	
	Constant	3.88	3.41–4.34	
Hemoglobin	Manganese (log)	−0.96	(−1.61)–(−0.31)	0.25
	Age	−0.02	(−0.03)–(−0.003)	
	Sex	−1.12	(−1.71)–(−0.54)	
	Sector distance	−0.31	(−0.47)–(−0.14)	
	Constant	19.49	17.07–21.93	

Note. All R^2 have $P < 0.05$.
[a] Adjusted R^2 is interpreted as the proportion of variance explained by the model.
[b] Sector dstance refers to the sampling sector, as for distance from the point source, 1 being the closest and 5 the farthest.
[c] In all analyses Community A was coded as 1 and B as 2.

considering chronic exposures rather than acute high ones, as peak doses may be rapidly excreted (42). This was our best estimator of exposure and well described by the exposure pathways. It is argued that the inhalation route is more efficient than the digestive route (4), the former considered by some to be 100% and the latter around 3–5%. Consistently, the air concentration was the main contributor to blood manganese in the exposure model in Table 8, having then home dust manganese and food manganese, both using the oral pathway, as additional contributing factors. A homeostatic mechanism has been shown for the oral route, where the body is protected against manganese toxiticy by low absorption and/or rapid presystemic elimination by the liver (43). This study supports the observation that manganese competes with iron metabolism, as hemoglobin was inversely related to it. There is a wide gradient of exposure, but none of the highest exposures were occupationally related since none of the selected subjects were miners; this is understandable since most of the mine workers come from outside the communities. Upper limits were well into the toxic levels identified in a report of an occupationally exposed population in Mexico where two intoxicated miners had blood manganese of 76 and

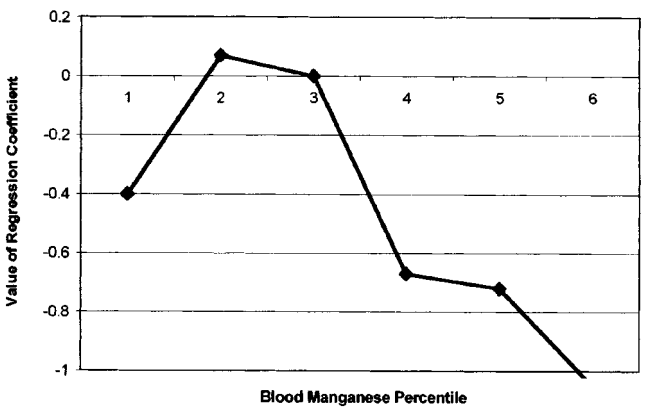

FIG. 3. Trend in log blood peroxide and log blood manganese.

TABLE 5
Regression Models for Manganese Exposure Effect with the Different Neuropsychologic Tests

Test	Coefficient[a]	Confidence interval	Model[c] adjusted R^2	Risk ratio[b]	Confidence interval
Attention and concentration					
Trail Making	1.00	0.40–1.59	0.20	1.68	
Direct digit retention	0.78	0.23–1.33	0.09	2.15	
Inverse digit retention	0.65	0.05–1.24	0.03	1.89	
Motor behavior					
Motor strength (continuous)	0.53	0.15–0.91	0.44	1.59	
Motor strength (categorical)			0.19	6.7	1.5–29.8
Finger tip touching	0.65	0.02–1.28	0.21	1.89	
Asymmetric rhythm	0.93	0.10–1.76	0.14	2.49	
Election reaction	0.58	0.20–0.97	0.26	5.40	
Symbolic actions	0.52	0.22–0.82	0.12	1.67	
Cognitive[d]					
Mini-Mental: concentration	2.00	0.56–3.42	0.34	7.11	
Memory	− 0.45	(− 0.85)–(− 0.05)	0.20	0.64	
Follow instructions	− 0.13	(− 0.25)–(− 0.00)	0.03	0.88	
Writing	0.43	0.07–0.80	0.04	1.52	
Total (continuous)	2.05	0.01–4.10	0.03		
Total (categorical score less than 17)			0.27	11.70	1.5–94.5
Clinical					
Hand numbness (categorical)			0.18	15.5	1.3–181.4

[a] Coefficients were estimated by least squares adjusted for other variables (most frequently age, schooling, community, alcohol, and occasionally age and sex). Only models with statistically significant coefficients associated with blood manganese are included; their confidence intervals exclude the null value. All models are with 90% C.I.

[b] Risk ratios, as estimated from the regression coefficients, are interpreted as how much risk increases when blood manganese is above the 75th percentile, in comparison to the lowest population 25th percentile level. Only statistically significant ratios are included.

[c] Interpreted as the proportion of variance explained by the model; in logisitc estimation we used the pseudo R^2. All R^2 have a $P < 0.05$.

[d] The Mini-Mental test is heavily influenced by education. The models for concentration, writing, and total Mini-Mental and categorical tests are adjusted by other variables and by education.

120 μg/L (44). The observed concentrations were inversely related to age. This suggests that we further seek to test the hypothesis of a preferential absorption of manganese at an early age (45–47).

Blood manganese concentrations were significantly related to the point source in the community, and the exposure model showed an important contribution of air sources; as with other metal exposures

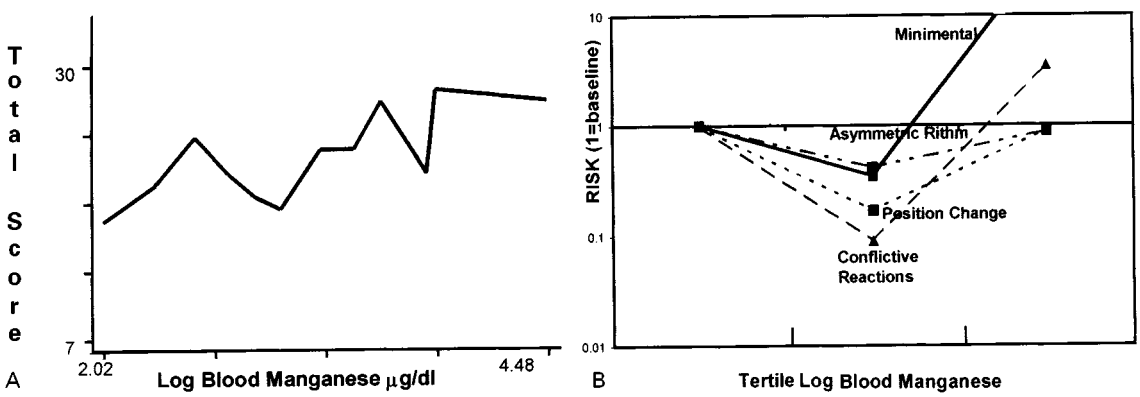

FIG. 4. Neuropsychologic test results for manganese. (A) Blood manganese and mini-metal score. Nonadjusted model. (B) Risk by tertile of blood manganese.

TABLE 6
Mini-Mental[a] Score Performance Deficit Risk Ratios

Variables in models[b]		Point estimate	90% Confidence interval
Model 1[c]	Blood manganese	2.82	0.94 to 8.52
Model 2	Schooling	11.15	1.88 to 66.22
Model 3	Blood manganese	4.92	1.39 to 17.38
	Schooling	16.37	2.53 to 105.65

[a] Mini-Mental: Total Score (categorical score less than 17).
[b] The first two logistic models are unadjusted; the last one is adjusted only for the variables shown.
[c] Models are estimated for risk of deficit when subject's blood manganese is above the median or when the subject has no schooling.

TABLE 8
Environmental Manganese Exposure Factors That Contribute to Integrating Human Population Exposure[a]

Variable	Coefficient	90% Confidence interval
Manganese in food	0.13	0.00–0.26
Outdoor air Mn in PM_{10} (log)	0.47	0.18–0.76
Manganese in home dust (log)	0.05	0.00–0.09

[a] Manganese tertiles as a dependent variable, adjusted for lead, hemoglobin, and socioeconomic index, and autocorrelation using Hildreth–Lu regression. Soil and well manganese were noncontributing. River manganese was only pertinent to Community B.

(48) home dust was an important determinant, possibly being introduced through air or through stepping into dry deposited manganese-polluted soil. Certainly both communities are different in their manganese contributions; both have manganese in the soil, although this is highest in Community B, and the air concentrations are much higher in A, further supporting the relevance of the air pathway. Historical outdoor air particles and manganese measurements were possibly underestimated by the survey measurements; there are company reports to the state environmental authority (49) of 1996–1997 data including median TSP and manganese concentrations of 35.19 and of 1.92 $\mu g/m^3$, respectively. Furthermore, a survey done a couple of weeks before our study by another group (50) showed Mn air concentrations with a geometric mean of 0.07 $\mu g/m^3$ in the $PM_{2.5}$ fraction and of 1.8 $\mu g/m^3$ in the PM_{15} fraction (PM_{10} fraction not reported). Therefore, our results should be taken as qualitatively more than quantitatively appropriate for cumulative exposure estimation. Longitudinal monitoring would provide a better assessment of manganese concentration in the river water as an important source of manganese in the downstream community. We believe that the exposure pathway is indirect since the local population does not drink this water. However, from the river they obtain freshwater shrimp, which feed from the river plankton deposited in the sediment where they reside, easily concentrating manganese, as phytoplankton and water invertebrates have been attributed bioconcentration factors of 2500–6300 and 10,000–40,000, respectively (2, p. 132). We were not able to analyze this food pathway. Manganese in well water was inversely related to B-Mn, possibly because the population does not use the closest water source—as this was the criterion to assign exposure—but the one with the lowest concentration and farthest from the point source. We had a very limited food consumption questionnaire and this might have accounted for the poor performance of the food pathway, rather than considering that this is unrelated. Our questionnaire was more geared toward lead-bearing food consumption than manganesebearing food consumption. Our food sampling included a single nonstandardized convenience sample at a high proportion of homes, and this should be improved in future research.

Blood manganese concentrations in Community A were 11% higher than those in Community B; we further took a convenience sample in far away communities and in clinic patients in Mexico City showing 27 and 74% higher concentrations. For comparison within Mexico, a probabilistic sample of Mexico City's pregnant women (to be published) had an average B-Mn of 7.72 (range 5.6 to 11.4), depicting generally higher blood manganese in Mexico; this might be because dietary factors make Mn ingestion in the upper range of the United States recommended intake (national daily average of 7.9 mg, with some states above 10). Geometric mean B-Mn in Community A was higher than the reported South Western Quebec concentration (51, 52), where MMT is currently being used as a gasoline octane

TABLE 7
Environmental Risk Perception Predictive Variables[a]

Variable	Point estimate	90% Confidence interval
Blood manganese (above mean)	0.26	0.07 to 0.92
Low schooling	0.06	0.01 to 0.28
Familiar with use of pesticides	2.5	0.73 to 8.56
Residence in Community A	5.57	1.60 to 19.37

[a] Ratios estimated from logistic linear regression.

booster; the highest concentrations were in the range of miners reported as occupationally intoxicated (44). Outdoor air manganese was twice the one reported in Soth Western Quebec and the reference United States concentrations (53), similar to the Canadian ones (54) and to the ones reported for Mexico City (55). Mn in soil was 3.6 times the average Canadian and 5.7 times the United States (45) concentrations. Well water manganese was 2.5 times higher than the South Western Quebec tap water. This clearly defines this population as a chronically intermediate level-exposed group. Still other exposure assessment limitations preclude a complete exposure assessment, such as the lack of analysis of time activity patterns in the study population, the external validation of the air sampling, and the need to differentiate recent exposure by using manganese in serum.

Of the expected health effects, both the respiratory symptoms and the radiological data fail to support an association with Mn exposure. It is possible that the intensity (time and concentration) is not as high as that in other settings. However, other clinical and biomarker effects were evident. Lipid peroxidation happened to be useful in identifying early effects and not so monoamine oxidase activity. The inverse U-shaped curve is consistent with the biological conceptual model for essential metals (56) consistent with the possible atioxidant activity of manganese (9). Plasma lipid peroxidation activity was greatly reduced in Community A versus B in spite of the not so high B-Mn difference (ratios being 3.4 and 1.09, respectively), possibly pointing toward the most effective effect of the inhaled route. The absence of association with MAO may be a useful differential diagnosis tool, but requires further examination as regards the time of onset of symptoms and validation. Several subjects had evident tremor and numbness, not requiring any further clinical measurements; some of these could not be assessed for neuropsychological tests because of their handicap. All the data on these subjects are consistent with the overall results but their relationship to the enzymes, possibly showing a different stage of the effects. All the testing was done with standardized procedures and well-trained personnel; still their sensibility could be improved by other computer-based tests (57). Although motor strength and coordination effects were clearly identified in the survey population, the mental deficiency identified by the Mini-Mental Examination showed the most worrisome result of this study. Mn and age are significantly and inversely related (-0.25), as is the case of education and age (-0.63), the latter with a stronger association as the older people had less chance for schooling. That is why the Mini-Mental model was adjusted for age and schooling. The Mn–Mini-Mental association held while considering socioeconomic and schooling covariates; their temporality is difficult to determine. The test has been relaxed in its cutpoint criteria from the score of 23 used internationally (19) to the rural Mexican cutpoint of 17, as adapted from locally developed studies. Further adaptations have included the query about locally familiar elements. The public health impact of this finding is of fundamental importance. Risk was 12 times higher in the upper concentrations versus that at the lower exposure concentrations. Still other, more sensitive effects (olfactory, sensitivity, balance, reflexes, and other neurological signs) were not explored, giving a partial figure of the potential neurological effects.

The B-Mn and hemoglobin inverse association found was interesting (58), although several issues have yet to be looked into. As with the Mini-Mental Examination, this association is socioeconomically and nutritionally related. The effect of Mn was higher than the known one of lead (59). The study did not assess nutritional status, and we cannot correctly assess iron intake, nor do we have information on serum iron since this element was not measured in the study. However, these findings are consistent with those in the literature (45, 59–61) and should be measured in future projects; the nutritional evaluation needs to be enhanced in order to address total exposure (62).

It was necessary to explore the lead interaction since this toxicant is highly prevalent in Mexican dish and cooking ware, and it has neurotoxic potential. The blood lead manganese concentrations are consistent with those of other Mexican rural community studies, where the main source of lead is low temperature-glazed pottery used for cooking and storing food (63). Other authors have identified an interaction of lead and manganese: In ther late 1970s Zielhius in Holland (64) identified this interaction, and later on Truckenbrodt found it when examining different occupations (65); furthermore, Saavedra (66), in a study of battery workers in Mexico, determined the same relationship. B-Pb and B-Mn were certainly correlated as they do not compete for metabolic pathways; however, in our study lead was seldom identified as related to neurotoxic effects: it was shown to be associated with the neuropsychological verbal word fluidity test, with some motor function tests (symbolic actions, conflictive reactions), and in the writing component of the Mini-Mental test in multivariate adjusted models.

This may be because manganese and lead have different brain sites of action.

The study limitations open up questions and improvements for future research. The findings of this study are biologically plausible and consistent with those in the literature. This study provides a cross-sectional picture of a population with lifetime exposure that warrants a longitudinal examination. This is in our immediate research plans; 10 to 30% of the population in the examined group suffer from different degrees of effects, and we expect these effects to be reduced or prevented in the younger population if exposure is limited in an important way. Although it is still premature to set a public health goal, including safety factors may lead to a goal of safe B-Mn concentration, in the range of 0.8–1.2 μg/L. Mean air manganese concentrations are at the currently discussed reference concentrations of EPA (53) and Canadian ones (54). A sanitary goal now can only be temporary since this has to be supported by improved exposure pathway assessment and more sensitive clinical tests. None of the mentioned limitations preclude the public health interventions that have been proposed locally and to federal authorities. This supports a precautionary approach to environmentally exposing the general population to manganese in any of its forms and media until a population-based description of the dose–response curve is obtained within a wider range of exposure and effects. While all the limitations of this study are addressed through future research, federal and state health and environmental authorities have determined to reduce air emissions to half their current levels, and have established several environmental hygiene actions in the community with greatest exposure. We strongly feel that an important risk is present which deserves further research and intervention for the benefit of local communities as well as for addressing fundamental scientific questions.

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