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### Lead in Hair of Children Exposed to Gross Environmental Pollution

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## LEAD IN HAIR OF CHILDREN EXPOSED TO GROSS ENVIRONMENTAL POLLUTION

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In the context of a cross-sectional study, concentrations of lead in hair (PbH) were determined by atomic absorption analysis among 263 children aged 1 to 9 years, living less than 900 m from a lead smelter in Santo Amaro City, Bahia, Brazil. PbH levels ranged from 20 to 4933 ppm; the median was 349 and the geometric mean was 313 ppm. The mean PbH level was increased among individuals with the following characteristics: female; younger age; shorter distance between residence and smelter; longer residence time in the area; racial group Dark; curly hair; being a child of a lead worker; high lead content in peridomiciliar area. The mean PbH level did not vary markedly according to the child's nutritional status, iron status and the habit of pica. The marked variations in pbH levels point out the feasibility of its use as an epidemiological index in situations of heavy environment pollution.

KEY WORDS: Lead, hair.

### INTRODUCTION

Lead in hair (PbH) concentration has been used as a biological parameter to assess environmental and occupational exposure.<sup>1-9</sup> Hair concentrates more lead per unit of mass than any other human tissue.<sup>10</sup> However, hair is not an important route for lead excretion.<sup>11</sup> One important limitation in using PbH is laboratory impossibility of providing separate information on endogenous (excreted) and exogenously incorporated from adsorbed onto hair surface lead.<sup>12-14</sup> Interpretation of PbH data remains controversial since they can reflect a complex, simultaneous process of exposure, absorption and excretion.

Since 1960 the population from Santo Amaro, State of Bahia, Brazil, has been exposed to gross environmental pollution as a result of atmospheric emissions of lead<sup>15-18</sup> and cadmium.<sup>19</sup> In addition, the industrial residue obtained from smelted ore, the so-called "smelter dross", was later presented as a gift to the

population and the local City Hall, and extensively used for paving private yards, gardens, schoolyards, streets and other public places.

This study aims at the determination of PbH concentrations among children living in the highly polluted area of Santo Amaro and to describe some epidemiologic characteristics associated with PbH variation in this population.

## MATERIAL AND METHODS

The Santo Amaro lead smelter is placed in a well-defined area in the outskirts of the city. The study area was delimited by a circle of 900 m radius, taking the smelter chimney as the central point. A census carried out in the third trimester of 1980 has identified 648 children, 1 to 9 years old, living in 237 dwellings in the area.<sup>20</sup> The study population comprised 263 children from whom blood and hair samples could be obtained. The person responsible for each child was interviewed in the home by two physicians and two students of medicine. Mothers gave information about each child; fathers were asked about their occupational histories.

Children were classified into three racial groups: Light, Medium and Dark, based on the degree of expression of the negroid phenotype.<sup>21</sup> Although necessarily subjective, this classification is based on hair colour and type, conformation of the nose and lips, and pigmentation of the skin.<sup>22</sup> The nutritional status was evaluated by the wasting (weight by height) index, expressed in standard deviation scores, in relation to the levels found in a reference population,<sup>23</sup> as recommended by Waterlow *et al.*<sup>24</sup> Detailed procedures on nutritional status evaluation were reported elsewhere.<sup>25</sup>

Blood was taken by venapuncture for the determination of lead in blood (PbB) concentration by flameless atomic absorption analysis, using a modification of the method described by Fernandez,<sup>26</sup> as reported elsewhere.<sup>20</sup> Serum iron (FeS) and Total Iron Binding Capacity (TIBC) were determined colorimetrically, using the Harleco<sup>27</sup> method. The index Per Cent of Transferrin Saturation (PTS) was calculated as follows:  $\text{FeS/TIBC} \times 100 = \text{PTS}$ . Iron deficiency was considered to occur when  $\text{PTS} \leq 15.9\%$ .

A hair sample weighing at least 1 g was taken from the nape of each child, hair strings being cut as close to the scalp as possible. Hair type was classified as either straight or curly by the same researcher, in the laboratory.

Each hair was treated independently. About 1 g of hair was washed with a 10% neutral Extran solution, first with a manual prewashing and then with ultrasound for 20 min, rinsed twice with deionized water—also with ultrasonic agitation—dried at 60°C and cut in small pieces. Portions of 0.1 g, weighed to 0.1 mg, were digested in 10 ml pyrex test tubes with 2.0 ml of concentrated HNO<sub>3</sub> at room temperature for 12 h and then at 80°C for 1–2 h in an aluminum heating block. 100 µl of the digested mixture were diluted to 1500 µl in Eppendorf tubes and analysed for Pb by ETAA (Perkin Elmer 460, HGA 2200) with 10 µl injections in pyrolysed graphite tubes utilizing deuterium background correction. The furnace program was:  $\lambda = 283.3 \text{ nm}$  from an EDL lamp, drying at 100°C for 20 sec,

charring at 2000 °C for 4 sec, clearing at 2600 °C for 2 sec. A Pb Merck Titrisol standard solution was used for matrix-matched calibration graphs. All glass and quartz labware was cleaned by treatment with low metal content concentrated HNO<sub>3</sub>. The reproducibility of digestion and analysis was always above 90% and was checked during the entire work at each 10 sample batch analyses, by using two hairs as control, one from a healthy person not occupationally exposed to Pb and another from a highly exposed one.

A soil sample could be taken from the peridomiciliar area of the dwellings of 225 children for the determination of its lead content (PbS). After removal of leaves and stones, the soil samples were dried in air, ground manually, and next, the fraction below 200 mesh was separated for analysis utilizing SPEX nylon sieves. About 0.30 g of each sample was digested with 2.0 ml of concentrated HNO<sub>3</sub> in PTFE-lined steel pressure decomposition vessels for 15 h at 120–130 °C. The digested mixture was diluted by weight to 50.0 g with deionized water in the same PTFE cup analysed for Pb by flame AAS (Perkin Elmer 460) at 283.3 nm, using an EDL lamp according to the manufacturer's instructions. Samples with concentrations exceeding the linearity range of the method were reanalysed with the burner head (10 cm, one slot) in a 30 °C position or after dilution with 4% HNO<sub>3</sub>.

The distributions of PbH and PbS were skewed and were always expressed in terms of geometric means and respective standard deviations. A set of statistical programs for computer developed by Hull and Nie<sup>28</sup> was used for data analysis. Comparisons between the means of two groups were made by using Mann–Whitney's test.

## RESULTS

PbH values among the 263 children ranged from 20 to 4933 ppm, with a median of 349, arithmetic mean of 588 (standard deviation = 664) and a geometric mean of 313 ppm (standard deviation = 3.2); 79% of the population had a PbH higher than 100 ppm, and 14.4% a PbH higher than 1000 ppm. PbH decreasing with increasing distance from child household to smelter chimney (Figure 1) and with age (Figure 2). PbH also showed a consistent trend to increase with the residence time in the area (Table 1).

For the whole group of 263 children, the PbB concentrations had an arithmetic mean and standard deviation of  $2.81 \pm 1.2 \mu\text{mol/l}$  and they were fairly well correlated ( $r = 0.38$ ;  $P < 0.00001$ ) with log PbH concentrations.

PbH levels showed a consistent increase with an increase of the lead content in the soil (Table 2). The correlation coefficient between PbH and log PbS values was  $r = 0.28$  ( $P < 0.00001$ ) among 225 children. The mean PbH level did not vary markedly ( $P > 0.05$ ) with the presence or absence of the habit of pica (refers to the act of a person to explore his environment by taking unedible materials like earth, paper, plaster, etc, to mouth; scavenging) in this population (Table 3).

Children with curly hair showed PbH mean value higher than those with

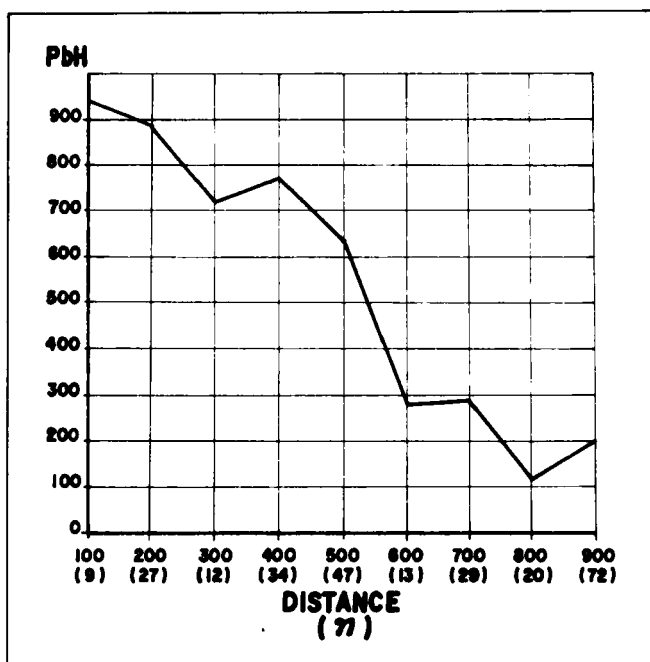


Figure 1 Geometric mean of PbH concentrations (ppm) as a function of the distance of a child's household to the smelter (in parentheses, the number of individuals in each group).

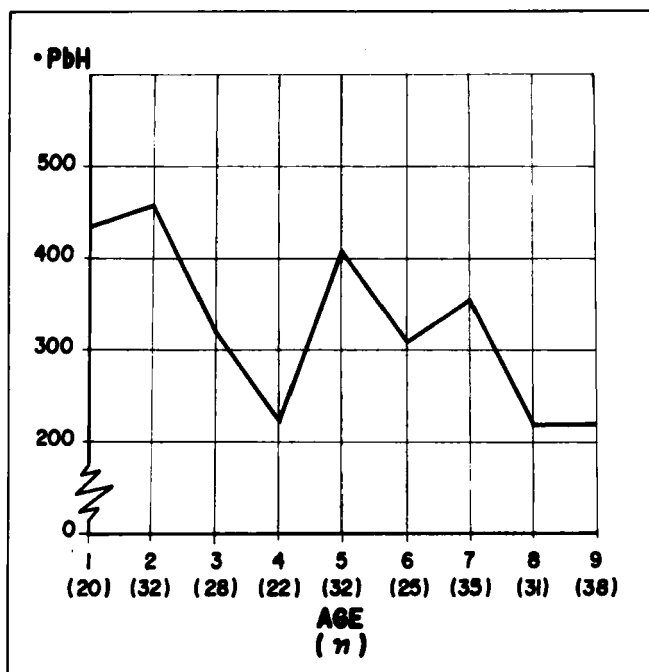


Figure 2 Geometric mean of PbH concentrations (ppm) as a function of children's age (in years; in parentheses, the number of individuals in each age group).

**Table 1** PbH concentrations as a function of children's residence time in area

Residence time (months)	PbH (ppm)		N
	GM <sup>a</sup>	SD <sup>a</sup>	
1-3	140	2.8	13
4-6	204	2.8	16
7-12	299	3.3	19
13-24	343	3.2	27
25-119	346	3.2	185
Total	316	3.2	260

<sup>a</sup>Geometric mean (GM) and standard deviation (SD).**Table 2** PbH concentrations as a function of the lead content in peridomiciliar soil (PbS)

PbS (ppm)	PbH (ppm)		N
	GM <sup>a</sup>	SD <sup>a</sup>	
0- 1 000	214	2.9	30
1 000- 5 000	249	3.0	81
5 000- 10 000	328	3.3	31
10 000- 30 000	417	3.5	63
30 000-108 000	445	3.3	20
Total	308	3.3	225

<sup>a</sup>Geometric mean (GM) and standard deviation (SD).**Table 3** Dependence of PbH concentrations on the habit of pica

Habit of pica	PbH (ppm)		N
	GM <sup>a</sup>	SD <sup>a</sup>	
No	311	3.2	238
Yes	338	2.7	27
Total	313	3.2	263

<sup>a</sup>Geometric mean (GM) and standard deviation (SD).

straight hair. The *P* value for the Mann-Whitney's test which compared these two means was 0.06 (Table 4).

PbH was the highest among Dark children, and the lowest among Light children, while those from the Medium group showed intermediate values. However, the three means did not show significant differences ( $P > 0.05$ ) at a Kruskal-Wallis one-way analysis of variance (Table 5). The girls showed mean PbH value significantly higher ( $P < 0.0005$ ) than the boys (Table 6).

PbH concentrations did not vary consistently with all five levels of nutritional status. The six children showing more severe malnutrition presented the lowest

**Table 4** PbH concentrations as a function of children's hair type

<i>Hair type</i>	<i>PbH (ppm)</i>		<i>N</i>
	<i>GM<sup>a</sup></i>	<i>SD<sup>a</sup></i>	
Straight	257	3.1	71
Curly	337	3.3	192
Total	313	3.2	263

<sup>a</sup>Geometric mean (GM) and standard deviation (SD).**Table 5** Dependence of PbH concentrations on racial group

<i>Racial group</i>	<i>PbH (ppm)</i>		<i>N</i>
	<i>GM<sup>a</sup></i>	<i>SD<sup>a</sup></i>	
Light	243	2.9	36
Medium	265	3.3	79
Dark	364	3.2	148
Total	313	3.2	263

<sup>a</sup>Geometric mean (GM) and standard deviation (SD).**Table 6** Dependence of PbH concentrations on sex

<i>Sex</i>	<i>PbH (ppm)</i>		<i>N</i>
	<i>GM<sup>a</sup></i>	<i>SD<sup>a</sup></i>	
Male	218	3.2	101
Female	393	3.0	162
Total	313	3.2	263

<sup>a</sup>Geometric mean (GM) and standard deviation (SD).**Table 7** PbH concentrations as a function of the nutritional status (standard deviation scores)

<i>Nutritional status</i>	<i>PbH (ppm)</i>		<i>N</i>
	<i>GM<sup>a</sup></i>	<i>SD<sup>a</sup></i>	
-5.95 to -3.00	185	3.7	6
-2.99 to -2.00	360	1.9	5
-1.99 to -1.00	326	2.9	23
-0.99 to -0.01	323	3.3	93
-0.00 to 2.63	310	3.4	127
Total	313	3.3	254

<sup>a</sup>Geometric mean (GM) and standard deviation (SD).

**Table 8** PbH concentrations as a function of the iron deficiency

Iron deficiency	PbH (ppm)		N
	GM <sup>a</sup>	SD <sup>a</sup>	
Yes	238	3.4	51
No	324	3.2	182
Total	302	3.3	233

<sup>a</sup>Geometric mean (GM) and standard deviation (SD).

mean PbH level (Table 7). Iron-deficient children showed lower mean PbH level than children with adequate iron stores, without these differences being statistically significant at the 5% probability level (Table 8).

Children of lead workers had a much higher ( $P < 0.0001$ ) mean PbH value than those of fathers having other occupations, as revealed by Table 9).

## DISCUSSION

To a certain extent, the lack of a children control population prevents one from reaching definitive conclusions concerning the levels of lead in the hair of children from Santo Amaro. However, these data can be compared with mean PbH levels among children living near lead smelters in the United States<sup>29</sup> ( $118 \pm 100$  ppm); England<sup>30</sup> (7.5 to 12.8 ppm); West-Germany<sup>31</sup> ( $9.52 \pm 5.36$  ppm) and the Netherlands<sup>6</sup> (6.8 ppm). From Santo Amaro and a non-industrial control area in Bahia, PbH levels are available for adult populations following the same laboratory methodology used in the present study. The mean PbH value was  $39.6 \pm 3.2$  ppm for the target population and  $6.9 \pm 3.2$  ppm for the control one.<sup>16</sup> Attention should be called to the fact that adults present systematically lower PbH values than do the children. On the other hand, PbH values higher than 100 ppm among children denote undue lead exposure/absorption and deserve a deeper toxicological investigation.<sup>10</sup> Among lead workers subject to stable lead exposure, it was estimated that a PbB level of  $60 \mu\text{g}/100 \text{ ml}$  ( $2.88 \mu\text{mol/l}$ ) corresponds to a PbH of about 70 ppm.<sup>4</sup> However, this kind of relationship between the lead content in blood and in hair can not be acritically applied to every environmental situation such as, for instance, that which prevails in Santo Amaro. In this area, children have been exposed to gross environmental pollution probably since intrauterine life.

Indicators of lead absorption and excretion can vary according to important adventitious sources of lead such as "smelter dross" and used "filters". At the time this survey was carried out, almost 500 pieces of a thick cloth, which were disposable parts of an antipollution device, were taken away from the smelter by the lead workers. These "filters" which contained high quantities of particulate lead, could be found in many households serving as carpets, rags and having other domestic uses.<sup>20</sup> This could partially explain why children of lead workers had such high levels of lead in their hair (Table 9).



**Table 9** Dependence of PbH concentrations of children according to father's job as a lead worker

Child of lead worker	PbH (ppm)		N
	GM <sup>a</sup>	SD <sup>a</sup>	
No	268	3.3	194
Yes	509	2.8	66
Total	316	3.2	260

<sup>a</sup>Geometric mean (GM) and standard deviation (SD).

Wibowo *et al.*<sup>9</sup> studied 71 Dutch children aged 1–3 years, which were subject to relatively low environmental lead exposure: the geometric mean of PbB was 0.72  $\mu\text{mol/l}$  (range, 0.24–1.69) and the mean PbH was 6.8 ppm (range, 0.8–114). PbB and zinc protoporphyrin (ZPP) concentrations were more strongly correlated with a series of parameters (lead in house dust, indoor and outdoor lead-in-air concentrations, and indoor and outdoor lead deposition) than was PbH. However, the correlation coefficient they found between PbH and PbB ( $r=0.22$ ;  $P<0.05$ ) was lower than that observed among children from Santo Amaro ( $r=0.38$ ;  $P<0.00001$ ).

The peridomiciliar soil in Santo Amaro, which is highly contaminated by lead, may also contribute to increase this metal content in hair to a greater extent than seen among the Dutch children. In Santo Amaro, PbH showed a good correlation with PbS, since the exposure level was much higher.

Soils highly contaminated by lead can cause distinct elevation in the metal levels in blood<sup>30,32–34</sup> and in hair.<sup>30,32</sup> Significant correlations have been reported between the habit of pica for soil and increased hair lead levels, but not with blood lead levels among children living in areas with highly contaminated soils.<sup>30,32</sup> In Santo Amaro, children with the habit of pica presented significantly higher ZPP levels than children without this habit.<sup>35</sup> However, the mean PbH level was not significantly elevated among children who presented the habit of pica (Table 3).

Children with curly hair and children from the racial group Dark had relatively high mean PbH levels. These facts are coherent with racial group classification and with the epidemiological studies which found Black children to be particularly vulnerable to lead poisoning.<sup>36,37</sup> However, an intriguing fact was that adult fishermen from Santo Amaro with straight hair showed significantly higher PbH levels than those with curly hair.<sup>16</sup>

Most studies<sup>6,8,38</sup> refer to boys presenting mean PbH values higher than girls, which contrasts with the findings for children from Santo Amaro. One possible explanation for these differences in PbH levels considering sex in the children study, and hair type and racial group in the fishermen study, takes into account the pattern of hair wash. Among fishermen from Santo Amaro, the poor-quality alkaline soap, commonly used by them could attack the hair surface and remove a certain amount of endogenous lead.<sup>16</sup> According to local culture, straight hair is a

beauty trait and, of course, deserves special care. Therefore, mothers would wash more frequently the hair of their Light daughters with straight hair. Usual tress styling for older girls with curly hair lasts for days and leads to less frequent washing. This hypothesis needs to be tested in future studies.

Lead absorption and retention is enhanced in rats with iron deficiency.<sup>39-41</sup> However, in this study, iron-deficient children did not show a significant increase in the levels of lead in the hair or blood,<sup>25</sup> zinc protoporphyrin<sup>15</sup> or cadmium in blood.<sup>19</sup>

Experimental studies<sup>42,43</sup> have shown the role of the nutritional status in lead absorption and toxicity, based on classical clinical observations that lead-poisoned children were generally malnourished.<sup>34,44</sup> Studies among child populations suggest the existence of a relationship between high lead levels in blood and impaired growth.<sup>44-47</sup> However, among the children from Santo Amaro, lead in blood was not significantly correlated with wasting levels,<sup>25</sup> as observed for PbH levels.

PbH levels varied similarly to PbB and ZPP levels in respect to well-known risk factors for lead poisoning such as young age, being a child of a lead worker, short distance between the child's household and the smelter, and a long residence time in the area.<sup>15, 25</sup>

In conclusion, the determination of the lead content in hair promises to be a useful epidemiological index of a complex process of exposure to, and absorption and excretion of lead. This fact indicates the feasibility of its use in situations of heavy environmental pollution.

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