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Cadmium Concentrations in Blood of Children Living near a Lead Smelter in Bahia, Brazil

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A prevalence study of cadmium absorption was carried out among 396 children aged 1 to 9 years living at less than 900 m from a primary lead smelter in Santo Amaro City, northeast Brazil. Geometric mean and geometric standard deviation of cadmium concentrations in blood (CdB) were 0.087 and 2.5 μmole/liter, respectively, ranging from 0.004 to 0.511 units. Ninety-six per cent of these children presented CdB higher than 0.0089 μmole/liter (or 1.0 μg/liter) which is usually taken as a reference level. Higher CdB levels were significantly associated with shorter distance from child's home to smelter chimney, residence time in the area greater than 7 months, racial groups Light and Medium, and heavy infection by hookworm. The variation in CdB levels was not associated with child's age, nutritional status, iron status, family per capita income, blood lead level, being a child of a lead worker, the habit of pica, and contamination of child's peridomiciliar environment by smelter dross.

INTRODUCTION

Since its initiation in 1960, a primary lead smelter has been polluting Santo Amaro City, northeast Brazil. The city has approximately 30,000 inhabitants (Brasil, 1980). The industrial process utilized ore containing 0.037 to 0.140% of cadmium which was completely discarded to the environment. The smelter management acknowledged that at least 250 tons were dumped into the Subaé River and other 150 tons were released to the air (Oliveira, 1977).

Waters from the Subaé River, collected at 100 m from the smelter presented mean ($\bar{X} \pm$ SD) cadmium concentration of 0.029 ± 0.022 ppm. Downstream, 5 km from the smelter, the level was 0.024 ± 0.009 ppm (Reis, 1975). The WHO (1977) tolerance limit for cadmium in drinking water is 0.01 ppm. Median concentration of cadmium in dust/dirt collected at the peridomiciliar environment of 221 households placed at less than 900 m from the smelter was 16 ppm, ranging from 0.4 to 335 ppm (Carvalho *et al.*, manuscript in preparation). Lettuce (*Lactuca sativa*), cabbage (*Brassica oleracea*), and okra (*Hibiscus esculentus*) collected at 200 m from the smelter presented mean cadmium concentrations of 11.80, 10.09, and 3.02 ppm, dry basis, respectively. On the other hand, mean cadmium concentrations in these vegetables bought in groceries from Salvador City (56 km remote from Santo Amaro City) were 0.18, 0.28 and 0.15 ppm, respectively (Petersen, 1982; Petersen and Tavares, 1981). Oysters (*Crassostrea rhizophorae*)

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from the Subaé River had mean cadmium concentration of 120 ppm, ranging from 80 to 135 ppm, dry weight basis (Souza *et al.*, 1978).

Geometric mean (GM) of cadmium concentrations in scalp hair of fishermen from Santo Amaro City was 1.01 ppm (geometric standard deviation (GSD) = 2.70), ranging from 0.18 to 9.80 ppm. Among fishermen from a reference area, the mean concentration was 0.15 ppm (GSD = 4.18) (Carvalho *et al.*, 1984b). The prevalence rate of proteinuria by the sulfosalicylic acid method was 7.0% among fishermen from Santo Amaro City and 4.8% among fishermen from a reference area (Carvalho *et al.*, 1983). Among 131 elderly women from Santo Amaro City, the prevalence rate of proteinuria was 7.6% and did not differ significantly from the rate of 10.7%, observed in a reference population (Carvalho *et al.*, 1984c).

This study determines the levels of cadmium in blood of children living near the Santo Amaro lead smelter and describes risk factors for increased cadmium absorption in this population.

MATERIALS AND METHODS

The Santo Amaro lead smelter is placed in a well-defined borough in the outskirts of the city. The borough was delimited by a circle of a 900-m radius, taking the smelter chimney as the central point (Fig. 1). The predominant wind is from the southeast, accounting for 58% of annual readings. The air is still for 24% of the time and mean wind speed is 1.7 m/sec (Brasil, 1981).

There were 648 children, 1 to 9 years old, living in 237 dwellings in the area. Blood samples were taken from 593 children during a study of lead poisoning in this population. However, cadmium concentrations could be determined in the blood of 396 children only. The person responsible for each child was interviewed in the home by two physicians and two students of medicine. Mothers gave the information about each child; fathers were asked about their occupational and migratory histories.

The industrial residue obtained from smelted lead ore contained 1 to 3% of lead (ABM Noticias, 1981) and a variable concentration of cadmium. The so-called "smelter dross" was freely given to the population and extensively used for paving backyards, gardens, streets and other public places. The presence of this smelter dross in each domicile was annotated since it could contribute to increase the level of cadmium in blood, mainly among children presenting pica for soil.

Children were classified into three racial groups: Light, Medium, and Dark (Oliveira and Azevedo, 1977) based on the degree of expression of the Negroid phenotype. Although necessarily subjective, this classification is based on hair color and type, conformation of the nose and lips, and pigmentation of the skin (Azevedo, 1975, 1980; Krieger *et al.*, 1965). 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 (Hamill *et al.*, 1977) as described by Waterlow *et al.* (1977).

Blood for determination of the concentration of cadmium was taken by venapuncture with sterile iron- and lead-free polyethylene syringes containing ethylene diamine–tetracetic acid as anticoagulant. One aliquot of blood had the

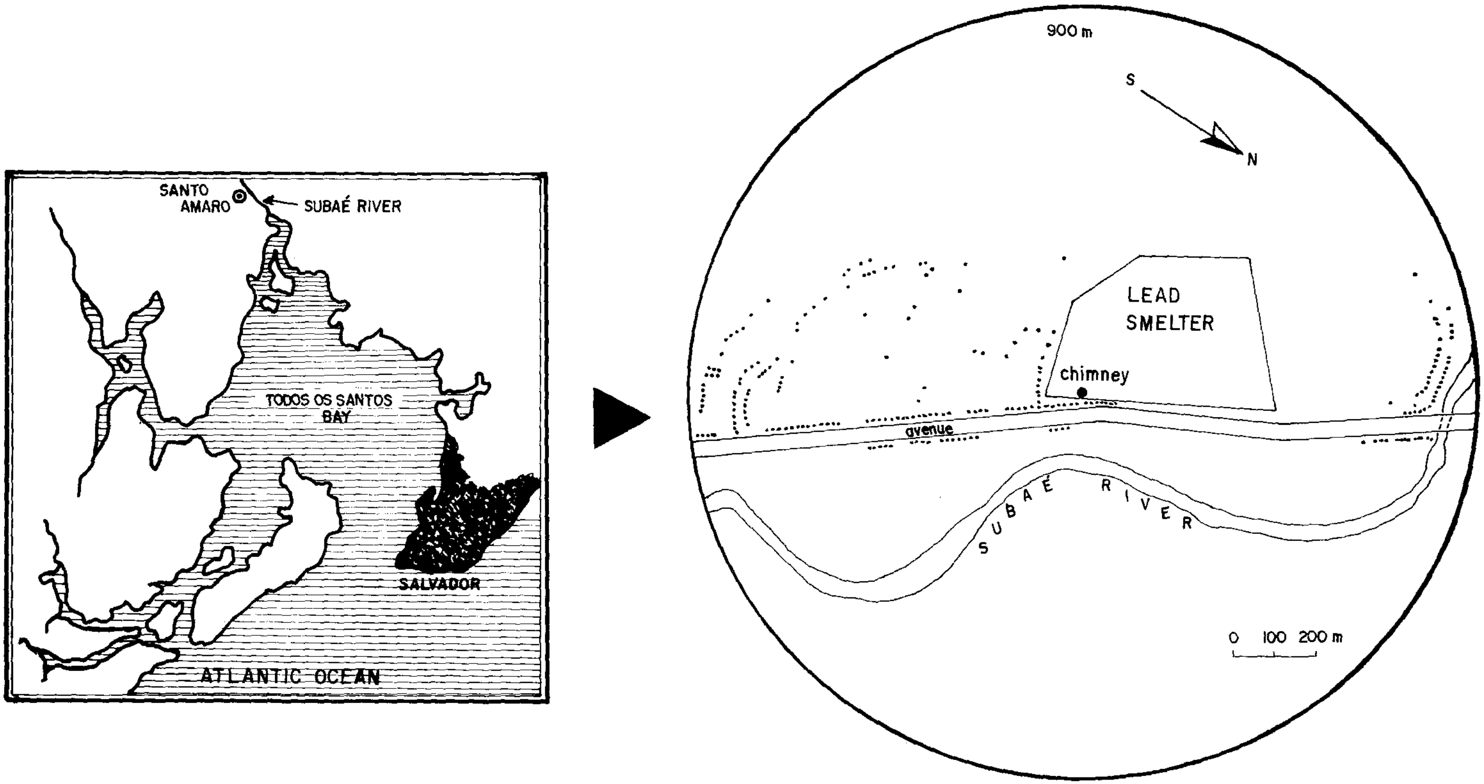

FIG. 1. Todos os Santos Bay, Santo Amaro City, and the study area where each dot represents one domicile.

serum separated for serum iron and total iron-binding capacity (TIBC) determinations and was kept at $-18°C$ until the analyses were performed.

CdB level was determined in a Zeeman Effect Hitachi 170-70 atomic absorption spectrophotometer. The graphite furnace program for 10 μl injection in pyrolysed tubes was $\lambda = 228.8$ nm, drying at 100°C for 20 sec, charring at 300–350°C for 10 sec, atomization at 2000°C for 4 sec, and cleaning at 2600°C for 2 sec. Cadmium Merck titrisol standard solution was used for matriz-matched calibration graphs. The HNO_3 was purified by subboiling distillation in a Hans Kürner quartz still. The used labware of glass and quartz was cleaned by rinsing with concentrated pure HNO_3. Cd-free yellow tips (Gilson C-20) for micropipets were used at all times. Argon of 99.999% purity was used throughout as purging gas for graphite furnace. Disposable plastic gloves were always used during blood sample manipulation.

Cd was liberated from blood by deproteinization and matrix modification with 1 M HNO_3, based on Stoeppler and Brandt (1980) as follows: to 500 μl of HNO_3 1 M in an Eppendorf tube was added 500 μl of whole blood. The tubes were manually vigorously shaken at once and then kept for 5 min in ultrasound, followed by centrifugation for 10 min. The supernatant (10 μl) was directly injected into the graphite furnace. Matrix-matched calibration graphs were obtained by addition of appropriate volumes of Cd standard solutions to 500 μl of HNO_3 1 M, using blood with relatively low Cd content. Two or three firings from each sample solution were performed and values of calibration curve were checked at every 10 sample determinations.

The performance of the analytical methodology had the following characteristics: linear range below 0.22 μmole/liter of blood; the blank value of 1 M HNO_3 was 0.007 μmole/liter; the determination limit for 10-μl injections (based on three standard deviations of blank) was 0.004 μmole/liter. The accuracy was checked against certified reference blood from the Behring Institute with assigned AAS value of 0.049 μmole/liter (=5.5 μg/liter). The results obtained were within 10% of the assigned confidence level.

Concentrations of lead in blood (PbB) were determined by flameless atomic absorption technique, using the method described by Fernandez (1975) modified, as detailed elsewhere (Carvalho *et al.*, 1984a). Serum iron (FeS) and total iron-binding capacity were determined photocolorimetrically, using the Harleco (1978) method. The index transferrin saturation (TS) was calculated as follows: FeS/TIBC $\times$ 100 = TS. CdB concentrations were expressed in SI units (WHO, 1979) (factor to convert CdB μmole/liter to μg/liter = $\times$ 112.4 and PbB μmole/liter to μg/100 ml = $\times$ 20.83).

Hookworm egg count in feces (hookworm) was performed by Kato's method as modified by Katz *et al.* (1972), using a commercially available kit (AK Industria e Comércio Ltda, Belo Horizonte, Minas Gerais, Brazil). A set of statistical programs for computer developed by Nie *et al;.* (1975) was used for data analysis. The distribution of CdB was very skewed and merited a logarithmic transformation, base 10, because the distribution of data was closer to log-normal than normal. Mean CdB levels were always expressed as geometric mean and its standard deviation.

RESULTS

Table 1 shows the frequency distribution of CdB levels in the population. The geometric mean was 0.087 μmole/liter (GSD = 2.5) and the arithmetic mean and its standard deviation were 0.12 ± 0.088 μmole/liter, ranging from 0.004 to 0.51 μmole/liter. In this population, 380 children (96%) had CdB level higher than 0.0089 μmole/liter (or 1.0 μg/liter.)

CdB levels tended to decrease with increasing distance from the child's home to smelter chimney (Table 2). Mean CdB levels were particularly high among children living at less than 0.5 km from the smelter. Mean CdB levels varied markedly according to children's residence time in the area, being the lowest among those living for less than 3 months in the borough (Table 3). Mean CdB levels did not follow a consistent trend according to age groups. The correlation coefficient (r) between log CdB and age was -0.04 ($P > 0.05$). Children aged 2 years presented the highest mean CdB levels: 0.11 μmole/liter (GSD = 2.6). Boys and girls presented similar mean CdB levels: 0.089 μmole/liter (GSD = 2.5) and 0.085 μmole/liter (GSD = 2.5), respectively.

Table 4 shows that Dark children had significantly lower mean CdB levels than those from racial groups Light ($P < 0.025$) or Medium ($P < 0.01$). Mean CdB levels did not vary markedly or consistently according to child's nutritional status (Table 5). Indeed, the correlation coefficient between log CdB and the wasting index was 0.03 ($P > 0.05$). Children presenting higher counts of hookworm eggs in feces (≥2000 eggs/g of feces) had mean CdB levels significantly higher ($P < 0.01$) than those with light infections (1 to 1999 eggs/g of feces) or those uninfected ($P < 0.025$) (Table 6). Table 7 shows that children who lived in households in which smelter dross was present had significantly higher ($P < 0.0001$) mean CdB levels than children in whose households smelter dross was not found.

The variation in CdB levels was poorly associated with family per capita income. The correlation coefficient (r) between log CdB and family per capita income was $r = 0.12$ ($P < 0.01$). Mean CdB level presented by 92 children of lead workers was 0.097 μmole/liter (GSD = 2.7). Among 301 children whose fathers did not work at the lead smelter mean CdB level was 0.084 μmole/liter (GSD = 2.5). These differences were not statistically significant at the 5% probability level. Forty-five (11.4%) out of the 396 children presented the habit of pica for

TABLE 1

FREQUENCY DISTRIBUTION OF CdB LEVELS OF CHILDREN FROM SANTO AMARO CITY, BRAZIL

CdB (μmole/liter)	N	%
0.004–0.049	73	18.4
0.050–0.099	129	32.7
0.100–0.149	74	18.7
0.150–0.199	50	12.6
0.200–0.249	37	9.3
0.250–0.511	33	8.3
Total	396	100.0

TABLE 2
CdB Levels according to Distance from Child's Home to Smelter Chimney

Distance ($\times 0.1$ km)	CdB (μmole/liter) GM[a]	GSD[b]	N
1	0.078	1.74	13
2	0.158	1.57	38
3	0.164	1.68	13
4	0.143	1.89	53
5	0.159	1.76	78
6	0.022	3.29	14
7	0.042	2.69	37
8	0.056	2.79	34
9	0.061	2.08	116
Total	0.087	2.52	396

[a] Geometric mean.
[b] Geometric SD.

soil or clay. Their mean CdB level was 0.088 μmole/liter (GSD = 2.5) and was very similar to that found for children who did not have this habit: 0.087 μmole/liter (GSD = 2.5).

Log CdB levels were poorly associated with TS levels ($r = 0.04$; $P > 0.05$). Among 75 iron-deficient children (TS < 15.9%) the mean CdB level was 0.083 μmole/liter (GSD = 2.4); among 275 children with adequate iron stores (TS ≥ 16.0%) the mean CdB level was 0.087 μmole/liter (GSD = 2.5).

Log CdB levels were associated with PbB levels of 383 children according to the following regression equation: log CdB = $-1.27737 + 0.07431$ (PbB). The correlation coefficient was $r = 0.23$ and the slope was statistically significant ($P < 0.00001$). the mean ($\overline{X} \pm$ SD) PbB level among these 383 children was 2.8 ± 1.2 μmole/liter.

A "stepwise" multiple regression equation (Table 8) showed that the variation toward higher log CdB levels was significantly associated with shorter distance from child's home to smelter chimney ($P < 0.00001$), residence time in the bor-

TABLE 3
CdB Levels according to Residence Time in the Area

Residence time (months)	CdB (μmole/liter) GM[a]	GSD[b]	N
1–3	0.053	3.26	15
4–6	0.075	1.86	20
7–12	0.095	1.45	23
13–24	0.067	3.35	50
25–119	0.093	2.44	284

[a] Geometric mean.
[b] Geometric SD.

TABLE 4
CdB LEVELS ACCORDING TO RACIAL GROUP

Racial group	CdB (μmole/liter) GM[a]	GSD[b]	N	P
Light	0.109	2.27	50	<0.025
Medium	0.101	2.24	122	<0.01
Dark	0.077	2.68	224	—

[a] Geometric mean.
[b] Geometric SD.

ough greater than 7 months ($P < 0.05$), heavy infection by hookworms ($P < 0.05$), and racial groups Medium ($P < 0.0005$) and Light ($P < 0.05$). The slopes of the variables of age, wasting, TS, PbB, being a child of a leadworker, presence of smelter dross at child's peridomicile, pica, and family per capita income (in cruzeiros, Brazilian currency, divided by 1000 to fit four decimal data presentation) were not statistically significant at the 5% probability level. Codes for "dummy" variables appear as footnotes to Table 8.

DISCUSSION

To a certain extent, the lack of a reference population prevents from the establishment of definitive conclusions concerning the magnitude of the levels of cadmium in blood of children from Santo Amaro City. However, it must be noted that 96% of these children had CdB levels higher than 0.0089 μmole/liter (or 1.0 μg/liter) which is usually taken as a "normal" reference level (Friberg *et al.*, 1979).

Among three children populations, aged 1 to 5 years, living near primary lead smelters in the United States of America, the mean ($\bar{X} \pm$ SD) CdB level was 0.019 ± 0.02 μmole/liter (Baker *et al.*, 1977). Studying 17 Dutch children aged 2–3 years who lived at less than 1 km from a secondary lead smelter, Zielhuis *et*

TABLE 5
CdB LEVELS ACCORDING TO WASTING INDEX

Wasting[a]	CdB (μmole/liter) GM[b]	GSD[c]	N
−5.95 to −3.00	0.099	1.79	7
−2.99 to −2.00	0.078	2.50	8
−1.99 to −1.00	0.092	2.26	42
−0.99 to −0.01	0.082	2.48	141
0.00 to 2.63	0.092	2.59	186

[a] SD.
[b] Geometric mean.
[c] Geometric SD.

TABLE 6
CdB LEVELS ACCORDING TO HOOKWORM INFECTION

Hookworm eggs per gram of feces	CdB (μmole/liter) GM[a]	CdB (μmole/liter) GSD[b]	N	P
0	0.088	2.68	276	<0.025
1–1,999	0.080	2.17	101	<0.01
2000–13,700	0.142	1.52	8	—

[a] Geometric mean.
[b] Geometric SD.

al. (1979) found a mean CdB level of 0.007 μmole/liter, ranging from 0.004 to 0.013 μmole/liter. Among 40 Belgian children aged 11 years, the mean ($\bar{X} \pm$ SD) CdB level was 0.010 ± 0.06 μmole/liter ranging from 0.002 to 0.037 μmole/liter (Roels *et al.*, 1978).

A marked effect of distance from child's home to smelter chimney was observed. Concomitantly, Table 7 showed that the presence of smelter dross in the child's peridomiciliar environment was associated with significantly higher ($P <$ 0.0001) CdB levels. However, most of the households showing this dross were placed nearer to the smelter (Carvalho, 1982). The multiple regression equation (Table 8) showed that most of the effect of the variable "smelter dross" on the variation of CdB levels was superseded by the effect of the variable "distance," which lowered its significance level markedly ($P > 0.05$). Further, the lead smelter has been increasingly changing from the use of the Brazilian lead ore to the imported one which contains a much smaller proportion of cadmium as a contaminant (Oliveira, 1977). These could be possible explanations because the smelter dross was not directly associated with increased cadmium absorption in a significant fashion.

For children living in the borough for more than 7 months, the multiple regression model predicted a mean CdB level 14% higher than that of children who lived in the borough for less than 6 months (Table 8). This finding agrees with the observations made by Kjellström and Nordberg (1978) concerning CdB levels of newly employed battery factory workers. Invariably, they observed an increase in blood cadmium concentrations during the first months of exposure.

TABLE 7
CdB LEVELS ACCORDING TO THE PRESENCE OF SMELTER DROSS IN CHILD'S PERIDOMICILIAR ENVIRONMENT

Smelter dross	CdB (μmole/liter) GM[a]	CdB (μmole/liter) GSD[b]	N
Yes[c]	0.104	2.46	184
No	0.075	2.50	212

[a] Geometric mean.
[b] Geometric SD.
[c] $P < 0.0001$.

TABLE 8

MULTIPLE REGRESSION EQUATION HAVING LOG CdB AS THE DEPENDENT VARIABLE FOR 324 CHILDREN FROM SANTO AMARO CITY, BRAZIL

Independent variable	CdB (μmole/liter) *B*	$SE_{(B)}$
Distance (0.1 km)***	−0.0569	0.0091
Residence time (months)[a],*	0.1435	0.0698
Hookworm (eggs/g of feces)[b],*	0.3124	0.1283
Racial group Medium[c],**	0.1557	0.0433
Racial group Light[c],*	0.1558	0.0660
Age (years)	0.0050	0.0085
Wasting (SD)	0.0015	0.1089
TS (%)	−0.0016	0.0023
PbB (μmole/liter)	0.0214	0.0185
Child of leadworker[d]	−0.0079	0.0417
Smelter dross[e]	0.0491	0.0417
Pica[f]	0.0357	0.0664
Family per capita income (Cruzeiros, Brazilian currency, ÷ 1000)	−0.0009	0.0206

Note. Intercept = −1.0004; R^2 = 23%. *B* = regression coefficient; $SE_{(B)}$ = SE of *B*. "Dummy" variables were coded as follows (figures in brackets represent the number of children in each group):

[a] Residence time: <6 (32) = 0; ≥7 (292) = 1.

[b] Hookworm: <1999 (316) = 0; ≥2000 (8) = 1.

[c] Racial group: dark (181) = 0; medium (105) = 1; light (38) = 1.

[d] Child of leadworker: no (249) = 0; yes (75) = 1.

[e] Smelter dross: no (171) = 0; yes (153) = 1.

[f] Pica: no (290) = 0; yes (34) = 1.

* $P < 0.05$.

** $P < 0.0005$.

*** $P < 0.00001$.

Early studies pointed out that lead-poisoned children were malnourished and iron-deficient (Watson *et al.*, 1958; Lin-Fu, 1977). Concerning increased cadmium absorption among children there is a lack of studies on the role of malnutrition (Bremner, 1979). Some studies focus on iron deficiency, which represents only part of the wider spectrum of malnutrition, and its effect on cadmium absorption. There are experimental evidences showing that cadmium is a potent inhibitor of intestinal iron absorption (Hamilton and Valberg, 1974) and that its retention is increased in iron deficiency (Valberg *et al.*, 1976). Concentrations of cadmium in blood was 13 times greater in iron-deficient rats than in controls (Ragan, 1977). In mice fed a low iron diet, the addition of low levels of cadmium to drinking water impaired growth and accentuated the development of anemia. In human subjects, cadmium absorption was much increased among iron-deficient individuals than among individuals with adequate iron stores (Flanagan *et al.*, 1978).

Among children from Santo Amaro, associations between CdB levels and malnutrition and between CdB levels and iron status were very weak. However, it is reasonable to assume that most of these children have been exposed to massive amounts of environmental lead since their earliest days of life and probably since intrauterine life. Iron deficiency, in turn, is supposed to occur later, in the first or second year of life, linked with nutritional deficiencies and developmental changes (Betke, 1970; Dallman *et al.,* 1980). If toxic effects of lead upon heme synthesis take place before the establishment of iron deficiency, serum iron concentrations are likely to increase (Waldron, 1980; Carvalho *et al.,* 1984a). Consequently, TS levels could be increased due to lead poisoning and the study of the relationship between TS and CdB levels becomes impaired.

For children from racial groups Medium and Light the multiple regression equation predicted approximate 16% elevations in mean CdB levels, compared with Dark children (Table 8). Concerning lead absorption in this population, the inverse relationship was found; that is, Dark children presented mean PbB levels higher than those from racial groups Light ($P < 0.05$) or Medium (Carvalho, 1982). Satisfactory explanations for these findings were not found.

For this population as a whole, the slope for the habit of pica for soil or clay was not significantly associated ($P > 0.05$) with the variation in log CdB levels (Table 8). However, 2-year-old children presented the highest mean CdB level compared to the rest of the population. Considering the high prevalence of iron deficiency (Dallman *et al.,* 1980) and pica (Barltrop, 1966) among toddlers plus the fact of living in an environment with soils heavily polluted by smelter dross one could suppose that the habit of pica should be strongly associated with increased CdB levels. Studying 11-year-old children (whose hand–mouth activity is certainly less intense than among toddlers), Buchet *et al.* (1980) estimated that 30% of circulating cadmium (CdB level) came from hand contamination by the metal, 30% from inhalation, and the remaining 40% from other sources (food, drink, redistribution from tissues, etc.). However, the small number of toddlers in the sample precluded the use of the multiple regression approach to this hypothesis.

Layrisse and Roche (1964) demonstrated that an infection represented by more than 2000 eggs of hookworm per gram of feces brought a good chance of produce anemia. Table 8 shows that heavy infection by hookworm was associated with a 31% increase in log CdB levels. However, the small number of individuals presenting such characteristic precludes a definitive confirmation of this finding. Increased log CdB levels among children with heavy infection by hookworm do not seem to be consequent to the iron deficiency state which usually happens in this condition of chronic blood loss (Roche and Layrisse, 1966). Hookworm infection as a risk for increased heavy metals absorption needs further investigation, mainly for its high prevalence among workers living in tropical areas with low levels of sanitation (Mendes, 1977). Hookworm infection and lead poisoning seem to act as additive, but not synergistic, factors in the causation of anemia among leadworkers from Santo Amaro (Loureiro *et al.,* 1983).

Being a child of a leadworker did not prove to be an important risk factor for increased cadmium absorption in this population (Table 8). However, children of leadworkers from Santo Amaro City had significantly higher blood lead ($P < 0.0001$) (Carvalho, 1982) and ZPP ($P < 0.05$) (Silvany-Neto, 1982) levels than children whose fathers had other types of jobs.

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