

## Urinary homovanillic acid and serum prolactin levels in children with low environmental exposure to lead

E. M. ALVAREZ LEITE<sup>1</sup>, A. LEROYER<sup>2</sup>, C. NISSE<sup>3</sup>, J. M. HAGUENOER<sup>3</sup>, C. Y. DE BURBURE<sup>4</sup>, J. P. BUCHET<sup>4</sup> and A. BERNARD<sup>4\*</sup>

<sup>1</sup> Faculdade de Farmacia da Universidade Federal de Minas Gerais – Bolsista do CNPq, Brazil

<sup>2</sup> Service d'Epidémiologie et de Santé Publique, Faculty of Medicine, Lille, France

<sup>3</sup> CERESTE-Laboratoire de Toxicologie, Faculty of Medicine, Lille, France

<sup>4</sup> Unit of Industrial Toxicology and Occupational Medicine, Faculty of Medicine, Université Catholique de Louvain, Belgium

*Received 4 April 2001, revised form accepted 21 June 2001*

Current evidence suggests that the neurotoxic effects of lead may partially be mediated through interference with the dopaminergic system. The aim of this study was to assess the levels of two peripheral dopaminergic markers – serum prolactin (Pro-S) and urinary homovanillic acid (HVA-U) – in children living around two lead smelters, who are presumed to be exposed to high environmental lead pollution ( $n = 200$ ), and compare their results with 200 age- and sex-matched controls living in an area unpolluted by heavy metals, giving a total of 400 children (200 boys and 200 girls). The influence of lead exposure on HVA-U and Pro-S was assessed by stepwise multiple regression, testing lead concentrations in blood (Pb-B), age, sex and area of residence as predictors. Though lead levels were significantly higher in boys and in the lead-polluted environment, mean Pb-B values were relatively low, indicating a low uptake of lead in the contaminated environment ( $39.5 \mu\text{g l}^{-1}$ , range  $4.6\text{--}165 \mu\text{g l}^{-1}$ ,  $n = 200$ ), and no significant correlation could be found with either Pro-S or HVA-U. However, when the subgroup of 121 children with Pb-B levels above  $50 \mu\text{g l}^{-1}$  were considered, a weak positive correlation was found between Pb-B and HVA-U ( $r^2 = 0.04$ ,  $p = 0.03$ ), whilst in the even smaller subgroup of 15 children with Pb-B levels above  $100 \mu\text{g l}^{-1}$ , Pro-S appeared to be positively correlated with Pb-B, though the numbers of children were too small for the correlation to reach statistical significance ( $p = 0.095$ ). These weak associations, probably not important in biological terms, indicate that Pro-S and HVA-U are not useful biomarkers at present exposure levels to lead in the environment. Nevertheless, the finding of subtle biochemical alterations in the dopaminergic system at Pb-B levels of around  $100 \mu\text{g l}^{-1}$  supports the recommended setting of the action level at this value.

**Keywords:** lead, neurotoxicity, biomarkers, homovanillic acid, prolactin

### Introduction

Though the neurotoxicity of lead has been extensively documented by numerous studies on occupationally or environmentally exposed populations, the mechanisms by which lead exerts its toxicity on the central nervous system remain poorly understood. Not only is lead possibly implicated in direct cellular toxicity and many cellular functions are directly or indirectly altered by its interactions, notably those with calcium-sensitive targets, several neurotransmitter systems can also be affected in different areas of the brain, sometimes in a dose-dependent and

\* Corresponding author: Alfred Bernard, Unit of Industrial Toxicology and Occupational Medicine, Université Catholique de Louvain, Clos Chapelle-aux-Champs 30, bte 3054, B-1200, Brussels, Belgium. Tel: (+ 32) 2 7643220; Fax: (+ 32) 2 7643228; e-mail: [bernard@toxi.ucl.ac.be](mailto:bernard@toxi.ucl.ac.be)

age-dependent manner (INSERM 1999). The cognitive function impairments described in children exposed to lead are a decrease of the IQ score by about 1 to 3 points with an increase in the lead concentration in the blood (Pb-B) from 100 to 200  $\mu\text{g l}^{-1}$  (WHO 1996), as well as deficiencies in short-term memory and reaction time performances, which jointly contribute to learning deficiencies (Dudek and Merez 1997).

The study presented here aimed to investigate the effects of living in a lead-polluted environment on the dopaminergic system of children. As suggested by a variety of *in vivo* and *in vitro* studies (Manini *et al.* 2000), the dopaminergic system could play an important role in the development of lead neurotoxicity. Lead may interfere with dopamine (DA) at different levels by inhibiting synaptosomal dopamine release, by altering production of dihydroxyphenylacetic acid and homovanillic acid in the mesolimbic and nigrostriatal regions, or by affecting the number and/or sensitivity of the dopaminergic receptors D1 and D2 in the tubero-infundibular region (Silbergeld and Chisolm 1976, Moresco *et al.* 1988, Pokora *et al.* 1996).

Two peripheral markers reflecting the integrity of the dopaminergic system – serum prolactin (Pro-S) and urinary homovanillic acid (HVA-U) – were selected in the hope that they would enable detection of the early neurological effects of lead exposure. Pro-S measurements are already used in the diagnosis of pituitary disorders and galactorrhoea and have been proposed for monitoring exposure to toxic agents known to affect the dopaminergic system such as styrene, manganese and aluminium (Mutti *et al.* 1984, 1996, Alessio *et al.* 1989). The experimental evidence that lead can inhibit the tubero-infundibular dopaminergic (TIDA) system, which controls prolactin secretion, offers an explanation for the positive relationships found between Pro-S and Pb-B levels in lead-exposed workers (Govoni *et al.* 1987).

HVA is one of the end-products of dopamine metabolism, produced as a result of the action of catechol-O-methyltransferase (COMT) on 3,4-dihydroxyphenylacetic acid. HVA-U already serves as a peripheral marker in the diagnosis of neuroblastoma and pheochromocytoma (Seviour *et al.* 1992, Tormey and Fitzgerald 1995) as well as in occupational exposure to carbon disulphide (Yang *et al.* 1996). Ong *et al.* (1989) reported a significant elevation of HVA-U in a lead-exposed group compared with controls. Likewise, Tang *et al.* (1995) found a positive correlation between HVA-U and Pb-B in lead-exposed workers. However, in recent study Manini *et al.* (2000) found that high levels of Pb-B (up to and above 900  $\mu\text{g l}^{-1}$ ) appeared to be negatively correlated with HVA-U.

With the exception of an early study by Silbergeld and Chisolm in 1976 on lead-intoxicated children, no study has so far used HVA-U and Pro-S as biomarkers to detect the early neurological effects of environmental lead exposure in children.

## Materials and methods

### Studied population

A total of 400 children (200 boys and 200 girls), aged 8.5–12.3 years, were studied. Of these, 200 children had lived since birth in the vicinity of two non-ferrous smelters, whereas 200 controls were recruited from an area unpolluted by heavy metals. The lead concentration in the soil of the polluted areas was on average higher than 200 p.p.m., with values as high as 1000 p.p.m. in the immediate vicinity of the factories. Subjects were recruited on a volunteer basis by sending a letter to their parents, via the schools, explaining the objectives and protocol of the study as well as the selection criteria (no

diabetes, no puberty and residence for at least 8 years in the area). Only children meeting these criteria and whose parents had given their written permission for examination of their child and sampling of blood and urine were considered for inclusion in the study. From these subjects we randomly selected a total of 200 boys and 200 girls, who constituted the final cohort for this study. Specific dietary information concerning the consumption of food either likely to be contaminated by heavy metals, such as fish, giblets, shellfish, etc., or rich in calcium was collected by means of a questionnaire. Samples from 12 children (10 girls and two boys) for whom all the biological parameters could not be determined were excluded from the analysis.

### Biological assays

Blood samples were collected and centrifuged, and 2 ml of serum was stored at  $-80^{\circ}\text{C}$  until analysis. For practical reasons, only untimed urine samples were collected. The urine samples were stored in tubes with 0.5 ml of phosphate buffer ( $1\text{ mol l}^{-1}$ ) containing 1% sodium azide.

### Analyses

HVA was assayed using an isocratic high performance liquid chromatography (HPLC) system (Gilson International model 802C manometric modules, model 305 piston pump, model 811B dynamic mixer) and an HPLC autosampler 465 (Kontron Instruments) with a Nucleosil 100-10 C18 column (Macherey-Nagel). The detector used was an ESA Coulochem II electrochemical detector with a standard model 5010 analytical cell and a model 5020 guard cell (ESA Coulochem II Multielectrode Detector, ESA Inc.). The mobile phase for isocratic elution was 100 mM citric acid solution containing 3.0% of acetonitrile, adjusted to pH 6.4. The flow rate was  $0.5\text{ ml min}^{-1}$ . HVA was extracted using 1 ml Sep-Pack Columns (Vac 3 ml C18 cartridges for solid phase extraction (Waters Corp.). The loaded column was washed with 4 ml of 10% methanol, pH 2.0. Elution was performed with 1 ml of methanol, and the eluate was evaporated at  $40^{\circ}\text{C}$  under nitrogen flow. The residue was dissolved in 1 ml of HPLC eluent (Seiler and Hiemke 1993). HVA concentrations were standardized for urinary creatinine concentration. Pro-S levels were determined using a chemiluminescent enzyme immunoassay (Immulite Prolactin, DPC). The analytical methods used to measure HVA-U and Pro-S showed a mean recovery of 92% and 107.7%, inter-assay coefficients of variation (CVs) of 18% and 8.2%, intra-assay CVs of 5% and 6.3%, and detection limits of  $0.125\text{ mg l}^{-1}$  and  $0.5\text{ }\mu\text{g l}^{-1}$ , respectively. The Pb-B assay was performed using furnace electrothermal atomic absorption spectrometry (Perkin Elmer 5100 Z), with the non-specific absorption corrected for the Zeeman effect. The measurements were made at 283.3 nm with a non-pyrolytic graphite tube (standard additions technique). The laboratory used is approved by the Ministry of Work and Solidarity to perform lead assays in blood and air. Control charts were prepared from measurements carried out on control samples with certified titres, as used by the Centre de Toxicologie du Québec and provided by UTAK laboratories (Seronom Whole Blood, levels I to III). For certified lead levels of 34, 385 and  $660\text{ }\mu\text{g l}^{-1}$ , we obtained values of 33 (CV = 9.5%), 390 (CV = 6.4%) and  $670\text{ }\mu\text{g l}^{-1}$  (CV = 5.0%) on 20 different days. Creatinine was measured in the urine using Jaffé's method.

### Statistical analysis

All variables were log transformed before statistical analysis, with the exception of age and categorical variables (sex: 0 for boys, 1 for girls; residence area: 0 for the control area, 1 for the polluted area). The need for log transformation was assessed by means of the Shapiro and Wilk test. The prevalence of Pb-B levels above 50 and  $100\text{ }\mu\text{g l}^{-1}$  in boys and girls in the two areas was studied using both the  $\chi^2$  and Fisher exact tests. The separate or combined influences of sex and area of residence (exposure) were assessed using two-way analysis of variance (ANOVA). Factors influencing HVA-U or Pro-S were also traced by stepwise multiple regression analysis, testing Pb-B, age and sex as independent variables. The stepwise multiple regression analysis was based on a  $p$  level of 0.15 for entry and of 0.05 for staying in the model. For other tests, the level of statistical significance was set at  $p < 0.05$ . The statistical package SAS/STAT (version 6, fourth edition; SAS Institute Inc., Cary, North Carolina, USA) was used for all the statistical analyses.

## Results and discussion

As shown in table 1, the children of both control and lead-polluted areas were well matched for age and sex. The Pb-B values were rather low (mean Pb-B of  $39.5\text{ }\mu\text{g l}^{-1}$  around the smelters versus  $30.5\text{ }\mu\text{g l}^{-1}$  in the control area), as illustrated in figure 1. Only 13 out of 200 lead-exposed children had a Pb-B value higher than  $100\text{ }\mu\text{g l}^{-1}$  compared with two out of 200 children in the control area ( $p = 0.006$ ,  $\chi^2$

Table 1. Biological variables in children from control and lead-polluted areas.

	Control area						Lead-polluted area					
	Girls			Boys			Girls			Boys		
	<i>n</i>	Mean $\pm$ SD <sup>a</sup>	Range	<i>n</i>	Mean $\pm$ SD <sup>a</sup>	Range	<i>n</i>	Mean $\pm$ SD <sup>a</sup>	Range	<i>n</i>	Mean $\pm$ SD <sup>a</sup>	Range
Age (years)	101	10.0 $\pm$ 0.60	8.5–11.5	99	10.2 $\pm$ 0.64	8.5–11.7	99	10.1 $\pm$ 0.70	8.5–12.3	101	10.1 $\pm$ 0.58	8.7–11.2
Pb-B ( $\mu\text{g l}^{-1}$ )	101	27.4 $\pm$ 2.00	1.6–125.8	99	33.9 $\pm$ 1.92	2.3–108.0	99	36.9 $\pm$ 1.73	7.8–165.0	101	42.2 $\pm$ 2.02	4.6–149.2
Pro-S ( $\mu\text{g l}^{-1}$ )	98	8.92 $\pm$ 1.79	2.40–38.3	97	7.39 $\pm$ 1.67	2.15–37.3	95	9.26 $\pm$ 1.72	2.75–42.0	101	8.15 $\pm$ 1.72	2.5–48.95
THVA-U ( $\text{mg g}^{-1}$ creatinine)	98	5.30 $\pm$ 1.71	0.58–18.7	99	5.58 $\pm$ 1.64	0.79–15.1	99	5.82 $\pm$ 1.76	0.22–35.1	101	5.30 $\pm$ 1.62	1.11–21.0
urinary creatinine ( $\text{g l}^{-1}$ )	99	0.90 $\pm$ 1.73	0.18–2.23	99	0.93 $\pm$ 1.60	0.19–3.07	99	0.90 $\pm$ 1.76	0.17–2.31	101	0.90 $\pm$ 1.62	0.27–2.59

<sup>a</sup>Arithmetic means for age; geometric means for Pb-B, Pro-S, HVA-U and urinary creatinine.

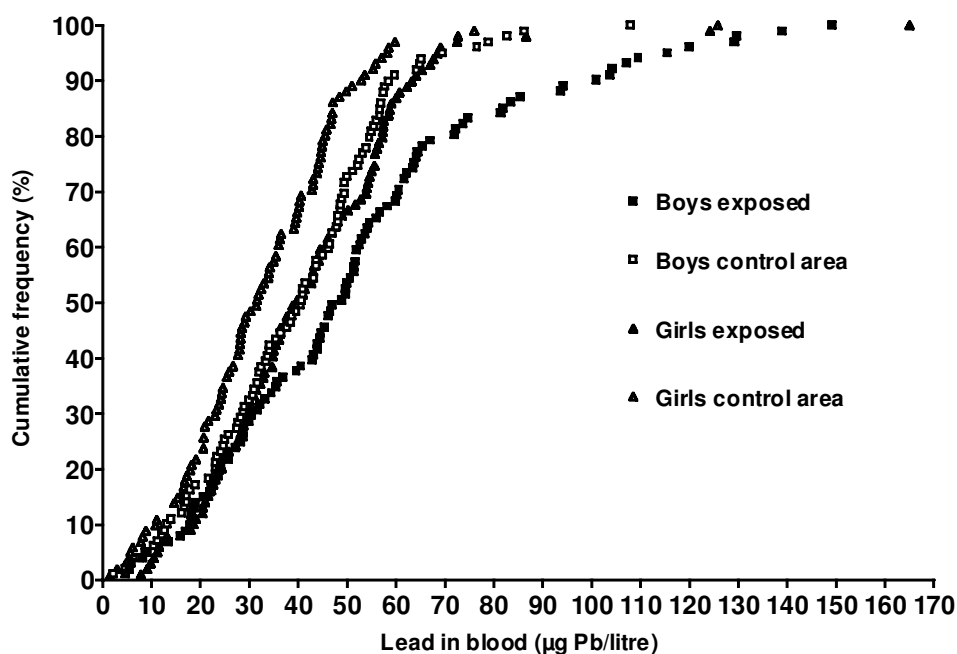


Figure 1. Cumulative frequency distributions of Pb-B levels in boys and girls in the lead-polluted and control areas (total 400 children).

test), whereas 81 and 40, respectively, had lead levels above  $50 \mu\text{g l}^{-1}$  ( $p = 0.0001$ ). These results are clearly indicative of a significant but limited uptake of the metal by the children, although soil contamination by lead exceeded  $1000 \text{ p.p.m.}$  in places. These low Pb-B values found in children living around smelters are very representative of the current situation in most areas of western Europe polluted by past emissions of lead and are thus of immediate relevance for assessing the health risks of exposed populations.

As expected from other studies (Walter *et al.* 1980, Junco Muñoz *et al.* 1996), boys had slightly higher Pb-B values than girls (table 1), probably as a result of behavioural differences since the information collected from the questionnaires did not reveal any gender difference in nutritional or environmental factors likely to influence lead uptake. Boys had similar levels of HVA-U to girls but, as expected, their Pro-S levels were lower than those of girls. The concentrations of HVA-U and Pro-S were also similar between the control and polluted areas. No interaction between sex and area of residence was found for these variables (table 2). Neither was there a difference between sex or area in the urinary creatinine concentrations, which allows the exclusion of any bias that might arise from the adjustment of HVA-U on the basis of this parameter.

The influence of lead exposure on HVA-U or Pro-S was assessed by stepwise multiple regression, testing Pb-B, age, sex and area of residence as predictors. When this analysis was performed on the whole population of children, a significant negative correlation was found with age both for HVA-U ( $r^2 = 0.03$ ,  $p = 0.0006$ ) and for Pro-S ( $r^2 = 0.02$ ,  $p = 0.0045$ ). As expected, sex emerged as a significant determinant of Pro-S ( $r^2 = 0.02$ ,  $p = 0.0071$ ). However, neither of the neurological biomarkers showed an association with either Pb-B or area of residence.

Table 2. Two-way ANOVA to assess the separate or combined influences of sex and area of residence (exposure).

	Exposure	Sex	Interaction
Pb-B	0.0001	0.0083	NS
Pro-S	NS	0.0044	NS
HVA-U	NS	NS	NS

NS, not significant.

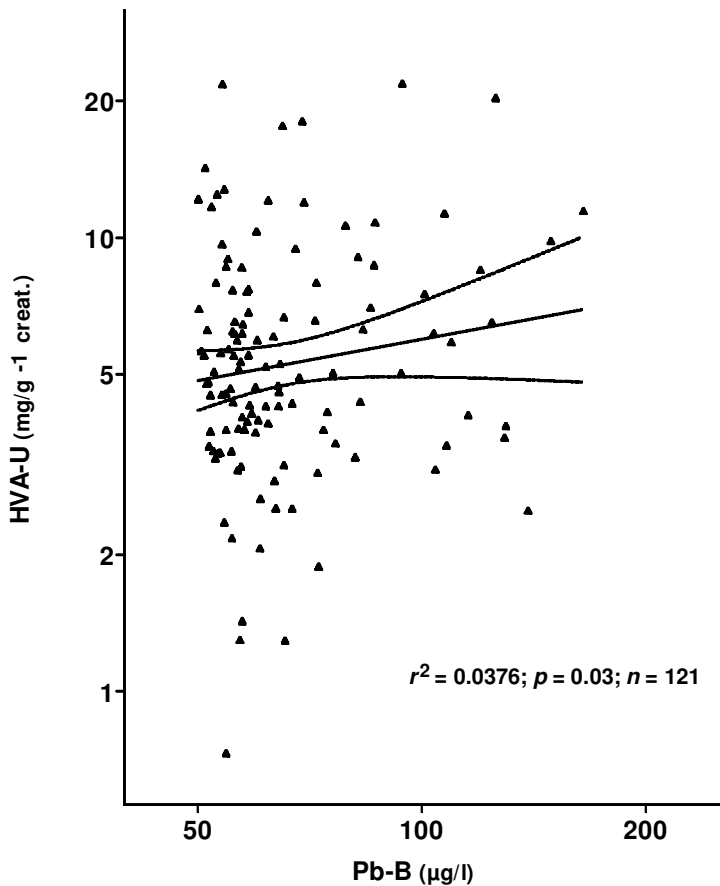


Figure 2. Correlation between HVA-U and Pb-B in children with Pb-B levels above 50 µg l<sup>-1</sup>. Values were adjusted for age (10 years) and sex (boys).

In order to further assess the possible influence of lead on these dopaminergic biomarkers, the analysis was repeated on only those children with a Pb-B higher than 50 µg l<sup>-1</sup> (121 in total, 40 of whom – 28 boys and 12 girls – lived in the control area) and then on those with a Pb-B greater than 100 µg l<sup>-1</sup>, a cut-off point that corresponds to the action level recommended by both the USA Center for Disease Control and the WHO guidelines based on population studies (see table 3). Interestingly, although Pro-S remained only influenced by sex in the subgroup of 121 children (standardized for a mean age of 10 years) with lead levels greater than 50 µg l<sup>-1</sup> (figure 2), HVA-U showed a weak but statistically significant

Table 1. Concentrations of HVA-U and Pro-S divided according to two thresholds of Pb-B (50 µg l<sup>-1</sup> or 100 µg l<sup>-1</sup>) and according to sex.

	50 µg l <sup>-1</sup> Pb-B threshold					100 µg l <sup>-1</sup> Pb-B threshold				
	≤ 50 µg l <sup>-1</sup>		> 50 µg l <sup>-1</sup>			≤ 100 µg l <sup>-1</sup>		> 100 µg l <sup>-1</sup>		
	n	Mean	n	Mean	p (t-test)	n	Mean	n	Mean	p (t-test)
HVA-U (mg g <sup>-1</sup> creatinine)	276	5.53	121	5.41	NS	382	5.46	15	6.31	NS
Pro-S (µg l <sup>-1</sup> )	271	8.57	120	8.00	NS	376	8.38	15	8.79	NS
Pb-B (µg l <sup>-1</sup> )	279	26.19	121	66.24	0.0001	385	33.03	15	120.90	0.0001
All boys										
HVA-U (mg g <sup>-1</sup> creatinine)	125	5.58	75	5.19	NS	188	5.44	12	5.40	NS
Pro-S (µg l <sup>-1</sup> )	123	8.10	75	7.25	NS	186	7.76	12	7.93	NS
Pb-B (µg l <sup>-1</sup> )	125	26.53	75	68.52	0.0001	188	35.23	12	117.15	0.0001
All girls										
HVA-U (mg g <sup>-1</sup> creatinine)	151	5.49	46	5.79	NS	194	5.50	3	11.70	0.0186
Pro-S (µg l <sup>-1</sup> )	148	8.98	45	9.43	NS	190	9.03	3	13.29	NS
Pb-B (µg l <sup>-1</sup> )	154	25.93	46	62.68	0.0001	197	31.07	3	137.12	0.0001

NS, not significant.

association with Pb-B (partial  $r^2=0.0376$ ,  $p=0.03$ ) that was independent of the association with age ( $r^2=0.0418$ ,  $p=0.02$ ). These findings are in keeping with previous studies (Ong *et al.* 1989, Tang *et al.* 1995), but have to be viewed in the light of the recent report by Manini *et al.* (2000), who, in agreement with experimental evidence that lead can inhibit DA production, described the opposite correlation for much higher values of lead (reaching up to 900 µg l<sup>-1</sup>). These contrasting findings could mean that lead has a biphasic dose-dependent effect on dopaminergic neurotransmission, as has been shown for glutamate receptor channels of the NMDA (N-methyl-D-aspartate) type (INSERM 1999), with or without combined effects on other co-transmitters (such as the effects of lead on glycine in glutamate neurotransmission). Although the association of HVA-U with Pb-B was weak (it contributed to only 3.7% of its variance) and is most probably biologically insignificant, one has to bear in mind that the strength of this association is of the same order of magnitude as associations that have been reported between IQ and Pb-B. In the even smaller subgroup of 15 children with Pb-B levels above the action level of 100 µg l<sup>-1</sup> (12 boys and three girls – one boy and one girl from the control area), only Pro-S was positively correlated with Pb-B, though the numbers of children were too small to reach statistical significance ( $r^2=0.198$ ,  $p=0.095$ ). However, this effect is again in keeping with previously reported studies (Govoni *et al.* 1987), and warrants further studies in children with lead levels around and above the action level of 100 µg l<sup>-1</sup>.

The potential biomarkers of lead-induced dopaminergic dysfunction were selected on the basis of previous studies showing associations between Pb-B and the levels of Pro-S and HVA-U in workers or children exposed to lead (Govoni *et al.* 1987, Ong *et al.* 1989, Tang *et al.* 1995). These studies, however, were characterized by exposure levels to lead that, on average, were at least one order of magnitude higher than the values found in the present population. This difference in exposure intensity probably explains why we could not find any significant association between Pb-B and the two biomarkers in the whole population studied, and only began to find associations when studying groups of children with lead

concentrations above  $50 \mu\text{g l}^{-1}$ . In addition, at such low levels, one cannot formally exclude the possibility of secondary associations generated by a third factor (nutritional, metabolic, genetic, etc.) influencing both biomarker metabolism and the intake/uptake of lead. It should also be noted that one of the biomarkers – HVA-U – was measured on untimed urine samples and expressed per gram of creatinine. Although this procedure is commonly adopted for most urinary biomarkers, it provides a less accurate estimate of the urinary output of HVA than a timed urine collection, and this might have decreased the association with Pb-B in our study. HVA-U and Pro-S thus remain potential biomarkers of lead effects, but at higher exposure levels than in the present study.

## Acknowledgements

This study was supported by the French Association for Metals and Health (AMSE) and the European Commission (ENV4-CT96-0171). A. Bernard is Research Director of the National Fund for Scientific Research (Belgium).

## References

- ALESSIO, L., APOSTOLI, P., FERIOLI, A., DI SPIO, I., MUSSI, I., RIGOSA, C. and ALBERTINI, A. 1989, Behavior of biological indicators of internal dose and some neuroendocrine tests in aluminium workers. *Medicina Lavoro*, **80**, 290–300.
- DUDEK, B. and MERECZ, D. 1997, Impairment of psychological functions in children environmentally exposed to lead. *International Journal of Occupational Medicine and Environmental Health*, **10**, 37–46.
- GOVONI, S., BATTAINI, F., FERNICOLA, C., CASTELLETTI, L. and TRABUCCHI, M. 1987, Plasma prolactin concentration in lead exposed workers. *Journal of Environmental Pathology and Toxicological Oncology*, **7**, 13–15.
- INSERM. 1999, Plomb dans l'environnement – quels risques pour la santé? Paris: INSERM, 461 pp.
- JUNCO MUÑOZ, P., OTTMAN, R., LEE, J. H., BARTON, S. A., RIVAS, F. and CERDA FLORES, R. M. 1996, Blood lead concentration and associated factors in residents of Monterrey, Mexico. *Archives of Medical Research*, **27**, 547–551.
- MANINI P., ANDREOLI, R., CAVAZZINI, S., BERGAMASCHI, E., MUTTI, A. and NIESSEN, W. M. A. 2000, Liquid chromatography-electrospray tandem mass spectrometry of acidic monoamine metabolites. *Journal of Chromatography B*, **744**, 423–431.
- MORESCO, R. M., DALL'OLIO, R., GANDOLFI, O., GOVONI, S., DIGNOVANE, S. and TRABUCCHI, M. 1988, Lead neurotoxicity: a role for dopamine receptors. *Toxicology*, **52**, 315–322.
- MUTTI, A., VESCOVI, P., FALZOI, M., ARFINI, G., VALENTI, G. and FRANCHINI, I. 1984, Neuroendocrine effects of styrene on occupationally exposed workers. *Scandinavian Journal of Work and Environmental Health*, **10**, 225–228.
- MUTTI, A., BERGAMASCHI, E., ALINOV, R., LUCCHI, R., VALENTI, G. and FRANCHINI, I. 1996, Serum prolactin in subjects occupationally exposed to manganese. *Annals of Clinical Laboratory Science*, **26**, 10–17.
- ONG, C. N., CHIA, K. S., KOH, D. and SAJJOH, K. 1989, Neurochemical effects of lead exposure: a study on catecholamine metabolism. *American Journal of Industrial Medicine*, **16**, 667–673.
- POKORA, M. J., RICHFIELD, E. K. and CORY-SLECHTA, D. A. 1996, Preferential vulnerability of nucleus accumbens dopamine binding sites to low-level lead exposure: time course of effects and interactions with chronic dopamine agonist treatments. *Journal of Neurochemistry*, **67**, 1540–1550.
- SEILER, W. and HIEMKE, C. 1993, Determination of homovanillic acid in human plasma using HPLC with electrochemical detection and automated solid phase extraction. *Journal of Liquid Chromatography*, **16**, 2813–2823.
- SEVIOUR, J. A., MCGILL, A. C., CRAFT, A. W., PARKER, L., BELL, S., COLE, M., SMITH, J., HAWKINS, E., BROWN, J. and DALE, G. A. 1992, Screening for neuroblastoma in the northern region of England. Laboratory aspects. *American Journal of Pediatric Hematology and Oncology*, **14**, 332–336.
- SILBERGELD, E. K. and CHISOLM, J. JR 1976, Lead poisoning: altered urinary catecholamine metabolites as indicators of intoxication in mice and children. *Science*, **192**, 153–155.



- TANG, H. W., LIANG, Y. X., HU, X. H. and YANG, H. G. 1995, Alterations of monoamine metabolites and neurobehavioral functions in lead-exposed workers. *Biomedicine and Environmental Science*, **8**, 23–29.
- TORMEY, W. P. and FITZGERALD, R. J. 1995, Pheochromocytoma – a laboratory experience. *International Journal of Medical Science*, **164**, 145–145.
- WALTER, S. D., YAKEL, A. J. and VON LINDERN, I. H. 1980, Age-specific risk factors for lead absorption in children. *Archives of Environmental Health*, **35**, 53–58.
- WHO 2000, *Air Quality Guidelines for Europe. Second Edition*. WHO Regional Publications, European Scenes No: 97, Copenhagen: WHO Regional Office for Europe, 49–153.
- YANG, X. F., LEE, B. L., NEW, A. L., ONG, H. Y., MA, L., ZHANG, Q. and ONG, C. N. 1996, Urinary homovanillic acid and vanillylmandelic acid in workers exposed to carbon disulfide. *American Journal of Industrial Medicine*, **29**, 269–274.