

**Universidade de São Paulo
Faculdade de Saúde Pública**

**Exposição a chumbo e comportamento anti-social
em adolescentes**

Kelly Polido Kaneshiro Olympio

**Tese apresentada ao Programa de Pós-Graduação em
Saúde Pública para obtenção do título de Doutor em
Saúde Pública.**

Área de Concentração: Saúde Ambiental

Orientador: Profa. Dra. Wanda Maria Risso Günther

Co-orientador: Prof. Dr. Etelvino José H. Bechara

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DEDICATÓRIA

Ao meu Senhor e Salvador **Jesus Cristo**

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RESUMO

Olympio KPK. **Exposição a chumbo e comportamento anti-social em adolescentes** [Tese de Doutorado]. São Paulo: Faculdade de Saúde Pública da USP; 2009.

Introdução- A intoxicação por chumbo é um conhecido problema de saúde pública e o envenenamento por este metal pode causar danos a vários órgãos, especialmente ao Sistema Nervoso Central de crianças em desenvolvimento. **Objetivo geral-** estudar a associação entre exposição a chumbo e comportamento anti-social (CAS) em adolescentes brasileiros. **Objetivos específicos:** a) analisar a associação entre exposição a chumbo e CAS / cometimento de atos infracionais (CAI); b) estudar potenciais fontes de exposição domiciliar a chumbo que mais estão associadas a altas concentrações de chumbo no esmalte dentário (CCED) e; c) avaliar o impacto de alterações metodológicas na técnica de microbiópsia ácida de esmalte dentário superficial (MAEDS) sobre CCED e profundidade da biópsia. **Métodos-** Um estudo transversal foi conduzido com 173 jovens (Bauru, SP). MAEDS foram realizadas nestes jovens por dois diferentes protocolos metodológicos. Além disso, questionários sobre comportamento dos adolescentes e exposição a possíveis fontes de contaminação por chumbo foram aplicados a pais e adolescentes. Análises de regressão logística, testes de Wilcoxon e testes t pareados foram aplicados aos dados. **Resultados-** *Odd ratios* ajustados para covariáveis indicaram que alta CCED está associada a risco aumentado de exceder o escore clínico para queixas somáticas, problemas sociais, comportamento de quebrar regras e problemas externalizantes (IC 95%). Alta CCED não foi associado com escores elevados de CAI. Os fatores de risco mais associados com alta CCED foram residir em área contaminada ou até 2 km da área contaminada e trabalhar na fabricação de tintas, pigmentos, cerâmicas ou baterias. A profundidade da biópsia, calculada pela fórmula da altura do cilindro, para

um dos protocolos, levou a resultados errôneos de profundidade da biópsia, confirmados por testes de perfilometria. **Conclusões-** A exposição a altos níveis de chumbo parece disparar o estabelecimento de CAS, o que alerta para a necessidade de desenvolvimento e implantação de políticas públicas de saúde que previnam o envenenamento da população por chumbo. Adolescentes foram expostos ao chumbo por algumas fontes estudadas, no Brasil. O esmalte dentário é um marcador fidedigno e a MAEDS é bastante útil e confiável. No entanto, CCEDs não podem ser comparadas entre resultados de pesquisas diferentes quando houver qualquer variação metodológica entre os estudos, havendo a necessidade da padronização do procedimento.

Descritores: Intoxicação por chumbo; Intoxicação do sistema nervoso por chumbo; Transtorno da conduta; Transtornos do comportamento social, Violência; Razão de chances; Fatores de risco; Esmalte dentário; Biópsia; Biomarcador; Chumbo; Saúde ambiental.

ABSTRACT

Olympio KPK. **Lead exposure and antisocial behavior in Brazilian adolescents** [Thesis]. São Paulo (BR): Faculdade de Saúde Pública da Universidade de São Paulo; 2009.

Introduction- Lead poisoning is a long known public health problem. Thus, lead exposure may cause damage to diverse organs, especially in the Central Nervous System of children in developing process. **Objectives-** a) to analyze the association between lead exposure and antisocial / delinquent behavior; b) to study the potential sources of lead home exposure more associated to high dental enamel lead levels (DELL) and c) to evaluate two distinct enamel biopsy protocols in relation to biopsy depth and DELL. **Methods-** A cross-sectional study was conducted with 173 adolescents (Bauru, SP, Brazil). Surface dental enamel (SDE) etch-acid microbiopsies were performed in upper central incisors of these youths by two different methodological protocols. In addition, questionnaires about adolescents' behavior and about possible sources of lead contamination were responded by youths and their parents. Logistic regression, Wilcoxon and paired *t* tests were applied to data. **Results-** *Odd ratios* adjusted for familial and social covariates indicated that high DELL is associated with increased risk of exceeding the clinical score for somatic complaints, social problems, rule-breaking behavior ($T \geq 70$) and externalizing problems ($T \geq 63$) (CI 95%). High DELL was not found to be associated with elevated SRD scores. The risk factors associated to high DELL were residing in contaminated area or close

proximity and working in paints, pigments, ceramic or batteries manufacturing. The biopsy depth, calculated by the cylinder formula, for Protocol II induced misleading results, as confirmed by profilometry tests.

Conclusions- It seems that exposure to high lead levels can indeed trigger antisocial behavior, which claims for public policies to prevent lead poisoning. Adolescents were exposed to lead, by some studied sources, in Brazil. SDE, measured by etch-acid microbiopsy, is a reliable biomarker, but DELL could not be compared when there is some methodological variation among the studies. A standardization of the procedure is necessary.

Descriptors: Lead poisoning; Lead poisoning, Nervous System; Conduct disorder; Social behavior disorders; Violence; Odds ratio; Risk factors; Dental enamel; Biopsy; Biomarker; Lead; Environmental health.

APRESENTAÇÃO

Esta tese de Doutorado é fruto de um longo e árduo trabalho desempenhado durante quatro anos. Como cirurgiã-dentista, interessada pelas questões de Saúde Pública, sempre senti necessidade de ter uma experiência maior de interdisciplinaridade, já que o meu Mestrado em Odontologia em Saúde Coletiva foi unicamente voltado para a Odontologia sem um aprofundamento na interface com outras especialidades, interface essa que a Saúde Pública apresenta como característica inerente da área. Quando decidi que iria fazer meu Doutorado na Faculdade de Saúde Pública da Universidade de São Paulo, resolvi que meu objetivo era ultrapassar a Odontologia em Saúde Coletiva, alcançando a Saúde Coletiva em sua essência. Mesmo assim, a princípio, nunca imaginei que este desejo seria transformado em realidade, cujo resultado não foi um trabalho interdisciplinar, mas um trabalho transdisciplinar, no qual as diversas áreas envolvidas contribuiriam tanto e na mesma proporção para que os esforços de todas estas subáreas unidas – Saúde Ambiental, Bioquímica, Química Analítica, Epidemiologia, Estatística, Toxicologia, Psicologia, Medicina, Odontologia, Sociologia, Matemática, se não me esqueço de mais nenhuma – resultassem nos quatro artigos apresentados neste volume.

De forma a melhorar a compreensão do presente estudo, é importante que seja entendida a morbidade psiquiátrica, cuja associação está sendo estudada com relação à concentração de chumbo no esmalte dentário. O

comportamento anti-social é considerado como sendo a sintomatologia central do transtorno da conduta, a mais freqüente categoria de transtornos psiquiátricos em crianças e jovens. O quadro clínico do transtorno da conduta é caracterizado por comportamento anti-social persistente com violação de normas sociais ou direitos individuais. Crianças e adolescentes com transtorno da conduta costumam agredir e maltratar pessoas e animais, destruir propriedade alheia, envolver-se em roubos e assaltos e violar as regras estabelecidas. Quando o quadro clínico é mais grave, pode ocorrer o cometimento de assaltos com a utilização de armas, estupros e homicídios (BORDIN 1996).

Inicialmente, o delineamento do projeto de pesquisa baseou-se em um estudo de caso-controle, onde os casos foram criteriosamente definidos como adolescentes julgados por cometimento de ato infracional e sentenciados a regime de internato na antiga Fundação para o Bem Estar do Menor (FEBEM), atual Fundação Casa. Três unidades foram selecionadas e tiveram a anuência da instituição para que o trabalho fosse realizado: unidade de Bauru-SP, Rio Dourado e Vitória Régia (Lins-SP). O trabalho de campo foi iniciado na unidade de Bauru-SP. Todos os adolescentes desta unidade foram examinados e entrevistados, assim como também foram entrevistados seus responsáveis. No entanto, a grande maioria das amostras de esmalte dentário coletadas na Fundação Casa apresentou contaminação por chumbo e muitas amostras não puderam ser consideradas na pesquisa. Na investigação da fonte de contaminação, constatei que os tubos utilizados na coleta de material apresentavam

chumbo em concentrações aleatórias. Esta foi uma grande perda, pois o trabalho na unidade havia sido extremamente desgastante e a descontaminação prévia dos tubos já havia sido discutida antes do início do trabalho, não sendo realizada e resultando no fato exposto. Além disso, neste cenário, a presidência da FEBEM (nomeada assim, na época) foi alterada e nossa permissão para entrada nas unidades foi suspensa. Mesmo após todos nossos apelos para que nos fosse autorizada a continuação da pesquisa, não pudemos concretizar o trabalho na instituição.

Nossos controles, segundo o projeto inicial, viriam de escolas da rede pública de ensino, adolescentes com características semelhantes aos casos, com exceção da condição de cometimento de ato infracional. A direção regional de ensino de Bauru, na pessoa da Sra. Vera Nilce L. Jarussi Gomes de Sá, negou a autorização para a realização da pesquisa nas escolas da rede, alegando que a pesquisa geraria “polêmica entre os familiares dos alunos e prejudicaria a rotina das secretarias das escolas com as dúvidas que seriam levantadas pelos pais, os quais procurariam as secretarias para tirar estas dúvidas”. A importância da pesquisa foi destacada, a manutenção da ordem da rotina escolar foi assumida como compromisso, mas a dirigente mostrou-se totalmente refratária a colaborar com o projeto, negando bruscamente a autorização para o desenvolvimento da presente pesquisa.

Após todos estes fatos, excluindo a opção de mudança de projeto, restou-me a opção de mudança de delineamento. O recrutamento de adolescentes de bairros com altos índices de criminalidade surgiu, neste contexto, como único cenário amostral possível. Assim, as visitas a projetos

sociais que atendiam jovens residentes destas áreas, com visitas domiciliares nos locais onde as excursões pelo bairro eram possíveis, agregando ainda jovens pela técnica epidemiológica da bola de neve foram as estratégias utilizadas para que adolescentes em situação de risco de apresentarem comportamento anti-social fossem encontrados para comporem nossa amostra. No bairro onde as visitas domiciliares representavam um risco à integridade dos pesquisadores, jovens da comunidade foram contatados para a realização destas visitas e, assim, foi composta a amostra apresentada nos artigos que compõem o capítulo de resultados e discussão deste texto.

Esta apresentação se faz necessária para que os leitores compreendam o que levou uma cirurgiã-dentista a se embrenhar por uma pesquisa de caráter tão diferente das outras que vinha realizando até então, apesar da Toxicologia estar presente nas minhas pesquisas, no que toca ao estudo do flúor como causa da fluorose dentária. Além disso, a exposição de todos os imprevistos ocorridos faz o leitor se lembrar dos caminhos acidentados pelos quais muitas pesquisas avançam, cujos esforços e méritos são coroados com a publicação dos resultados destas pesquisas em revistas reconhecidas pela comunidade científica, ultrapassando a impressão de volumes, os quais, muitas vezes, só servem para se juntar a outros em prateleiras de bibliotecas, sendo consultados em uma freqüência ínfima quando comparada à visibilidade dada a estes mesmos textos transformados em artigos científicos.

Este volume é composto por quatro artigos submetidos ou que serão submetidos a periódicos científicos. Um artigo de revisão de literatura (*Manuscrito I*) substitui a introdução da tese. Esta revisão de literatura foi redigida após a constatação de que há poucas publicações científicas que se dedicaram a discutir o estabelecimento de comportamento anti-social associado à exposição ao chumbo, além da necessidade do desenvolvimento de políticas públicas que protejam a população brasileira deste perigo. Esta revisão também é resultado de um trabalho realizado no início do meu Doutorado para que eu me inteirasse do assunto e pudesse estudar o conteúdo envolvido com o meu problema de pesquisa. Como era nosso objetivo levantar a discussão entre os profissionais de Saúde Pública e formuladores de políticas públicas de saúde, este artigo será publicado na Pan American Journal of Public Health / Revista Panamericana de Salud Pública, a qual é uma publicação da Organização Panamericana de Saúde Pública (OPAS).

Os capítulos referentes aos resultados e discussão, estão sendo representados por três artigos: *Manuscrito II*) "Surface dental enamel lead levels and antisocial behavior in Brazilian adolescents" (preparado para a Neurotoxicology and Teratology, FI=2,444); *Manuscrito III*) "Risk factors associated with high lead levels measured in the surface dental enamel from Brazilian youths" (submetido ao Bulletin of the World Health Organization, FI=4,019); e *Manuscrito IV*) "Methodological alterations of surface dental enamel microbiopsies for lead body burden measurement" (submetido à Toxicological Sciences, FI=3,814). O primeiro destes artigos é fruto do nosso

problema de pesquisa principal – o estudo da associação entre exposição a chumbo e comportamento anti-social. Como nós aplicamos um questionário referente à identificação de possíveis fatores de confusão, aproveitamos também para investigar possíveis fontes de exposição a chumbo que estivessem presentes na rotina familiar daqueles adolescentes, o que resultou no segundo artigo apresentado. No transcorrer do trabalho, diferenças metodológicas e de resultados foram sendo detectados entre o nosso trabalho e de outros grupos de pesquisa, o que gerou a curiosidade científica de se estudar mais especificamente o esmalte dentário como marcador biológico para chumbo, gerando o quarto artigo fruto da tese e terceiro artigo do capítulo de resultados e discussão.

Espero com esta apresentação e após a realização desta pesquisa, realizados com todo o rigor científico e carinho pessoal, poder ter contribuído para a Ciência, além de tornar mais agradável o trabalho do leitor que se aventurar a avaliar criticamente ou consultar displicentemente este volume.

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LISTA DE ABREVIATURAS

ADM -	Assessment Data Manager
AFQT -	Armed Forces Qualification Test
AIP -	Acute Intermittent Porphyria
ALA -	5-aminolevulinic acid
ALAD -	Delta-aminolevulinic acid dehydratase
CAS-	Comportamento anti-social
CAI-	Cometimento de atos infracionais
CBCL -	Child Behavior Checklist
CCED -	Concentração de Chumbo no Esmalte Dentário
CDC -	Centers for Disease Control and Prevention
CEP -	Cumulative Exposure Project
CETESB -	Companhia de Tecnologia de Saneamento Ambiental
CI -	Confidence Interval
CNPq -	Conselho Nacional de Desenvolvimento Científico e Tecnológico
CNS -	Central Nervous System
CONAMA -	Conselho Nacional do Meio Ambiente
DELL -	Dental Enamel Lead Levels
DNA -	Deoxyribonucleic acid
DSM -	Diagnostic and Statistical Manual of Mental Disorders
EC-THGA -	End-Capped Transverse-Heated Graphite Atomizers

EIA-RIMA -	Estudo de Impacto Ambiental – Relatório de Impacto Ambiental
EPA -	Environmental Protection Agency
FAPESP -	Fundação de Amparo à Pesquisa do Estado de São Paulo
FI -	Fator de Impacto
GABA -	Ácido gama-aminobutírico
GFAAS -	Graphite Furnace Atomic Absorption Spectrometry
HCl -	Cloridric acid
HNO ₃ -	Nitric acid
5-HIAA -	5-hydroxyindoleacetic acid
IC -	Intervalo de Confiança
ICP-OES -	Inductively Coupled Plasma Optical Emission Spectrometry
IP -	Instituto de Psicologia
IQ -	Intelligence Quotient
KHN -	Koop Hardness Number
KH ₂ PO ₄ -	Potassium dihydrogen phosphate
Km -	Kilometer
LTCIP -	Laboratório de Terapia Comportamental do Instituto de Psicologia
MAEDS-	Microbiópsia ácida de esmalte dentário superficial
Mg -	Magnesium
Mg(NO ₃) ₂ -	Magnesium nitrate
NHANES -	National Health and Nutrition Examination Surveys

NLSY -	National Longitudinal Survey of Youth
NMDA -	N-Metil-D-Aspartato
OR -	Odds ratio
P -	Phosphorus
Pb -	Lead
ppb -	Parts per billion
ppm -	Parts per million
Pb(NO ₃) ₃ -	Lead nitrate
PNS -	Peripheral Nervous System
Pd (NO ₃) ₂ -	Palladium nitrate
SD -	Standard Deviation
SDE -	Surface Dental Enamel
SP -	São Paulo
SRD -	Self-Reported Delinquency
UN -	United Nations
USA -	United States of America
USP -	Universidade de São Paulo
Zn -	Zinc
WHO -	World Health Organization

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*"(...) Portanto, não importa quão pequeno
seja o começo; o que é bem feito uma
vez, está feito para sempre."*

Henry David Thoreau

Neurotoxicity and aggressiveness triggered by low-level lead in children – a review

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Abstract

Lead is a ubiquitous, silent and devastating metal toxin, used since ancient times. Lead-induced neurotoxicity acquired by low-level long-term exposure to the metal has special relevance for children. A plethora of recent reports has demonstrated a direct link between low-level lead exposure and deficits in the neurobehavioral-cognitive performance manifested from childhood through adolescence. In many studies, aggressiveness and delinquency have also been suggested as symptoms of lead poisoning. Several environmental, occupational and domestic sources of contaminant lead and consequent health risks are largely identified and understood, but the occurrences of lead poisoning remain numerous. There is an urgent need for public health policies to prevent lead poisoning so as to reduce individual and societal damages and losses. In this paper we point out unsuspected sources of contaminant lead, discuss the economic losses and urban violence possibly associated with the metal and review the molecular bases of lead-induced neurotoxicity, emphasizing its effects on children's and teenagers' social behavior, delinquency and IQ.

Key words: lead poisoning, neurotoxicity syndromes, oxidative stress, juvenile delinquency.

Introduction

Lead (Pb) is known to be toxic to human health since ancient times. In 200 BC, Dioscorides stated prophetically that “lead makes the mind give way” (1). Indeed, lead is now recognized as a devastating neurotoxin. The widespread contamination of the environment by lead, the metal’s propensity to cause a wide spectrum of toxic effects, and the millions of people affected worldwide, both in poor and developed nations, make this insidious and ubiquitous neurotoxicant a public health problem of global magnitude and concern (2).

The levels of lead considered tolerable and putatively non-toxic for children have been repeatedly lowered over the last three decades (3,4,5). Using venous blood lead levels as a lead poisoning marker, the upper acceptable limit for children was 60 µg/dL in the early 1960s, a level capable of engendering overt physical symptoms (6). In 1970, after the recognition that even lower blood lead levels may not produce overt physical symptoms but can cause brain damage (7), the lead threshold was reduced to 40 µg/dL. Since then, the upper limit of “acceptable” blood lead levels has been successively lowered. In 1975, it was reduced to 30 µg/dL, in 1985 to 25 µg/dL, and finally, in 1991, the Centers for Disease Control and Prevention (CDCs) definition of childhood lead poisoning set 10 µg/dL as the screening action guideline. According to Bellinger (8), although this value only intends to serve for a risk guidance and management tool, it probably permeates biological significance for the individual child. Indeed, 10 µg/dL blood may be

taken as a threshold; therefore, a level of $<10 \mu\text{g/dL}$ can be viewed as safe and a higher level as toxic.

In truth, no single number can be cited as a threshold without considering contextual factors such as endpoint of interest, the age of the individual at exposure and assessment, the duration of blood level elevation, and characteristics of the child-rearing environment (8). Recently, studies by Lanphear et al (9) and Canfield et al (10) have shown intellectual impairment in children with blood lead concentrations below $10 \mu\text{g/dL}$, and Chiodo et al (11) have demonstrated child neurobehavioral deficits linked to $3 \mu\text{g/dL}$ concentrations.

We point out herein unexpected sources of contaminant lead and review the molecular bases of the neurotoxicity induced in children by low-level lead, emphasizing its effects on social behavior, criminality and IQ. Finally we discuss the urgent need for public health policies designed to prevent lead poisoning.

Lead toxicity: a short history

According to Needleman (5), childhood lead poisoning was first recognized only one century ago. He formally divided the temporal accumulation of scientific information on lead poisoning into four stages. First, reports on lead-poisoned children in Brisbane (Australia) in 1892, although having reached epidemic proportion, were received with widespread disbelief. Many of the homes in Brisbane were raised on piles, with large

wooden-enclosed verandas that served as play areas for children. The rails were painted with white lead, which chalked and powdered under the hot Brisbane sun (12). The origin of the epidemic - lead-containing paint - was established in 1904, and lead paint was banned for household use in Brisbane in 1920 (5). Today, the Environmental Protection Agency (EPA, USA) establishes that paints must not contain more than 0.06% of lead in their formulation.

The first report of infantile lead poisoning in the United States was presented by Blackfan (12), in 1914, according to Needleman (5). In this second stage of the lead poisoning history, only two outcomes were recognized: death or complete recovery without any sequelae. This misconception was refuted in 1943 with the first follow-up of children who had recovered from acute toxicity. Previously, Levinson and Harris (13) recommended that children should perhaps be followed on a long-term basis to ascertain possible neurobehavioral disturbances. In 1943, lead poisoning sequelae were well documented by Byers and Lord, who studied 20 children who had symptomatic lead poisoning in early childhood and were followed until school age. They found that 19 out of the 20 children presented aggressive, antisocial, and uncontrollable behavior (14). Thus, in this third stage, it was generally accepted that lead toxicity caused long-term neurological impairments, although these deficits were thought to occur only in children who had displayed clinical signs of encephalopathy during the acute episode. The fourth stage began in the 1970s, when studies of children

with no clinical signs of toxicity showed deficits in IQ scores, attention, and language (15,16,17).

Major sources of lead exposure

Lead is naturally encountered in the Earth lithosphere at concentrations of c.a. 13 mg/kg. From the early sixth millennium BC, several ancient civilizations were already employing lead to manufacture tableware, trays and other decorative objects. The Romans believed that lead - the “oldest” metal - was a precious gift of the Gods’ father, Saturn (Khronos, for the Greeks), as they used lead to construct aqueducts to draw water from the hills to Rome and to prepare lead acetate, a sweetener of wine daily consumed by the Roman aristocrats. The name *saturnism* for lead poisoning was coined after Saturn.

Lead occurring naturally or anthropogenically is encountered in all environmental compartments, including air, water, soil, biota and human beings. Inhalation, intake and dermal contact are described as distinct pathways of human exposure. Lead may be ingested directly from contaminated water, air, and soil and indirectly by consuming animals, plants and their derivatives. Environmental or occupational exposure may be aggravated by inadequate protective behaviors, habits as well as socio-economic factors. Lead is encountered in food, batteries, solders, plastics, household paints and gas, but also in pottery utensils, glass nursing-bottles, toys, glazed pottery, granite floors, calcium supplements, herbal medicines,

wild game, facial make-up and cigars. Lead affects the brain, kidneys, liver, blood and testicles, leading to disturbances of learning, attention, IQ, memory, hearing, sociability, hypertension, anaemia, nephropathies, sterility and encephalopathies, in a lead level-dependent poisoning degree (Figure 1). Human uses of lead increased during the Industrial Revolution and in the early 20th century, when there was high demand for anti-knock leaded gas, lead-containing paints, canned foods, and car batteries. Importantly, the population of American children with blood lead levels over 10 µg/dL declined by 80% since lead was banned from gas, solder in canned food, and house paint (18). The World Health Organization recommends constant research on the various “silent” sources of lead exposure (19). Vigilance is crucial and must be shared through community awareness, and by the better control of the use of products suspected to contain lead as well as stricter surveillance/testing of imported goods (20).

According to Goyer (21), exposure to lead-contaminated food was most likely to have occurred from cans containing lead solder in the joints. Outlawing of lead solder in canned food is estimated to have reduced the average dietary intake of lead in 2-year-old children from 30 µg/day in 1982 to approximately 2 µg/day in 1991 (21,22,23). While banned in the US, lead solder continues to be used in other countries, resulting in elevated lead levels in some imported canned foods (22,23). Additional but less common than other sources of exposure to dietary lead are ethnic food, dietary

supplements, folk medicines and moonshine. Hair/eyelash/eyebrow dyes can be sources of dermal lead poisoning (23,24).

Ingestion of lead does not occur solely through dietary sources. Currently, lead-containing paint sold in the USA between 1884 and 1978 is the major source of lead ingestion in young American children (22,23). Although banned in household paint since 1971, 80% of US houses built before 1950, or 23 million units, contain leaded paint (5). In 2002, it was reported that 65% of 38 million housing units in the United States were painted with products considered lead-based hazard (25). The replacement of highly toxic lead compounds in white paints by very expensive titanium oxide-based paints and, hopefully in the near future, for non-toxic and inexpensive aluminum phosphate or polyphosphate-based pigments (Biphor[®], Bunge Co.) (26) represents novel technologies to prevent lead poisoning from paints.

Drinking water can also be contaminated by lead, either at the source due to deposition from environmental sources or in the water distribution system. The U.S. National Primary Drinking Water Regulations for Lead and Copper state that water is unsafe if 10% of a municipality's test sample is determined to have lead levels greater than 15 ppb (27,25). Several authors have pointed out that the municipal infrastructure distribution systems contain components that may leach lead, such as lead service lines that connect the water main to the consumer's residence, lead pipes that supply water to the inside of the residence, copper supply pipes that have been joined using lead

solder and lead-containing brass pipes and fixtures that can contain up to 8% lead (28,29). A recent survey of the Washington, DC area by the CDC (30), the DC Department of Health and the US Public Health Service estimate that 18% of over 30% of the analyzed population residing in this area had blood lead levels above 5 µg/dL, leading to cognitive deficits in children (9,10).

In Brazil, Teixeira *et al* (31) have found that 11% of pipes used in the water distribution systems of 100 schools of São Paulo present lead levels higher than the limit considered safe by the WHO. In 2% of the water samples, the lead level was found to be five-fold higher than the recommended value (<10 µg/L, according to the WHO), thereby threatening children's neuropsychiatric health. This information raises the possibility of lead-containing pipes being used in the water distribution system of São Paulo's schools. According to the São Paulo State Health Department (18), about 80% of the lead found in urban air samples before 1982 was derived from leaded gas. Car battery manufacture is the main source of secondary lead (32), but other sources cannot be discarded.

Several studies have shown that high blood lead levels of preschool children are strongly correlated with high lead levels in house dust (33,34,35). This association has been attributed to dust intake from the frequent hand-to-mouth behavior of young children. Flaking lead-based paint, road dust, garden soil and airborne lead-bearing particles are believed to be the sources of lead in household dust (36).

Leaded gas has caused more exposure to the metal than any other source worldwide (37). Thus, it is not surprising that there is consensus among international bodies, such as the World Bank, WHO and the UN Commission on Sustainable Development, that countries must give up leaded gas for the sake of public health. In 1994, the UN commission called on governments worldwide to switch from leaded to unleaded petrol. Nevertheless, up to year 2000, only 42 countries, including China, New Zealand, the US, some Western and Eastern European countries, and several Latin American countries, had phased out or were phasing out lead from gas. India and a dozen or more countries in Latin America and Western Europe were committed to making the shift by 2005, while the remaining 150 or so countries in the world have still not had decided (38).

Biochemical mechanisms of lead toxicity

Lead is a heavy metal with no apparent biological function. In spite of extensive documentation of the toxic effects of lead on human health, the molecular mechanisms underlying its poisonous effects on the central nervous system (CNS) have yet to be clarified (39,40).

Bioactive lead is a divalent cation that binds strongly to sulfhydryl groups of cysteine residues of proteins and enzymes. Lead toxicity can be largely attributed to conformational changes undergone by enzymes and structural proteins upon binding the lead ion, but this versatile toxic agent has other targets as well. For example, lead interferes in the endogenous opioid

system (41) and efficiently breaks the ribosyl phosphate group of tRNA (42). Many toxic properties of lead are putatively due to the metal's capacity to mimic and compete with calcium and zinc ions in finger proteins dependent on these metals. (43).

Recent studies have also focused on the heme biosynthetic pathway, where many lead interference sites are encountered. Thus, lead poisoning can be considered a chemical or acquired porphyria (44). The thiol enzymes δ -aminolevulinic acid dehydratase (ALAD) and ferrochelatase of this pathway are extremely sensitive to lead. Inhibition of these enzymes increases, respectively, ALA and protoporphyrin IX concentrations in urine, blood and other tissues. ALA has long been known to compete with γ -aminobutyric acid (GABA), a neurotransmitter in the cortex, hypothalamus and other tissues of the CNS and the peripheral nervous system (45).

An increase of ALA in the blood circulation and brain areas could contribute to triggering behavior disorders in patients carrying genetic porphyrias, including acute intermittent porphyria (AIP) and hereditary tyrosinemia type 1, and also in lead poisoned individuals. This hypothesis is based on the fact that ALA has been shown *in vitro* to exhibit pro-oxidant properties towards biological molecules (proteins, membranes, DNA) and supramolecular structures (mitochondria, synaptosomes), as well as, *in vivo*, in brain, liver and red muscles of ALA- or lead-treated rats and in the blood of lead exposed workers (46,47,48,49,50) (Figure 2). Of utmost importance was the finding that ALA-driven oxidative injury to GABA receptors in synaptic membranes, synaptosomes, and GABA-rich brain slices leads to a two-fold

increase of the dissociation constant of the receptor-GABA complex (51) and a significant decrease of GABA receptor population (52).

Fundamental questions about the molecular bases of the ALA-induced neurological lesions remain unanswered. It is worth noting that acute porphyric attacks of inborn and acquired porphyria patients correlate with elevation of blood and urinary ALA and that lead exposed subjects with high levels of lead ($> 60 \mu\text{g/dL}$) and ALA ($> 1 \mu\text{M}$) in the blood present neurological manifestations similar to those in AIP (53,54,55).

Lead poisoning of the infant nervous system

Studies in several countries have estimated that about 4% of their children have high blood lead levels (19). Children living in inner-city areas of the United States may reach even higher prevalence. According to data collected between 1976 and 1980, 17% of children presented lead blood levels above $15 \mu\text{g/dL}$; 5.2% higher than $20 \mu\text{g/dL}$ and 1.4% higher than $25 \mu\text{g/dL}$ (56). Lead poisoning is not considered a significant environmental risk for children in rural areas of developing countries. However, in a study with children living in the rural Philippines, 21% (601 of 2861 children) had blood lead levels higher than $10 \mu\text{g/dL}$. Blood lead levels were associated independently with age, hemoglobin concentration, water source, roofing material, expenditures and history of breastfeeding. The authors evaluated possible environmental exposures among a sub-sample of children with elevated blood lead levels and found multiple potential sources, such as

fossil-fuel combustion, lead paint (in or around 38% of homes) and household items (57).

Children are more sensitive to lead than adults for many reasons. Their exposure to lead is favored by the habit of taking things to the mouth (*pica* habit). A child's intestine absorbs lead much faster than the adult's and the developing infant CNS is more vulnerable to toxic agents than the mature CNS, especially in the case of undernourished children. Neural proliferation, differentiation and plasticity are strongly impaired by lead.

In the United States, overall childhood blood lead levels have declined as a result of federal regulatory measures to reduce population exposure to environmental lead. Screening data from the late 1960s and early 1970s found that 20% to 45% of children tested had blood lead levels ≥ 40 $\mu\text{g/dL}$. Between 1976 and 1980, the weighted geometric mean of blood lead among 1 to 5-year-old children in the US was 15 $\mu\text{g/dL}$ (58). Data from the Third National Health and Nutrition Examination Survey (NHANES III), phase 1 (1988 – 1991), showed a decline in the geometric mean of lead level to 3.6 $\mu\text{g/dL}$ (58). NHANES III (1991 – 1994) showed a further decline in this biomarker to 2.7 $\mu\text{g/dL}$. The NHANES III, phase 2 data indicated that ~4.4% of 1- to 5-year-old children (~890,000 children) had blood levels ≥ 10 $\mu\text{g/dL}$ (59). Bernard et al (28) analyzed the data from NHANES III (1988-1994) and found that the overall prevalence of blood levels ≥ 5 $\mu\text{g/dL}$ was 25.6% although most (76%) of these children had lead levels < 10 $\mu\text{g/dL}$.

Including Latin American experience data, in Argentina, studies carried out in Cordoba and Buenos Aires showed that between 10 and 40% of

children aging less than 15 presented blood lead levels higher than 10 µg/dL (60). In Uruguay (61), at beginning of 2001, blood lead levels higher than 25 µg/dL were detected in children in La Teja, Montevideo. In this place, several metal smelting plants and other industrial segments were operating during the last 50 years. Because that, Public Health Ministry created a special team that carried out another study, which showed that 61% of the 2351 studied children presented blood lead levels higher than 10 µg/dL.

In Brazil, studies on environmental lead exposure are rare, limiting a comprehensive understanding about its impact on the Brazilian public health (62). Silvany-Neto et al (63) found blood lead levels mean of 36.7 ± 20.7 µg/dL in children living close to a primary lead smelting plant in Santo Amaro, Bahia. In 1996, the same authors (64), using the Zn-protoporphyrin method, found lead levels as high as 65.5 µg/dL in children in the same area. These levels have remained abnormally high since 1980 due to the contamination of soil by lead. In 2003, those authors found blood lead levels of *c.a.* 17 µg/dL, which were 5 µg/dL greater among children with *pica* habit, but independent of age, visible presence of scum surrounding the home, employment status of the father, family history of lead poisoning, and malnutrition (65).

In Cubatão (São Paulo), one of the most industrialized areas in Brazil, Santos Filho et al. (66) found lead levels in the population blood averaging 17.8 µg/dL. Paoliello (67) assessed the blood lead level in children living in the upper Ribeira do Iguape river valley and found a blood lead level median value of 11.25 µg/dL. Freitas (2004) carried out an evaluation of lead exposure in a contaminated area of Bauru which revealed that 311 out of 850

analyzed children presented blood lead levels above the limits established by WHO (68).

In the mentioned investigation (Bauru, São Paulo), a battery recycling plant contaminated its neighboring residential area with lead oxides during the last 8 years. The environmental lead contamination has been assessed by CETESB (State Authority for Environmental Control). The plant had its activities suspended in 2002. Over half of the 311 studied children presented blood lead levels between 15 and 19 µg/dL, 21% had levels between 20 and 39 µg/dL, and less than 1% (three children) showed blood lead levels of 40 µg/dL or higher. Comparing to other lead contaminated areas in Brazil, blood lead levels found in Bauru study were rather low (median of 7.3 µg/dL) (69).

In Brazil, there are no public policies designed to establish official procedures for sampling and analyzing lead in human tissues, nor to screen lead in school children, even in the case of those who present psychomotor and learning disabilities (18).

Lead effects on IQ and social behavior

Denno (70) traced the behavioral patterns of 987 African-American youths from birth to age 22. She found that among the dozens of sociologic and biologic correlates of delinquency, lead poisoning was among the strongest for male subjects.

Cognitive function, measured by psychometric IQ tests, has been the major focus of most studies on lead exposure in childhood. There are persuasive reasons to believe that cognitive dysfunction may not be the most important effect of lead and that we may be entering a fifth stage of understanding the biochemistry of lead toxicity, including the molecular mechanisms by which lead triggers anti-social attitudes in children and adults (5).

Lead-induced aggressiveness is not an entirely new notion. Parents have frequently reported that after recovery from an episode of acute lead poisoning, their children's behavior changed dramatically to restless, inattentive and aggressive conduct (5). In 1943, Byers and Lord reported attention deficits and aggression in a sample of lead-poisoned children on follow-up (14).

Needleman et al. (71) studied 301 primary-school students and found that children with elevated bone lead levels scored higher on the attention deficit, aggression, and delinquency clusters of the Child Behavior Checklist after adjustment of covariates. Dietrich et al (72) found that prenatal lead exposure was associated with parents' reports of delinquency and aggression, and postnatal lead exposure was associated with self-reports of delinquent acts. A case-control study of 195 arrested and convicted delinquent youths, conducted by Needleman et al. (73) on 194 adjudicated youths (aged 12-18) and 146 non-delinquent controls, revealed an increased risk of delinquency associated with bone lead concentrations measured by X-ray fluorescence. The covariate-adjusted odds ratio was 4 (95% CL 1.4-

11.1). The population-attributable risk for delinquency due to lead exposure ranged from 11% to 38% in this sample. In Brazil, a case-control study survey of a connection between lead in the tooth enamel of adolescent inmates and their anti-social behavior is presently underway in Brazil's youth re-educational system, Fundação Casa.

Recently, Chiodo et al. (74) showed a relation between blood lead level and neurobehavioral outcome in African American 7-year-old children. Among the studied variables, social problems, delinquent behavior and total behavior problem were associated with blood lead levels ($\beta=0.10^*$, $\beta=0.09^*$ and $\beta=0.09^*$; $*p<0.05$, respectively).

A number of recent ecological investigations have correlated leaded gasoline sales and lead in air particulates with crime rates, strongly indicating an association between lead exposure and crime. Stretesky and Lynch (75), when comparing homicide rates in 3111 counties in the United States and adjusting 15 covariates, reported a four-fold increase in homicide rates in those counties with the highest air lead levels compared to controls. Nevin (76) correlated sales of leaded gasoline with violent crime rates and, adjusting for unemployment and percentage of population in the high-crime age group, found a statistically significant association between lead and crime. It has been speculated that one of the reasons for the recent decline in crime rates in the United States can be attributed to the adoption of public policies to ban lead in paints, gas, and canned food, therefore reducing exposure to lead. In 2007, Nevin (77) carried out single and combined nation regressions, identifying "best-fit" lags for each crime analyzed, with the

highest significance (t-value) for blood lead and percent of crime rate variation explained (R^2). The results presented a strong association between preschool blood lead and subsequent crime rate trends over several decades in nine countries. Furthermore, regression analysis of average 1985-1994 murder rates across USA cities suggests that murder could be especially associated with more severe cases of childhood lead poisoning.

Many studies provide evidence of an inverse relationship between lead exposure and cognitive ability (78). There is, however, disagreement about the IQ to blood lead slope (IQ points lost/one $\mu\text{g}/\text{dL}$ increase in blood lead) and the influence of confounding variables (79,80). There is strong evidence that young children face the greatest risk of IQ losses due to lead exposure, especially during the first three years of life, when basic cognitive abilities develop (79). This information is very important because many painted toys may contain lead, becoming a risk for children, especially because children introduce toys into their mouths, sometimes biting them. Cognitive losses due to lead exposure during the first three years of life appear to be most evident in IQ tests carried out some years later, around age 10 or older, when IQ scores are more stable and predictive of future outcomes (78,79). There is no consensus, however, on whether lead exposure is more strongly associated with verbal IQ, mathematical skills or performance IQ. However, despite the similarity with a pattern of outcomes typical of brain injury, detailed comparisons of children's deficits indicate that lead, like most other causes of brain injury, does not produce exactly the same set of impairments in each patient (81).

Although data on yearly changes in IQ are unavailable, temporal data are available for specific types of social behavior associated with lower IQ scores. Herrnstein and Murray (82), in their controversial book *The Bell Curve*, cite data showing that individuals with lower IQ levels account for a disproportionate share of violent crime and unwed births. Herrnstein and Murray (82) associated IQ with social behavior based on data from the National Longitudinal Survey of Labor Market Experience of Youth (NLSY), a representative national sample of American youths. When the NLSY began in 1979, the 12,686 participants were aged 14 to 22. In 1980, 94% of these youths were given the Armed Forces Qualification Test (AFQT) in the IQ metric (a mean of 100 and a standard deviation of 15), and these results are referred to as IQ scores. To justify the use of IQ, the authors showed that this test has a very high correlation with other IQ tests available for some NLSY participants. In addition to the NLSY figures, the authors cite research from Britain, Sweden, Denmark and New Zealand and conclude that the data as a whole indicate that incarcerated offenders have an average IQ approximately 92 (eight points below the mean) (82).

In 1960, in the United States, the accepted threshold for blood lead in children was 60 $\mu\text{g}/\text{dL}$. Follow-up studies carried out in American cities showed 10 to 20% of the children of these cities presenting lead levels of up to 40 $\mu\text{g}/\text{dL}$. This finding increased the conjecture that some learning difficulties and behavior disorders (sub-clinical manifestations) may be attributed to lead. Five studies on lead levels and behavior in children without overt signs or symptoms of lead poisoning were carried out in the 70s. Three

of them related an association between lead and IQ (15,17,16) and two did not confirm this connection (83,84). These studies had limitations of design: the number of patients in each study was small, and blood lead was used as a biomarker. Blood lead can only be reliable as a biomarker of short term exposures, as the metal has a half-life of 36 days in the blood (85).

Three meta-analysis studies confirmed that exposures to low levels of lead may be associated with IQ deficiency (86,79,80). In response to these data, in 1991, the Centers for Disease Control and Prevention – CDC- reviewed the acceptable levels for blood lead and reduced the 60 µg/dL blood threshold established in 1970 to the present accepted value of 10 µg/dL. More recent data point toward the manifestation of deficiencies in cognition, attention and behavior in children presenting lead levels of between 3 and 5 µg/dL of blood (9). In 2007, Chiodo et al (74) presented new data showing that none of those studied neurobehavioral outcomes evidenced a threshold below which blood lead levels do not be associated with harmful outcomes. The authors suggest a reduction of the “acceptable” level, considering the recent scientific evidences.

Dudek and Merezcz (87) found that the most rapid deterioration of IQ was observed at blood lead levels between 5-10 and 11-15 µg/dL, consistent with the Schwartz’s (79) finding of an increased slope at lower blood lead levels (76).

In recent decades, the social problems of violent crime and unwed pregnancies have been associated with teenagers and young adults in poor urban areas. Average blood lead for poor children in urban areas probably

began to rise as early as the 1940s, due to the addition of tetraethyl lead to gasoline combined with use of high lead-based pigments in house paints. Therefore, temporal data on leaded gasoline consumption might serve as a rough indicator for changes in blood lead in poor urban children from 1940 to 1987. If childhood lead exposure affects IQ, and IQ affects population rates for crime and unwed pregnancy, then changes in crime and unwed pregnancy rates from 1960 to the late 1990s could reflect changes in IQ associated with temporal trends in leaded gasoline consumption from 1940 through the early 1980s (76).

Primary prevention: benefits for public health

A cost-benefit analysis carried out by the US Public Health Service estimated the cost of abatement of old houses painted with lead-containing paints over a 30-year period at \$33.7 billion, in 1991. The estimated benefit from avoided health care costs and increased income due to raised IQ was \$ 61.7 billion. This cost analysis may be conservative, as it does not include avoided delinquency and cardiovascular disease, both demonstrated effects of lead exposure, among other health effects (4).

According to Needleman (5), current analyses also demonstrate that primary lead exposure prevention yields large economic benefits. Grosse et al (88) calculated that each present-day preschool child's IQ was increased by 2.2-4.7 points above what it might have been if leaded gasoline and blood lead had not been reduced. From this, they calculated the IQ-related increase in income and estimated that the economic benefit for each year's

birth cohort was between \$110 billion and \$319 billion. Landrigan et al (89), assuming no threshold for the lead-IQ association, estimated the loss of future earnings for the one-year cohort of children aged 5 in 1997 at \$43.4 billion.

The monetary cost associated with the ubiquitous exposure of fetuses and children to lead industrialized societies has been also calculated by Schwartz (90) estimating the benefits of a 1 µg/dL reduction in the population mean blood lead concentration (Table 1). The analysis was based on the monetary savings of reducing lead levels in children with blood lead concentrations between 10 and 20 µg/dL. Schwartz (90) estimated medical costs associated with treatment of children with undue lead exposure, the increase in remedial education, and the costs associated with reduced birth-weight and reduced gestational age, among other factors. The largest single cost is earnings lost as a result of decreased intellectual capability. In a later similar analysis using the comprehensive National Longitudinal Survey of Youth database to give monetary value to the effect of decreased cognitive ability on earning capacity, the estimated gain in earnings would be 7.5 billion U.S. dollars per year for a decrease in blood lead levels of 1 µg/dL in the U.S. population (91). It is obvious that the effect of lead on IQ is reflected in an enormous cost to society in terms of lost potential and increased need for medical care and special education.

According to Needleman (5), the evidence that lead toxicity extends down to the lowest measurable levels, that pharmacological therapies are

ineffective at preventing sequelae in those with low levels, and that reduction of exposure yields huge economic as well as health benefits provides a strong argument in favor of a systematic program of abatement of lead from the single remaining major source in the United States: lead in older homes. On the other hand, an association has been firmly established between air lead concentrations and levels of lead in the body (92,93,94,95,96). To determine if data on potential lead exposure drawn from the Environmental Protection Agency's Cumulative Exposure Project (CEP) were correlated with blood lead levels at the county level, Stretesky and Lynch (75) collected data from the Ohio Department of Health on children younger than 6 years who had blood lead levels above 10 µg/dL during 1998. The Centers for Disease Control and Prevention (CDC) (Atlanta, GA) identified the data from Ohio as more valid than data from most states because a large proportion of children across all Ohio counties are tested for lead poisoning. The authors found that across Ohio's counties, CEP-estimated air lead concentrations were positively and significantly correlated with the percentage of children (of those children screened) who had elevated blood lead levels (Pearson correlation coefficient, 0.44; $P < .001$; $n = 88$). This relationship persisted (Pearson correlation coefficient, 0.48; $P < .001$; $n = 88$) after adjustments from the percentage of houses built before the 1950s (1990 census estimate), reinforcing previous findings suggesting that lead exposure may result from a variety of contamination sources, including lead-contaminated air.

An important and interesting example of primary prevention is that of the city of Hartford, Connecticut, USA. As part of a citywide effort to increase lead poisoning awareness, the Hartford Health Department implemented a multifaceted public health campaign involving several novel elements and partnerships, including the use of municipal sanitation trucks to disseminate lead-poisoning prevention messages throughout the city. Key results were as follows: recall of campaign components ranged from 21.5 to 62.6%, with newspaper advertisements and signs on buses and billboards recalled most often and a video broadcast on public-access television recalled least often; more than 45% of respondents reported that they took steps to prevent lead poisoning because of at least one of the campaign components, with the newspaper advertisements being the most effective component in terms of prompting lead-poisoning prevention behavior; respondents' awareness was particularly low in terms of becoming more informed as to how medical personnel and procedures can and cannot detect and prevent lead poisoning in children. This campaign prompted caregivers to take steps to prevent lead poisoning and may help public health professionals in other communities to develop novel ideas by which to embark on similar initiatives (97).

In Brazil, according to the São Paulo State Health Department some measures have been adopted for protecting the population, despite the lack of an official program for environmental lead exposure prevention. Since 1978, tetraethyl lead has no longer been added to gasoline as an anti-detonator. In addition, there are regulations for acceptable lead levels in food

and water (98,99). Regarding acceptable levels in humans, only occupational exposure is regulated.

Among the non-regulated sectors, some factories follow the internationally accepted lead parameters. According to the Brazilian Association of Paints Manufacturers, there is a trend that began in the 1990s to substitute lead pigments in paints. At present, Brazilian domestic paints are free of lead. However, lead is still used as an anti-corrosive, such as red lead in iron gates, refrigerators, cars, stoves, bicycles, and many other goods. In this case a covering paint should be applied over the red lead (18). The aforementioned non-toxic and inexpensive Biphor[®] white pigment, based on aluminum phosphates and polyphosphates, will greatly decrease paint related lead poisoning (26).

Studies conducted at the Adolfo Lutz Institute (100) (Brazil) concluded that lead may be found in pencils, pens, colored paints, erasers and other school supplies. This study recommended that there should be regulatory guidelines for the manufacture of such products.

The canned food manufacturers in Brazil have also substituted lead-based solders. Regarding the contamination of fresh food by lead, Sakuma (101) found in Brazil secure lead levels for human consumption. Glazed ceramic containers can be a source of lead poisoning when lead leaches into stored beverages, especially in the case of acidic fruit juices such as those made from grapes and citrus fruit (102). Lead from glazed ceramics is

promptly dissolved by the tartaric and citric acids present in the juices, respectively, due to the chelation of the metal by these acids.

Since 1986, an Environmental Impact Report (EIA/RIMA) has been officially required for the approval of potentially polluting industrial plants, as an obligatory document to license these companies (103). The strategies proposed in this report to minimize the pollution by the plant must be analyzed and approved (99). Nevertheless, the companies built before 1986 are not obliged to follow this protocol, unless they have caused environmental damage (104).

According to information from the Sanitary Vigilance Center, São Paulo State Health Department (18), several small companies and domestic sources of lead contamination do not issue warnings to prevent lead exposure

Thus, considering that “the child is father of the man” and that a healthy social tissue can be seriously harmed by lead, it is extremely important to establish public policies against lead contamination and guarantee an adult population that is socially well-balanced and productive. In a recent paper, provocatively named “Childhood lead poisoning prevention – too little, too late”, Lanphear (105) called attention to the importance of preventing lead poisoning for the good of individuals and of society. In this context, it is tempting to state that there is a high probability that many young delinquents are actually victims of lead poisoning and not necessarily genetic

or social criminals. We conclude with a plea for public health policies to prevent lead poisoning in underdeveloped and developing countries, such as those that have long been adopted in the U.S.A., Europe, and Japan (www.cdc.gov, www.fda.gov, www.epa.gov).

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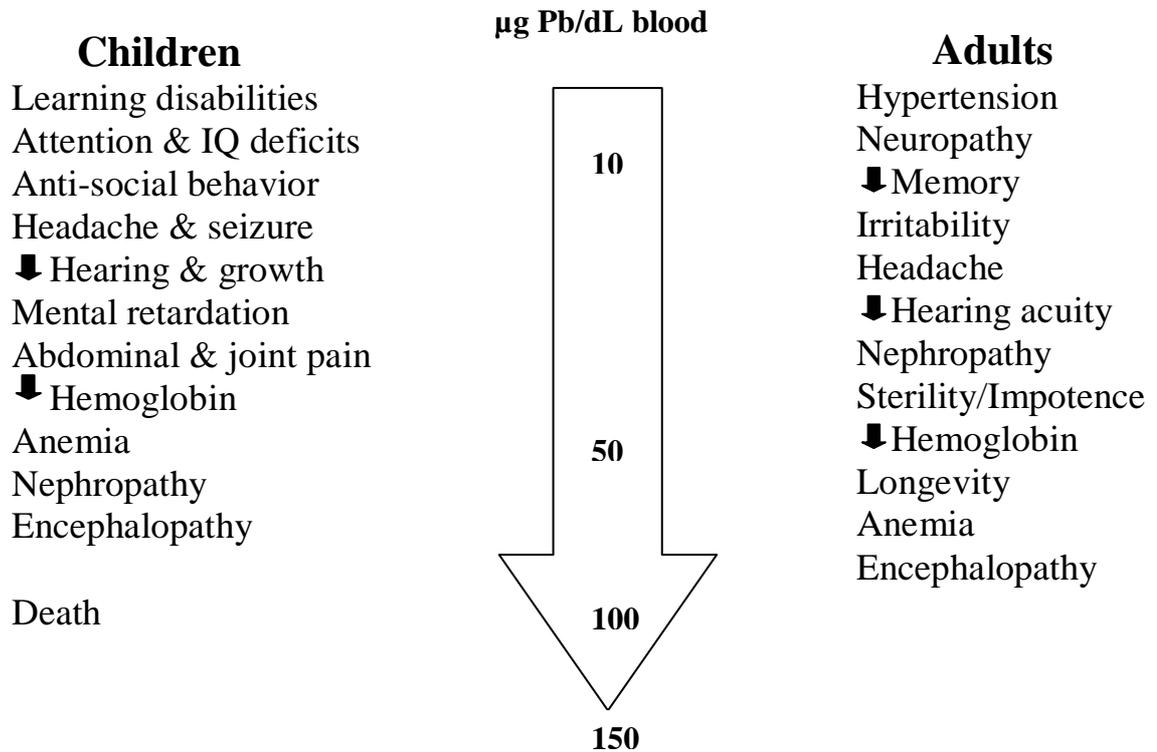


Figure 1. Effects of lead poisoning on human health. Adapted from Gurer and Ercal, 2000 (48). ↓ = reduction

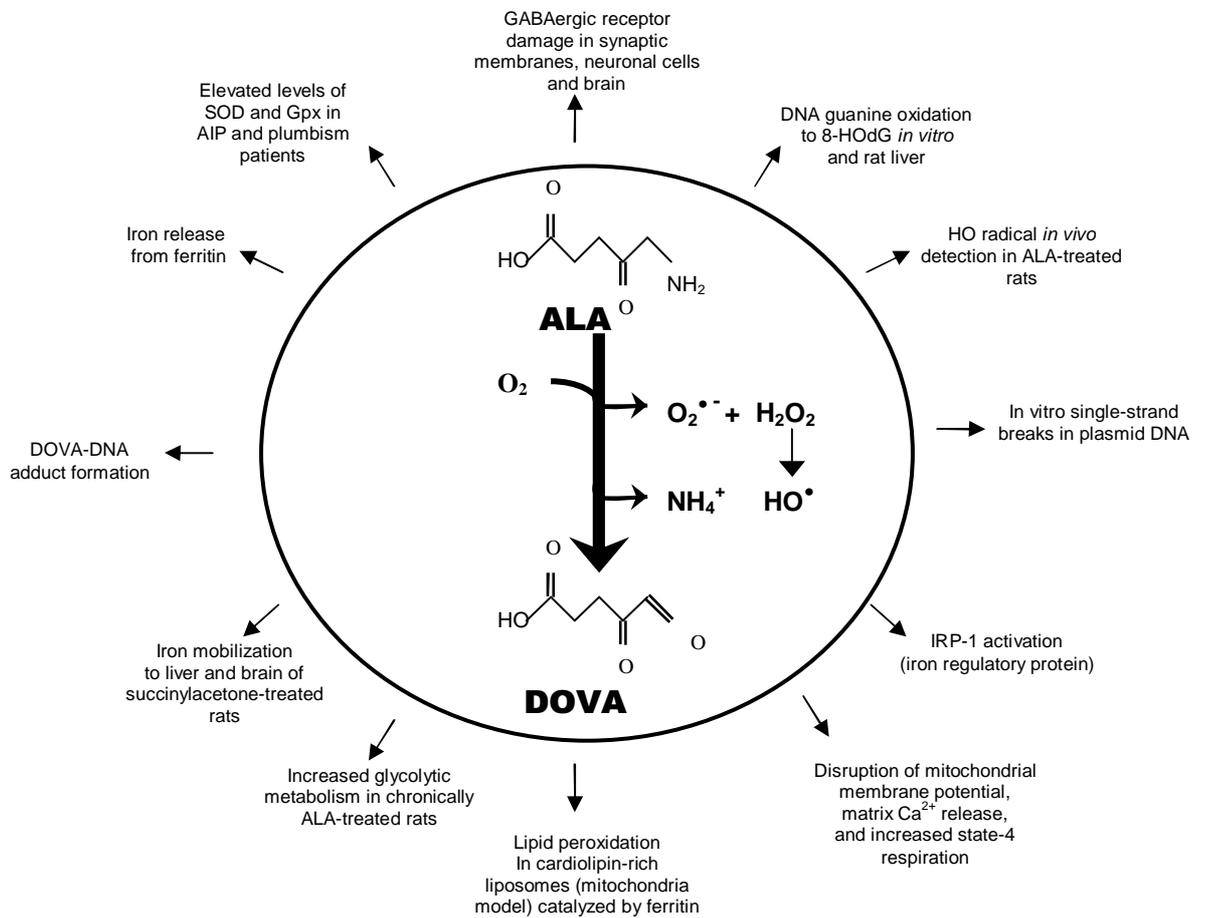


Figure 2. Production of reactive oxygen species by the aerobic oxidation of δ -aminolevulinic acid (ALA), a heme precursor accumulated in lead poisoning, ALA-driven oxidative damage to biomolecules, and biological consequences observed in ALA-treated rats and lead poisoned individuals (106).

Table 1. Annual benefits of a 1 µg/dL reduction in the mean blood lead concentration of US infant population, according to Schwartz (1994) (90).

Annual Benefits	US\$, million
Medical costs	189
Compensatory education	481
Infant mortality	1,140
Neonatal care	67
Earnings	5,060
Total	6,937

2 OBJETIVOS

Objetivo geral:

- Estudar a exposição ao chumbo associada a comportamento anti-social em adolescentes brasileiros e desenvolver metodologias para determinação de biomarcadores que retratem a exposição ao chumbo.

Objetivos específicos:

- Avaliar a associação entre altas concentrações de chumbo presentes no esmalte dentário superficial e estabelecimento de comportamento anti-social / cometimento de atos infracionais em adolescentes (Manuscrito II);
- Investigar potenciais fatores de risco domiciliares à contaminação por chumbo mais associados a altas concentrações de chumbo no esmalte dentário (Manuscrito III);
- Verificar o impacto de alterações metodológicas da técnica de microbiópsia ácida de esmalte dentário superficial nos valores obtidos para a profundidade da biópsia, calculada pela fórmula da altura do cilindro, e sobre conseqüentes interpretações das concentrações de chumbo no esmalte dentário (Manuscrito IV).

3 MÉTODOS

Este estudo foi aprovado pelo Comitê de Ética em Pesquisa da Faculdade de Saúde Pública da Universidade de São Paulo (Proc. No. 244/05). Os modelos das cartas de informação e termos de consentimento livre e esclarecido assinados pelos adolescentes e responsáveis estão apresentados no ANEXO 1.

Os procedimentos adotados para atender aos objetivos da pesquisa estão resumidamente descritos abaixo. Todos os procedimentos são detalhados nos manuscritos que compõem o capítulo RESULTADOS e DISCUSSÃO. Assim, para facilitar a leitura do texto, o manuscrito e o número da página onde a respectiva metodologia pormenorizada pode ser encontrada estão indicados entre parênteses.

Um estudo transversal foi conduzido com 173 jovens residentes na cidade de Bauru, SP, Brasil. MICROBIÓPSIAS ÁCIDAS DE ESMALTE DENTÁRIO SUPERFICIAL foram realizadas nos incisivos centrais superiores destes jovens por dois diferentes protocolos metodológicos. O Protocolo I (n=114) consistiu em uma biópsia circular de 4 mm de diâmetro, 35 s de exposição do dente ao ácido [HCl 1,6 M em 70% de glicerol], 10 µL HCl (Manuscritos II (p. 95), III (p. 125) e IV (p. 150)) e o Protocolo II (n=59) consistiu de uma biópsia de 1,6 mm de diâmetro, 20 s de exposição ao mesmo ácido, 5 µL HCl (Manuscrito IV, p. 150). Todos os adolescentes participantes da pesquisa receberam profilaxia dentária, raspagem e

alisamento dentário, quando necessário, e aplicação tópica de flúor gel, quando indicado clinicamente.

Além disso, QUESTIONÁRIOS SOBRE COMPORTAMENTO DOS ADOLESCENTES (*Child Behavior Checklist - CBCL* e *Self-Reported Delinquency - SRD*) (Manuscrito II, p. 93) e EXPOSIÇÃO A POSSÍVEIS FONTES CONTAMINADORAS DE CHUMBO (Manuscrito III, p. 125) foram aplicados aos pais e aos adolescentes.

As ANÁLISES QUÍMICAS foram realizadas no Laboratório de Química Analítica do Instituto de Química da USP/SP. As amostras foram analisadas para o fósforo (P) por espectroscopia de emissão ótica com plasma indutivamente acoplado e, para o chumbo, por espectrometria de absorção atômica com forno de grafite (Manuscritos II, p. 96; III, p. 126 e IV, p. 152).

Para atender aos objetivos tratados no Manuscrito IV, TESTES DE PERFILOMETRIA (p. 154) foram realizados em blocos de esmalte dentário bovino, após realização de biópsias de esmalte utilizando-se, para isso, perfilômetro da Clínica de Odontologia Preventiva, Periodontia e Cariologia da Universidade de Zurique, Suíça.

QUESTIONÁRIOS

Para a avaliação do comportamento social, foram utilizados dois questionários: “Child Behavior Checklist” (CBCL) (ACHENBACH, 2001) (ANEXO 2), validado no Brasil e adaptado transculturalmente por BORDIN et

al., 1995, e “Self-Reported Delinquency” (SRD) (LOEBER et al., 1989) (ANEXO 3).

O SRD é um auto-relato de comportamento composto por 36 questões no qual, para cada questão, o jovem atribui uma escala de 0 a 4, onde os escores significam: 0= nunca; 1= uma vez; 2= 2-5 vezes; 3= 6-10 vezes e 4= mais de 10 vezes, referindo-se ao número de vezes que o adolescente praticou cada ação questionada nos últimos 6 meses. O escore final é obtido pela soma total de todos os 36 escores.

O CBCL é um questionário empiricamente baseado, ou seja: a) os dados levantados são baseados nas experiências de pessoas que vêem o adolescente em um determinado contexto, no caso desta pesquisa, o contexto familiar; b) “os dados obtidos para uma grande amostra de clientes foram analisados estatisticamente para identificar os padrões de problemas que realmente acontecem nas pontuações dadas por diferentes avaliadores” (Laboratório de Terapia Comportamental do Instituto de Psicologia da Universidade de São Paulo (LTCIP-IP-USP, 2006)); e c) “os padrões derivados das análises estatísticas foram usados para construir as escalas síndromes para marcar os conjuntos que co-ocorrem” (LTCIP-IP-USP, 2006).

Os dados obtidos a partir de itens de competência social são convertidos em escores de 0 a 4, segundo as instruções do manual do CBCL (ACHENBACH, 2001). Estes escores são chamados de “escores crus” que, registrados em escalas, fornecem o perfil social da criança ou do adolescente. O perfil social aplica-se à faixa etária de 6 a 18 anos de idade e

conta com três escalas individuais: atividades, sociabilidade e escolaridade. A soma dos escores crus obtidos nas escalas sociais individuais indica a Competência Social Total do indivíduo.

Os 113 itens relativos a problemas de comportamento constituem descrições de comportamentos que podem estar presentes ou ausentes na vida da criança ou adolescente. O informante pode classificar tais comportamentos de acordo com três variáveis: item falso ou comportamento ausente (escore=0); item parcialmente verdadeiro ou comportamento às vezes presente (escore=1) ou comportamento freqüentemente presente (escore=2).

Os escores de 0 a 2 para problemas de comportamento são chamados de “escores crus” que, registrados em escalas, fornecem o perfil comportamental da criança ou adolescente. O perfil comportamental aplica-se à faixa de 6 a 18 anos de idade e é constituído de oito escalas. As oito escalas correspondem às seguintes síndromes: I. Ansiedade / Depressão, II. Isolamento / Depressão, III. Queixas Somáticas, IV. Problemas sociais, V. Problemas de pensamento, VI. Problemas de atenção, VII. Comportamento de quebrar regras, VIII. Comportamento agressivo. As escalas I, II e III são chamadas de problemas internalizantes, quando consideradas em conjunto e as escalas VII e VIII são nomeadas problemas externalizantes, quando agrupadas. A soma dos escores crus obtidos nas escalas comportamentais individuais corresponde ao total de problemas de comportamento (BORDIN et al., 1995).

Os fatores de confusão foram avaliados por meio de perguntas sobre endereço; tempo de residência no endereço atual; locais anteriores onde o jovem residiu; número de pessoas que moram na casa (crianças e adultos); se os pais moram juntos; escolaridade do pai e da mãe; se fumante ou não (pais e filhos); local de trabalho dos pais; se alguém da casa trabalhou em fábricas de baterias, pigmentos, tintas e cerâmica; utilização doméstica de cerâmica vitrificada, brinquedos pirateados e de baixa qualidade, baterias de carros e zarcão (ANEXO 4). Este questionário foi baseado na tese de Doutorado de FREITAS, 2004.

Todos os entrevistadores foram selecionados e treinados pela pesquisadora responsável (KPKO), anteriormente ao início das entrevistas, de maneira a padronizar os procedimentos adotados. Entre os entrevistadores havia estudantes de Relações Públicas (UNESP-Bauru) e uma cirurgiã-dentista, Mestre em Odontologia em Saúde Coletiva (USP-Bauru).

A Figura 1 mostra a urna utilizada para colocação das respostas do SRD, o que foi realizado com o intuito de diminuir possíveis constrangimentos dos adolescentes em relatar cometimento de atos infracionais.

Figura 1- Local onde o adolescente respondia ao auto-relato de cometimento de atos infracionais (*Self-Reported Delinquency*).



A urna foi utilizada para que o adolescente não se sentisse constrangido em entregar suas respostas diretamente para a pesquisadora.

MATERIAL UTILIZADO PARA OS PROCEDIMENTOS CLÍNICOS

Figura 2 - Instrumental utilizado durante o exame clínico do adolescente (A) e para posterior coleta de esmalte dentário (B).

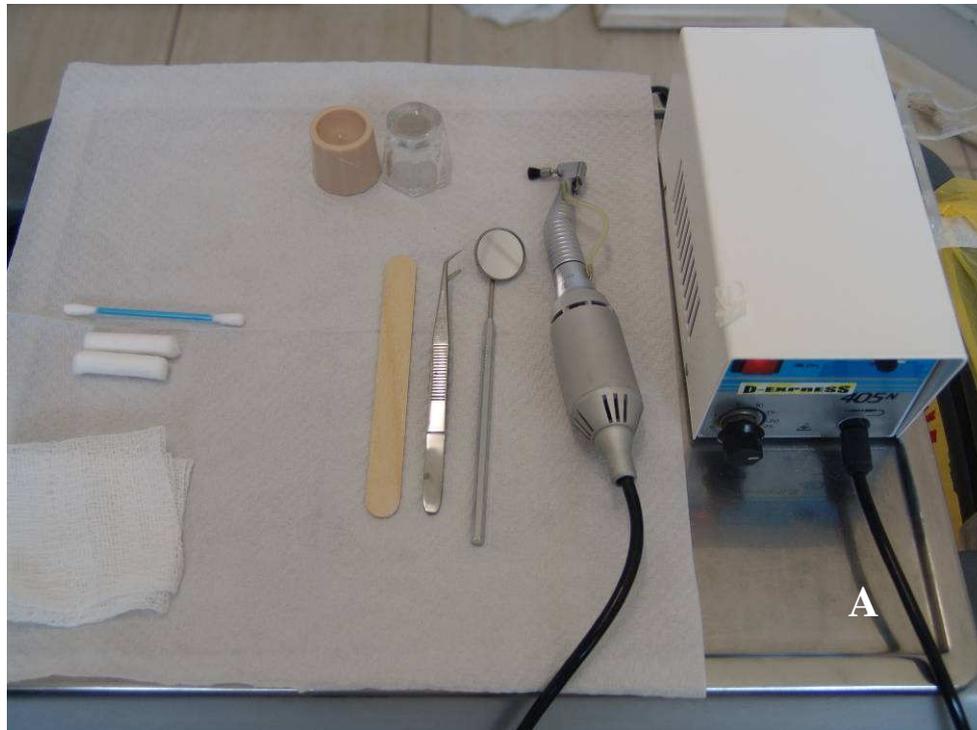
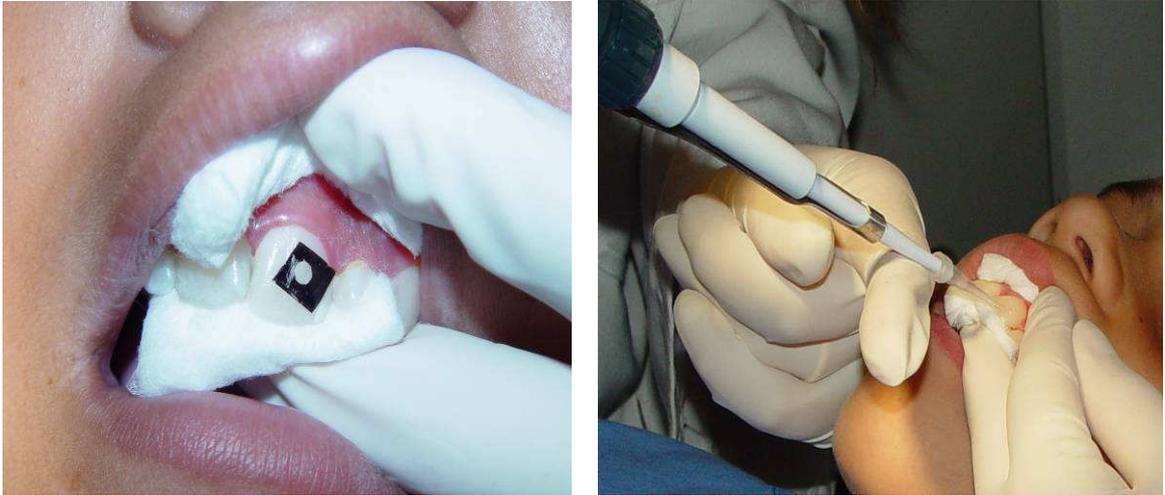


Figura 3 - Coleta da amostra de esmalte dentário.



A fita foi colorida de preto para melhor visualização da imagem. Originalmente, a fita adesiva é transparente. Após a realização da profilaxia, o dente a ser biopsiado é isolado com roletes de algodão, a fita perfurada é fortemente aderida à superfície dentária e a biópsia é realizada (Fotografias gentilmente cedidas pela Profa. Dra. Maria Fernanda Borro Bijella).

BAIRROS ONDE OS ADOLESCENTES RESIDEM E LOCAIS DE
COLETAS DE AMOSTRAS

Figura 4 – Vista do Bairro Ferradura Mirim, Bauru, SP.



Figura 5 - Centro Irmã Adelaide, localizado no Bairro Ferradura Mirim.



Neste local, projetos sociais são desenvolvidos como o Programa Primeiro Emprego voltado aos jovens de 15 a 17 anos de idade. A maioria deles integrou a presente pesquisa.

Figura 6 – Vista do Núcleo Habitacional Fortunato Rocha Lima, Bauru, SP.



Este núcleo habitacional é oriundo de um projeto de desfavelamento da cidade de Bauru – SP. Neste local, funcionam o Projeto Girassol e a Creche São José, ambos atendendo a comunidade carente que lá reside.

Figura 7 – Tipo de residência presente no Núcleo Fortunato Rocha Lima.



ANÁLISE ESTATÍSTICA

Análises de regressão logística foram realizadas para verificação das síndromes psiquiátricas (p. 98) e fontes de exposição (p. 128) mais associadas a altas CCED. Para verificação da diferença da CCED e profundidade de biópsias obtidas por protocolos metodológicos diferentes (I e II), testes de Wilcoxon e testes t pareados foram aplicados aos dados (p. 155).

4 RESULTADOS e DISCUSSÃO

Artigos:

Manuscrito II. “Surface dental enamel lead levels and antisocial behavior in Brazilian adolescents” (última versão redigida para submissão à *Neurotoxicology and Teratology*, FI=2,444);

Manuscrito III. “Risk factors associated with high lead levels measured in the surface dental enamel from Brazilian youths” (versão submetida ao *Bulletin of the World Health Organization*, FI=4,019);

Manuscrito IV. “Methodological alterations of surface dental enamel microbiopsies for lead body burden measurement” (versão submetida à *Toxicological Sciences*, FI=3,814).

Surface dental enamel lead levels and antisocial behavior in Brazilian adolescents

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Abstract

Lead poisoning is reportedly linked to a high risk for learning disabilities, aggression and criminal offenses. Thus, to study the association between lead exposure and antisocial/delinquent behavior, a cross-sectional study was conducted with 173 Brazilian youths aged 14-18 and their parents (n=93), living in impoverished neighborhoods of Bauru-SP, with high criminality indices. Self-Reported Delinquency (SRD) and Child Behavior Checklist (CBCL) questionnaires were used to evaluate delinquent/antisocial behavior. Body lead burdens were evaluated in surface enamel acid-etch microbiopsies. The dental enamel lead levels (DELL) were quantified by graphite furnace atomic absorption spectrometry (GFAAS) and the phosphorus content was measured using inductively coupled plasma optical emission spectrometry (ICP-OES). Logistic regression was used to identify associations between DELL and each scale defined by CBCL and SRD scores. *Odds ratios* adjusted for familial and social covariates, considering a group of children exposed to high lead levels of lead (≥ 75 percentile), indicated that high DELL is associated with increased risk of exceeding the clinical score for somatic complaints, social problems, rule-breaking behavior ($T \geq 70$) and externalizing problems ($T \geq 63$) (CI 95%). High DELL was not found to be associated with elevated SRD scores. In conclusion, it seems that exposure to high lead levels can indeed trigger antisocial behavior, which calls for public policies to prevent lead poisoning.

Keywords: lead poisoning; dental enamel; biopsy; antisocial behavior; juvenile delinquency; aggressiveness, d-aminolevulinic acid.

1. Introduction

Lead is a long known devastating neurotoxicant [Toscano and Guilarte, 2005]. The specific biological mechanisms underlying lead's effect on social behavior have not been completely established. Many toxic properties of lead are putatively due to the metal's capacity to mimic and compete with calcium and zinc ions in finger proteins dependent on these metals [Markovac and Goldstein, 1988]. Studies have also focused on the heme biosynthetic pathway, where many lead interference sites are encountered. Thus, lead poisoning can be considered a chemical or acquired porphyria [Chisolm, 1971].

The thiol enzymes delta-aminolevulinic acid dehydratase (ALAD) and ferrochelatase of this pathway are extremely sensitive to lead. ALA has long been known to compete with γ -aminobutyric acid (GABA), a neurotransmitter in the cortex, hypothalamus and other tissues of the Central Nervous System (CNS) and the peripheral nervous system (PNS) [Monteiro *et al*, 1991]. An increase of ALA in the blood circulation and brain areas could trigger behavior disorders in patients carrying genetic porphyries [Bechara, 1996; Costa *et al*, 1997; Gurer and Ercal, 2000; Aykin-Burns *et al*, 2003; Rocha *et al*, 2003]. Of utmost importance was the finding that ALA-driven oxidative injury to GABA receptors in synaptic membranes, synaptosomes, and GABA-rich brain slices leads to a two-fold increase of the dissociation constant of the receptor-GABA complex [Demasi *et al*, 1996] and a significant decrease of the GABA receptor population [Adhikari *et al.*, 2006].

Three meta-analysis studies confirmed that low lead levels exposures were associated with reduced IQ [Needleman and Gatsonis, 1990; Schwartz, 1994; Pocock *et al.* 1994]. More recent data indicated cognition, attention and behavior disturbances in children presenting lead levels in order of 3 - 5 µg/dL [Lanphear *et al.* 2000; Chiodo *et al.*, 2004; Chiodo *et al.*, 2007]. In the recent years, ecological [Denno, 1990; Nevin 2000; Stretesky; Lynch, 2001; Nevin, 2007] and observational [Needleman *et al.*, 1996; Dietrich *et al.*, 2001; Needleman *et al.*, 2002; Whright *et al.*, 2008] studies relating lead exposure to antisocial, aggressive, delinquent and criminal behavior have been carried out. These studies showed an association between these disorders and lead intoxication.

Thus, considering evidence linking lead exposure to a higher risk for aggression and antisocial problems [Olympio *et al.*, 2009], the aim of this study was to determine lead concentrations in poverty-stricken Brazilian adolescents using a biological marker of chronic exposure (surface dental enamel) to evaluate the association between lead exposure and antisocial/delinquent behavior in Brazilian youths.

2. Methods

2.1. Subjects

Volunteers were youths residing in a settlement of shacks (Ferradura Mirim); in a housing complex, originated from an urban renovation project to remove shantytown squatters from the city (Fortunato Rocha Lima); and in

the Fundação Casa (a unit serving court-ordered juvenile delinquents). All these sites are located in the city of Bauru, Southeast Brazil, and were selected for this study due to the high criminality indices. In Ferradura Mirim, a census was recently carried out by the School of Sciences of the University of the State of São Paulo and, considering these data, only the houses with adolescents aged 14-18 years were visited. In Fortunato Rocha Lima, where no prior census data existed, two social projects (Girassol Project and Youth Agent) that youths attended were visited and the adolescents who participated of these projects were enrolled. These youths were encouraged to invite other adolescents resident in the same area to compose the sample (snowball technique sampling). In both areas, meetings were held with the parents and youths to explain the purpose of the research. Afterwards, informed consent forms were presented and the adolescents whose parents signed them were scheduled for an interview.

The Ethical Committee of the School of Public Health (University of São Paulo) reviewed and approved this research (Proc. No. 244/05). All youths participants received a dental cleaning and neutral fluoride gel application, if indicated. A clinical exam was carried out and the conditions of oral health were informed to the adolescent. When a curative treatment was necessary it was explained to the participant and a written orientation was provided with a list of local public institutions offering treatment. This procedure was adopted for all subjects participating in this study.

2.2. Measures of antisocial / delinquent behavior

The Self-Reported of Delinquency (SRD) [Loeber *et al*, 1989] was administered to the adolescents. SRD is a 36-item inventory of delinquent acts committed over the past 6 months, scaled from 0 to 4 depending on the frequency of acts committed. To prevent disingenuous answers to questions that may be possibly embarrassing or incriminating, the interviewer (KPKO) gave a copy of the SRD to the adolescent and stayed distant from him or her. All the questions were then read aloud by the interviewer and, if the adolescent had any questions while filling out the inventory, the interviewer was available. When the inventory was completely filled out, the youth deposited it into a slot in a large sealed box. The volunteers were cleared up that the inventories were codified and it was not necessary to write their names on the paper.

The CBCL/6-18 is a revision of the CBCL/4-18 [Achenbach, 1991]. It is an inventory containing 113-item, with a three point scale (scored “never, some, often”), used widely in diagnosis and assessment of psychopathology [Achenbach; Rescorla, 2001]. CBCL/6-18 investigates competence items, illness, disabilities and behavior, emotional and social problems. In the present study, parents or guardians answered this inventory. As the literacy and comprehension level of the parents were low, the interviewer read the questions and the respondent followed the reading with a copy of the inventory. The answers were given in a clear audible voice and the interviewer recorded them. This process was adopted to avoid disconcertedness or confusion and it is recommended by Achenbach and Rescorla (2001).

2.3. Covariates

To allow for social and familial context variables in the analysis, we included in this study questions about maternal educational level, occupation of the head of the household (Hollingshead, 1958), number of children and number of people present in the house, age and sex of the adolescent, and parents living together or not.

2.4. Dental enamel biopsy

To measure the dental enamel lead levels (DELL), surface enamel acid-etch microbiopsies were performed. To prevent possible prior contamination, all vials and polypropylene flasks used to prepare and store solutions were cleaned with detergent solution, rinsed with 10 % (v/v) HNO₃ overnight, rinsed with deionized water, dried and stored in a closed polypropylene container. High purity water provided by a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used throughout. Analytical-grade Tritisol solutions of 1000 mg L⁻¹ of Pb (Merck, Darmstadt, Germany) was used to prepare the reference analytical solutions. All reagents used were analytical-grade. The biopsy procedure was performed at the dental clinic. Adolescents were positioned on the dental chair and all the procedures were performed by a dentist (KPKO). The teeth were cleaned with a rotary brush and pumice slurry, washed and dried. The maxillary right incisor was isolated with cotton rolls prior to the biopsy and an adhesive tape (Magic Tape, 810 Scotch -3M) containing a circular perforation of 4.0 mm in diameter was firmly pressed on to the labial surface of one of the central

maxillary incisors (11), delimiting the biopsy site. The sampling site was etched once according to the following procedure: 10 μL 1.6 mol/L HCl in 70% (v/v) glycerol were applied to the area for 35 s [Cleymaet *et al*, 1991]. The biopsy solution was then transferred to centrifuge tube (Axygen Scientific, Inc., Union City, USA), containing 200 μL high purity water. The surface was then rinsed twice for 10 s with 10 μL high purity water, which was quantitatively transferred to the centrifuge tube, making a final volume of 230 μL . The tape was then removed and the tooth was washed with water for 30 s and dried with an air jet to receive a topical fluoride application. Biopsies were also performed on various surfaces at the dental clinic to evaluate lead contamination in the environment where the procedures were carried out.

2.5. Chemical Analysis

2.5.1 *Pb* determination

A SIMAA-6000 graphite furnace atomic absorption spectrometer with a longitudinal Zeeman-effect background correction system, Echelle optical arrangement, solid state detector, end-capped transversal heating graphite tubes (EC-THGA) with integrated pyrolytically coated platforms (Perkin-Elmer, Norwalk, CT) and hollow cathode lamp was used for the Pb determination. Solutions were delivered into the graphite tube by means of an AS-72 autosampler. The instrumental conditions for the spectrometer were 15 mA of current lamp, 0.7 nm of bandpass and 283.3 nm of wavelength. The heating program consisted of 5 steps (temperature/°C,

ramp/s, hold/s): 1 (130, 10, 10); 2 (200, 5, 20); 3 (800, 5, 20); 4 (2100, 0, 5); and 5 (2400, 1, 2). Aliquots of 10 μL of samples or analytical solutions were introduced into the graphite furnace with 10 μL of chemical modifier (5 μg Pd + 3 μL Mg). This chemical modifier was prepared using suprapur solutions of 10 g/L Pd in 15 % (v/v) HNO_3 and 10 g/L Mg, from the salts $\text{Pd}(\text{NO}_3)_2$ and $\text{Mg}(\text{NO}_3)_2$ (Merck, Darmstadt, Germany), respectively.

The calibration curve (2 – 40 $\mu\text{g/L}$) was constructed using analytical-grade Tritisol solutions of 1000 mg L^{-1} of Pb ($\text{Pb}(\text{NO}_3)_3$), conveniently diluted in 1.6 mol/L HCl in 70% glycerol (v/v).

The samples analyses were performed without previous pretreatment. Dilution procedure with deionized water (2-5 times) were done for samples with high concentrations of Pb (>40 $\mu\text{g/L}$). For each sample, the analytical signals were obtained in triplicate.

The accuracy of analytical procedure was checked by analysis of standard reference material of animal bone (H-5, IAEA from Austria). The comparison between Pb concentrations obtained experimentally (3.08 ± 0.16 mg/kg) and the certified value (3.10 ± 0.18 mg/kg) showed good agreement, considering Student's *t* test at significance level of 95 %.

2.5.2 P determination

A Modula ICP optical emission spectrometer (Spectro Analytical Instruments, Kleve, Germany) equipped with radial-viewed plasma torch was used for phosphorus determination. The setting of the instrumental conditions for the analyses is 1400 W of power, cross-flow nebulizer, double pass (Scott-type) spray chamber, 12 L/min of outer gas flow, 1.0 L/min of

intermediate and nebulizer gas flow, 1.5 mL/min of sample uptake rate and 213.618 nm of atomic P analytical wavelength.

The calibration curve was obtained using analytical-grade Tritisol solutions of 1000 mg/l of P (KH₂PO₄) from Spex (Spex Sample Preparation, Metuchen, USA) after appropriate dilution (20 times) in water. The analytical range was 0.5-10 mg/L. For each sample, the analytical signals were obtained in triplicate.

2.6. Statistical Analysis

The Assessment Data Manager (ADM) software (ASEBA, Burlington, VT) was used to type and analyze data obtained from inventories. Syndromes scales (withdrawn/depressed (counterpart of the 1991 Withdrawn Scale), somatic complaint, anxious/depressed, social problems, thought problems, attention problems, rule-breaking behavior (counterpart of 1991 Delinquent Behavior Scale), aggressive behavior), internalizing, externalizing problems, and DSM (Diagnostic and Statistical Manual) - oriented scales (affective problems, anxiety problems, somatic problems, attention deficit/hyperactivity problems, oppositional defiant problems and conduct problems) scored from the CBCL and the SRD scores were considered as dependent variables. The independent variables were DELL, maternal educational levels, head of the household's occupational status, adolescent's sex and age, presence of two paternal figures at home (if living together or not). CBCL clusters were dichotomized in clinical (score T exceeding the clinical score) or normal profile.

To deal with the large standard deviation in DELL, and considering that there is not an established prevalence of DELL in the population, we decided to consider the 75th percentile (217.35 ppm) as a cut-off point to more appropriately study the extreme cases. Thus, DELL was analyzed as a dichotomic variable (DELL \geq 217.37 ppm = high exposed-lead group and DELL $<$ 217.35ppm = low exposed lead group).

The SRD scores were also dichotomically analyzed [\geq 75th percentile (SRD \geq 15) or $<$ 75th percentile (SRD $<$ 15)], because our sampling was not previously selected to find people presenting higher rates of criminal infractions, except for the selection of the areas recognized as having higher criminality indices. Bivariate analyses were performed to identify associations between the independent variables and the outcomes. To take into account the influence of potential confounding factors, multiple logistic regression models were used.

The software Stata, version 9.1, was used for the analysis.

3. Results

During the entire period, 313 adolescents were invited to participate in this study. Of these eligible subjects, 234 (75%) parents signed the informed consent. Nevertheless, 94 parents did not attend the interviews and 6 adolescents did not attend the exams, even after a minimum of three attempts by home visit or telephone. Four volunteers could not be submitted to lead exams because they presented dental caries or orthodontic appliances on both the maxillary central incisors. Fifty one adolescents were

not examined for lead because the Fundação Casa canceled the authorization for the research with its adolescents due to a change in the institution administration. Thus, 173 (55%) subjects were examined for lead and filled out the Self-Reported of Delinquency (SRD). Out of 140 (45%) parents who answered the Child Behavior Checklist (CBCL), 93 children were examined for lead. Table 1 shows the data characterizing the sample.

Table 2 shows the mean \pm SD of DELL for all volunteers and for sex subgroups. Subjects presenting clinical scores had significantly higher DELL than those with normal scores. There were no significant statistically differences between sex subgroups ($p > 0.05$).

Tests χ^2 were applied and the Table 3 shows the number and percentage of normal and clinical subjects distributed in the low- and high-lead groups. There were significant differences for somatic complaints, somatic problems, social problems and externalizing between low and high-lead groups ($p < 0.05$).

Because the biopsy depth is critical to evaluate the DELL, all models were adjusted for biopsy depth. Table 4 presents the odds ratios adjusted only for biopsy depth and adjusted for biopsy depth, familial and social variables. The only influential variable was “parents living together” in the models with the following dependent variables: conduct problems, thought problems, attention problems, SRD, aggressive behavior, rule-breaking behavior, and externalizing problems. The other covariates were not statistically significant. The bold variables in the table showed the stronger associations, adjusted only for biopsy depth and adjusted for depth biopsy,

familial and social variables. Adolescents' SRD was not significantly related to DELL.

4. Discussion

The results of the present study support the previous findings [Needleman *et al*, 1996; Dietrich *et al*, 2001; Needleman *et al*, 2002] indicating an association between exposure to high lead levels and antisocial/delinquent behavior. In all the models, where the associations were found significant, DELL was the strongest risk factor with small effects of the covariates (Table 4). Despite that Byers and Lord's early case studies identified severe behavior problems as prominent outcomes of lead poisoning, epidemiological studies have only recently begun to focus in detail on psychopathological outcomes (Bellinger, 2008).

“From a public health standpoint, a major concern is a possible “silent pandemic” [Grandjean and Landrigan, 2006] of neurodevelopmental disorders resulting from children's continuing exposure to low lead levels” [Bellinger, 2008]. The form in which neurodevelopmental toxicity is expressed depends on factors such as: age at exposure, coexposures to other neurotoxicants, nutritional status, genotype and the characteristics of the home environment [Hubbs-Tait *et al*, 2005; Weiss and Bellinger, 2006].

Unfortunately, we could not continue the study with arrested and adjudicated adolescents because the Fundação Casa institution canceled the authorization previously conferred to perform the study there. If we could have continued the study there, a case-control design would have been

performed. Thus, we choose to study adolescents living in adverse socioeconomic conditions because we hypothesized that maybe the so many social problems present in their lives, such as low-income; bad conditions of dwelling-place; biological parents living separated; conflicting, aggressive and violent familial environment [Loeber, 1990; Farrington, 1995] could be more significantly associated to behavior problems than exposure to lead. However, even in these severe conditions, the lead exposure was found to be associated with somatic complaints, social problems, rule-breaking behavior and externalizing problems. In this respect, it is important to mention that externalizing is a grouping consisting of the two syndromes that comprise problems that mainly involve conflicts with other people (aggressive behavior and rule-breaking behavior) [Achenbach and Rescorla, 2001 and 2007]. In addition, the rule-breaking behavior scale, in this CBCL version used, is the counterpart version of the 1991 Delinquent behavior scale. Conduct problems is a DSM-oriented scale that group problems such as vandalism, rule-breaking, fighting and stealing, to name a few.

Recently, Wright *et al.* (2008) published a prospective study, in which they followed subjects measuring blood lead levels from pregnancy until the adulthood. In their conclusions, prenatal and postnatal blood lead concentrations were associated with higher rates of total arrests and/or arrests for offenses involving violence, being the first study that reported an association between developmental exposure to lead and adult criminal behavior. Following this same cohort, Dietrich *et al.* (2001) had previously

showed an association between lead exposure and antisocial behavior, independent on other social and biomedical covariates.

An experimental study supports the controversial [McCall *et al.*, 2004] association between lead exposure and aggressiveness. This study used a validated animal model to test the hypothesis that there is a causal relationship between lead exposure and aggression in the absence of confounding variables. The results showed that lead exposure enhances predatory aggression in the cats and provided experimental support for a causal relationship between lead exposure and aggressive behavior in humans (Li *et al.*, 2003). Previously, another experimental study indicated this causal relationship. Golden hamsters exposed to lead became faster and more likely to attack and bite their intruders [Delville, 1998]. These results are very important because epidemiological studies have reported associations between environmental lead exposure and aggressive behavior in children, but could not establish a causal relationship.

In spite of extensive documentation of the toxic effects of lead on human health, the molecular mechanisms underlying its poisonous effects on the central nervous system (CNS) have yet to be clarified [Toscano and Guilarte, 2005]. The specific biological mechanisms underlying lead's effect on aggression and impulsivity are not completely known. "Lead acts on a large number of CNS sites, some which are involved in impulse control. Lead interferes with synaptogenesis [Averill and Needleman, 1980], reduces the inhibition of brain phosphokinase C [Markovac and Goldstein, 1988], and decreases norepinephrine-induced inhibition [Taylor *et al.*, 1978] and lowers

brain levels of serotonin or 5-HIAA [Lasley *et al.*, 1984; Widmer *et al.*, 1991]. Lead exposure is associated with increased levels of D-aminulevulinic acid, which may antagonize GABA inhibition [Meredith, 1978]. Lead also enhances both D1 and D2 dopamine sensitivity, and alters NMDA receptor sensitivity [Cory-Slechta *et al.*, 1992]” (Needleman *et al.*, 2002).

Recently, the neuroanatomical basis for deficits in cognition, executive functions, social behaviors and motor abilities was studied through the relationship between childhood lead exposure and adult brain volume using magnetic resonance imaging. Higher mean childhood lead concentrations were associated with significant decrements in brain volume. The most affected regions were frontal gray matter, specifically the anterior cingulate cortex, responsible for executive functions, mood regulation, and decision-making. [Cecil *et al.*, 2008].

In this study, no association was found between self-reported delinquency, measured by SRD, and DELL. In a case-control study, adjudicated delinquents had significantly higher mean concentrations of lead in their bones than controls and the adjusted odds ratio was 4.0 (95% CI: 1.4-11.1) [Needleman *et al.*, 2002]. However, in a previous cohort study, Needleman *et al.* (1996) found an association between SRD and bone lead levels, but this finding was slightly altered by entering covariates into the model ($p=0.07$). Some reasons may explain why high DELL had presented itself as a protection factor for high SRD in the present study. Firstly, we used the snow ball technique, but, even so, the participants resided in districts with high indices of criminality; the participation in the study was voluntary, which

may explain the small number of offenders in the sample; increasing the need for a larger sample size. Secondly, although great care was taken to avoid possible embarrassment in responding to questions about offences committed, some youths may have omitted facts, which would diminish the SRD score.

A limitation of this study was the small number of participants whose parents responded to the CBCL. It is important to remember that statistical significance and the width of the confidence intervals are strongly dependent on the sample size [Szklo and Nieto, 2007]. Thus, a smaller number of clinical conditions of a given syndrome could explain why an association such as, for example, conduct problems vs DELL was not found to be statistically significant. However, inferring that there is no association when the association is not significant or when the confidence interval overlaps the null hypothesis value fails to consider the important fact that the likelihood of the values within the confidence interval is the maximum for the point estimate [Szklo and Nieto, 2007].

To our knowledge, this is the first study showing an association between antisocial/delinquent behavior and exposure to high lead levels in Brazilian adolescents. In Brazil, only recently a law setting the maximum limit of lead in the manufacturing of varnishes, furniture, toys and other materials used by children in educational settings was approved [Brazil, 2008]. Considering the current high criminality indices existing in Brazil and the results of our study, we substantiate the need for establishing a strong primary prevention policy for preventing lead poisoning in the country. The

scientific evidence presented is more than sufficient to justify this urgent social action.

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Table 1. Characteristics of the entire study sample according to sex subgroups

Variable	All (n)	Boys (n)	Girls (n)	p-value
Mean ± SD of age	15.6±1.3 (179)	15.5±1.3 (103)	15.6 ± 1.2 (75)	0.51
Mean ± SD of schooling (years)	8.1±1.8 (111)	8.0±1.9 (81)	8.5±1.7 (30)	0.16
Mean± SD and Median of maternal schooling	1.77± 1.46(159) Median= Up to 4 years	1.98±1.45 (90) Median = Up to 4 years	1.51±1.43 (69) Median= Up to 4 years	0.04
Occupation of the head of the family* (Median)	Unskilled work (160)	Unskilled work (90)	Unskilled work (70)	0.60
Mean ± SD of number of children living at home	1.4±1.2 (157)	1.3±1.3 (90)	1.5±1.2 (67)	0.21
Mean ± SD of number of people living at home	5.3±1.6 (158)	5.3±1.7 (89)	5.3±1.6 (69)	0.70
Percentage of parents living together	57.5% (160)	57.8% (90)	57.1% (70)	0.08
Self-Reported Delinquency Score	12.4±16.0 (173) Median = 7	15.9±18.8 (97) Median = 10	8.0±9.9 (76) Median = 5	0.001

p-values express the statistical differences between boys and girls.

*Hollingshead classification

Table 2. Mean ($\mu\text{g/g}$, $\pm\text{SD}$) (n) of dental enamel lead concentrations (75th percentile) for clinical and normal subjects, considering somatic, social, conduct and externalizing problems and rule-breaking behavior.

CBCL cluster	Clinical			Normal		
	All	Boys	Girls	All	Boys	Girls
Somatic problems	197.1 \pm 157.0 (22) Median= 188.8	206.0 \pm 159.0 (18) Median= 226.8	156.6 \pm 163.5 (4) Median= 102.6	177.5 \pm 347.0 ^p (71) Median= 89.0	138.1 \pm 136.3 (59) Median= 81.0	126.8 \pm 133.7 (12) Median= 101.1
Social problems	231.1 \pm 482.6 (32) Median= 114.7	240.5 \pm 503.4 (29) Median= 148.5	140.3 \pm 220.3 (3) Median = 22.3	156.4 \pm 164.04 (61) Median= 89.5	159.9 \pm 174.0 (48) Median= 88.7	143.3 \pm 125.3 (13) Median= 122.0
Rule-breaking behavior	293.56 \pm 632.25 (18) Median= 120.0	288.0 \pm 651.3 (17) Median= 76.9	387.4 (1) Median= 387.4	155.37 \pm 159.38 (75) Median= 89.5	162.6 \pm 166.8 (15) Median= 94.8	126.4 \pm 125.8 (15) Median= 80.4
Externalizing	232.6 \pm 445.6 (38) Median= 156.4	236.0 \pm 467.2 (34) Median= 156.4	203.8 \pm 216.3 (4) Median= 207.2	147.2 \pm 163.3 (55) Median= 82.0	154.1 \pm 176.3 (43) Median= 82.0	122.4 \pm 106.7 (12) Median= 101.2

Mann-Whitney test (between clinical and normal groups): Somatic problems $p=0.01$; Social problems $p=0.09$; Rule-breaking behavior $p=0.08$; Externalizing $p=0.47$

Mann-Whitney test (between clinical boys and girls): Normal somatic problems $p=0.78$; Clinical Somatic problems $p= 0.60$; Normal Social problems $p= 0.99$; Clinical Social problems $p= 0.49$; Normal rule-breaking

behavior $p= 0.40$; Clinical rule-breaking behavior $p=0.14$; Normal externalizing $p=0.79$; Clinical externalizing $p=0.77$.

Table 3. Number and percentage of normal and clinical subjects, according to the CBCL profiles and the SRD scores, in the low- and high-lead groups.

CBCL cluster	Low-Lead Group		High-Lead Group		p
	Normal	Clinical	Normal	Clinical	
Withdrawn	33 (35.5%)	33 (35.5%)	12 (12.9%)	15 (16.1%)	0.63
Somatic complaints	45 (48.4%)	21 (22.6%)	11 (11.8%)	16 (17.2%)	0.01
Somatic problems	55 (59.2%)	11 (11.8%)	16 (17.2%)	11 (11.8%)	0.01
Anxious/depressed	28 (30.1%)	38 (40.9%)	11 (11.8%)	16 (17.2%)	0.88
Social competences	46 (49.5%)	20 (21.5%)	15 (16.1%)	12 (12.9%)	0.19
Social problems	44 (47.3%)	22 (23.7%)	12 (12.9%)	15 (16.1%)	0.04
Thought problems	43 (46.2%)	23 (24.7%)	17 (18.3%)	10 (10.8%)	0.84
Attention problems	52 (55.9%)	14 (15.1%)	19 (20.4%)	8 (8.6%)	0.38
Self-Reported Delinquency	100 (57.8%)	34 (19.7%)	30 (17.3%)	9 (5.2%)	0.77
Aggressive behavior	51 (54.8%)	15 (16.1%)	18 (19.4%)	9 (9.7%)	0.29
Rule-breaking behavior	56 (60.2%)	10 (10.8%)	19 (20.4%)	8 (8.6%)	0.11
Conduct problems	54 (58.1%)	12 (12.9%)	19 (20.4%)	8 (8.6%)	0.22
Internalizing	14 (15.1%)	52 (55.9%)	3 (3.2%)	24 (25.8%)	0.25
Externalizing	44 (47.3%)	22 (23.7%)	11 (11.8%)	16 (17.2%)	0.02

Table 4. Association between dental enamel lead concentration and Child Behavior Checklist (CBCL) profiles or Self-Reported Delinquency (SRD) scores.

CBCL Cluster or SRD Score	OR (95% CI)	
	Adjusted for biopsy depth	Adjusted for biopsy depth, familial and social variables
Withdrawn	1.38 (0.54 – 3.49)	1.18 (0.44 – 3.20) ^a
Somatic complaints	3.09 (1.20 – 7.98)	2.93 (1.09 - 7.91)^b
Anxious/depressed	1.15 (0.45 – 2.96)	1.13 (0.41 – 3.09) ^c
Social problems	2.61 (1.00 – 6.84)	3.04 (1.07 - 8.64)^d
Thought problems	1.06 (0.39 – 2.83)	0.99 (0.34 – 2.88) ^e
Attention problems	1.42 (0.49 – 4.08)	1.47 (0.47 – 4.62) ^f
SRD	0.93 (0.40 – 2.20)	0.84 (0.32 – 2.22) ^g
Aggressive behavior	1.51 (0.54 – 4.20)	1.31 (0.42 – 4.09) ^h
Rule-breaking behavior	2.77 (0.90 – 8.51)	3.72 (0.99 – 14.04)ⁱ
Conduct problems	2.38 (0.79 - 7.17)	3.01 (0.83 - 10.89) ^j
Internalizing	2.40 (0.61 – 9.46)	2.17 (0.54 – 8.83) ^l
Externalizing	3.06 (1.17 – 8.04)	2.87 (1.05 – 7.85)^m

Odds ratios (OR) and their 95% confidence interval (CI)^a adjusted for biopsy depth, sex, age, number of children at home and head of the family's occupation; ^b adjusted for biopsy depth, head of the family's occupation and parents living together; ^c adjusted for biopsy depth, age, parents living together and number of children at home; ^d adjusted for biopsy depth, sex, head of the family's occupation, parents living together and number of children at home; ^e adjusted for biopsy depth and parents living together; ^f adjusted for biopsy depth, parents living together, head of the family's occupation and number of children at home; ^g adjusted for biopsy depth, parents living together and

maternal schooling; ^h adjusted for biopsy depth, parents living together, head of the family's occupation and number of children at home; ⁱ adjusted for biopsy depth, parents living together, head of the family's occupation and number of children at home; ^j adjusted for biopsy depth, parents living together, head of the family's occupation and number of children at home; ^l adjusted for biopsy depth, parents living together and maternal schooling; ^m adjusted for biopsy depth and parents living together.

Risk factors associated with high lead levels measured in the superficial dental enamel from Brazilian youths

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Abstract

Objective To investigate the domestic risk factors associated with high lead levels in the surface dental enamel (SDE).

Methods A cross-sectional study was conducted with 179 Brazilian youths aged 14-18 and their parents living in impoverished neighborhoods (Bauru-SP). The parents responded a questionnaire on exposure to possible exposure sources of lead. Body lead burdens were evaluated in SDE acid-etch microbiopsies. The dental enamel lead levels (DELL) were quantified by graphite furnace atomic absorption spectrometry (GFAAS) and the phosphorus content was measured using inductively coupled plasma optical emission spectrometry (ICP-OES). Logistic regression was used to identify associations between DELL and each risk factor evaluated (A, residing in a contaminated area or close proximity; B, working with paints, pigments, ceramic or battery manufacturing; C, to do home-based use of lead-glazed ceramic, low-quality pirated toys, anticorrosive enamel in gates and/or selling of used car batteries, and D= smoking. Social and familial covariates were entered in the models.

Findings The risk factors associated with high DELL were A (OR=4.49; 95%CI=1.69-11.97); B (OR=3.43; 95%CI= 1.31-9.00). C and D were not found to be associated with high DELL (OR=1.31; 95%CI= 0.56-3.03) and (OR=1.66; 95%CI=0.52-5.28), respectively.

Conclusion This work demonstrated that Brazilian youth continue to be exposed to lead by the studied sources. The companies that utilize lead in their manufacturing process must prevent their employees from carrying contamination home on their bodies, clothes or shoes. The surveillance on these companies must be reinforced so that people living near of them are not contaminated and undergo the lead poisoning.

Introduction

Lead poisoning is a long known public health problem. There is not a lead dose considered safe for human health¹. Thus, lead exposure may cause damage in diverse organs, especially in the Central Nervous System (CNS) of children in developing process.

Many countries still add tetraethyl lead to gasoline. In Brazil, although tetraethyl lead is no longer added to gasoline as an anti-detonator since 1978, and there are also regulations for acceptable lead levels in food and water^{2,3}, regarding acceptable levels in humans, until a recent past, only occupational exposure was regulated. Recently, a law was approved fixing the maximum limit of lead in the manufacturing of toys and other material for children in educational settings, as well as varnishes and furniture⁴.

Among the non-regulated sectors, some factories follow the internationally accepted lead parameters. According to the Brazilian Association of Paint Manufacturers, there has been a trend beginning in the 1990s to substitute lead pigments in paints. At present, Brazilian domestic paints are lead free. However, lead is still used as an anti-corrosive, such as red lead in iron gates, refrigerators, cars, stoves, bicycles, and many other goods. In this case a protective paint coating should be applied over the red lead⁵.

The canned food manufacturers in Brazil have also substituted lead-based solders. Glazed ceramic containers can be a source of lead poisoning when lead leaches into stored beverages, especially in the case of acidic fruit

juices such as those made from grapes and citrus fruits⁶. Lead from glazed ceramics is promptly dissolved by the tartaric and citric acids present in the juices, respectively, due to the chelation of the metal by these acids.

Since 1986, an Environmental Impact Report (EIA/RIMA) has been officially required for the approval of potentially polluting industrial plants, as an obligatory document to license these companies⁷. The strategies proposed in this report to minimize the pollution by the plant must be analyzed and approved⁸. Nevertheless, the companies built before 1986 are not obliged to follow this protocol, unless they have caused environmental damage⁸. Unfortunately, several small companies and domestic sources of lead contamination do not issue warnings to prevent lead exposure⁵.

Thus, this study aimed to measure the lead levels present in surface dental enamel from Brazilian adolescents and to compare them between youths exposed and non-exposed to potential risk factors, such as: a) residing in contaminated area or close proximity; b) having someone from their family who worked/is working in paint, pigment, ceramic or battery manufacturing; c) home-based use of glazed ceramic kitchenware, low-quality/pirated toys, anticorrosive enamel (red lead) in iron gates without protective paint coating, and/or selling used car batteries; and d) smoking.

Methods

Subjects

Volunteers were youths aged 14-18, residents in the municipality of Bauru, Southeast Brazil. Some of these adolescents attended a social project called “First Job”, offered by the Irmã Adelaide Center and resided in a shantytown (Ferradura Mirim), in a neighborhood 2 km from a battery recycling plant (Tangarás). These two areas were considered “contaminated or close proximity”. A housing state, originating from an urban renewal project aimed at removing shantytowns from the city (Fortunato Rocha Lima) was considered a non-contaminated area. This housing state is located around 11 km from the battery recycling plant.

In Ferradura Mirim, a census was recently carried out by the School of Sciences of the University of the State of São Paulo and, considering the results of this census, only the houses where adolescents aged 14-18 years resided were visited. In Fortunato Rocha Lima, previous data did not exist, two social projects (“Girassol Project” and “Youth Agent”) that youths attended were visited, and the adolescents that participated in these projects were enrolled. These youths were motivated to invite other adolescent residents in the same area to join and compose the sample (snowball technique sampling). In both areas, meetings were held with the parents and youths to explain the purpose of the research. Afterwards, informed consent forms were distributed and those who signed them were scheduled for an interview.

The Ethical Committee of the School of Public Health, University of São Paulo, reviewed and approved this research (Proc. No. 244/05).. All youth participants received a dental cleaning and neutral fluoride gel

application, if indicated. A clinical exam was carried out, and when a curative treatment was necessary, it was explained to the study participants and a written orientation was delivered with a list of local public institutions offering treatment. This process was adopted for all subjects participating of this study.

Measures of risk factors

Parents of the adolescents completed a questionnaire focusing on: location of actual and prior residences (risk factor A); whether someone from home had worked or works in ceramic, paint, pigment or battery manufacturing (risk factor B); whether their home contains/contained glazed ceramic for hot/acidic food/beverage, low-quality/pirated-toys, anticorrosive enamel on gates without other enamel covering it, and or someone who sells used car batteries (risk factor C); and if the adolescent was a smoker (risk factor D).

Covariates

To allow for social and familial context variables in the analysis, we included in this study questions about maternal educational level, occupation of the head of the family (Hollingshead classification⁹), number of children and number of people present in the house, age and sex of the adolescent.

Dental enamel biopsy

To measure the dental enamel lead levels (DELL), surface dental enamel acid-etch microbiopsies were performed. Material used during the procedure was cleaned in 10% nitric acid (v/v) to avoid possible prior contamination. All reagents were tested for contamination with lead. The biopsy procedure was performed at the dental clinic. Adolescents were positioned on the dental chair and all the procedures were performed by a dentist (KPKO, first author). The teeth were cleaned with a rotary brush and pumice slurry, then washed and dried. The maxillary right incisor was isolated with cotton rolls prior to the biopsy and an adhesive tape (Magic Tape, 810 Scotch -3M) containing a circular perforation of 4.0 mm in diameter was adhered on to the labial surface of the tooth, delimiting the biopsy site. The sampling site was etched once according to the following procedure: 10 μL 1.6 mol/L HCl in 70% glycerol (v/v) were applied to the area for 35 s¹⁰. The biopsy solution was then transferred to a 0.2 mL centrifuge tube (Axygen Scientific, Inc., Union City, USA) containing 200 μL ultrapurified water. The surface was then rinsed twice for 10 s with 10 μL ultrapurified water which was then transferred to the centrifuge tube, making a final volume of 230 μL . The tape was then removed and the tooth was washed with water for 30 s and dried with an air jet to receive a neutral topical fluoride application. Biopsies were also performed on various sites at the dental clinic bench or at a centrifuge tube rack to evaluate lead contamination in the environment where the procedures were carried out.

Chemical Analysis

Lead (Pb) determination

A SIMAA-6000 graphite furnace atomic absorption spectrometer with a longitudinal Zeeman-effect background correction system, Echelle optical arrangement, solid state detector, end-capped transversal heating graphite tubes (EC-THGA) with integrated pyrolytically coated platforms (Perkin-Elmer, Norwalk, CT) and hollow cathode lamp was used for the Pb determination. Solutions were delivered into the graphite tube by means of an AS-72 autosampler. The instrumental conditions for the spectrometer were 15 mA of current lamp, 0.7 nm of bandpass and 283.3 nm of wavelength. The heating program consisted of 5 steps (temperature/°C, ramp/s, hold/s): 1 (130, 10, 10); 2 (200, 5, 20); 3 (800, 5, 20); 4 (2100, 0, 5); and 5 (2400, 1, 2). Aliquots of 10 µL of samples or analytical solutions were introduced into the graphite furnace with 10 µL of chemical modifier (5 µg Pd + 3 µL Mg). This chemical modifier was prepared using suprapur solutions of 10 g/L Pd in 15 % (v/v) HNO₃ and 10 g/L Mg, from the salts Pd(NO₃)₂ and Mg(NO₃)₂ (Merck, Darmstadt, Germany), respectively.

The calibration curve (2 – 40 µg/L) was constructed using analytical-grade Tritisol solutions of 1000 mg L⁻¹ of Pb (Pb(NO₃)₃), conveniently diluted in 1.6 mol/L HCl in 70% glycerol (v/v).

The samples analyses were performed without previous pretreatment. Dilution procedure with deionized water (2-5 times) were done for samples with high concentrations of Pb (>40 µg/L). For each sample, the analytical signals were obtained in triplicate.

The accuracy of analytical procedure was checked by analysis of standard reference material of animal bone (H-5, IAEA from Austria). The comparison between Pb concentrations obtained experimentally (3.08 ± 0.16 mg/kg) and the certified value (3.10 ± 0.18 mg/kg) showed good agreement, considering Student's *t* test at significance level of 95 %.

Phosphorus (P) determination

A Modula ICP optical emission spectrometer (Spectro Analytical Instruments, Kleve, Germany) equipped with radial-viewed plasma torch was used for phosphorus determination. The setting of the instrumental conditions for the analyses is 1400 W of power, cross-flow nebulizer, double pass (Scott-type) spray chamber, 12 L/min of outer gas flow, 1.0 L/min of intermediate and nebulizer gas flow, 1.5 mL/min of sample uptake rate and 213.618 nm of atomic P analytical wavelength.

The calibration curve was obtained using analytical-grade Tritisol solutions of 1000 mg/l of P (KH_2PO_4) from Spex (Spex Sample Preparation, Metuchen, USA) after appropriate dilution (20 times) in water. The analytical range was 0.5-10 mg/L. For each sample, the analytical signals were obtained in triplicate.

2.6. Statistical Analysis

The data from questionnaires were typed using the software Excel 2003 (Microsoft Corporation, Redmond, WA, USA). Each risk factor evaluated was dicotomically analyzed. For risk factor A, to reside in Ferradura Mirim, Tangarás or close to companies that possibly contaminate

the environment with lead were considered as 1 and residing in Fortunato Rocha Lima or locals where there are not industries was considered as 0. For risk factor B, when someone at home had worked/ was working in factories that possibly use lead in their manufacturing process, it was considered 1 and, when nobody had worked in this type of work, it was considered 0. For risk factor C, 0 was adopted for none use of glazed ceramic kitchenware, pirated toys, anticorrosive enamel in gates without covering paint, and selling of used car batteries and 1 when one or more of those items was/were utilized. For risk factor D, smokers were considered 1 and non-smokers were designated 0. Afterwards, dental enamel lead levels (DELL) were considered as a dependent variable and each risk factor as independent variables.

To deal with the large standard deviation in DELL, and considering that there is not an established DELL prevalence in the population, we decided to consider the 75th percentile (217.35 ppm) as a cut-off point to study more appropriately the extreme cases. Thus, DELL was analyzed as a dichotomic variable (DELL \geq 217.37= high exposed-lead group and DELL $<$ 217.35= low exposed-lead group). All the models were controlled by the biopsy depth.

Bivariate analyses were performed to identify associations between the independent variables and the outcome. To take into account the influence of potential confounding factors, multiple logistic regression models were used.

Mann-Whitney tests were used to analyze the difference between sex subgroups; and between exposed and non-exposed groups ($p < 0.05$).

The software Intercooled Stata version 9.1 was used for the analysis.

Results

During the entire period, 262 adolescents were invited to participate in this study. Of these eligible subjects, 183 (70%) parents signed informed consent forms. Nevertheless, 6 adolescents did not attend the exams, even after a minimum of three attempts at home visits or telephone contact. Four volunteers could not be given lead exams because they presented dental caries or orthodontic appliances on both the maxillary central incisors. Thus, 179 (68%) subjects were examined for lead and 160 parents (61%) filled out the questionnaires. The principal motives of the youth who did not want to participate in the study were: the adolescent had left home; the adolescent could not attend the exam because he/she worked the whole day or did not have interest in participating.

Table 1 shows the covariates structure and data characterizing the sample. As can be seen, the only variable significantly different between males and females was the mean of the maternal educational level ($p = 0.04$).

DELL only was statistically different between exposed and non-exposed subjects, when risk factor A was analyzed ($p = 0.0006$). In relation to risk factor B, although the medians found for adolescents whose parents worked in ceramic, pigment, battery or paint manufacturing were found to be higher than DELL median from adolescents whose parents did not work in

this type of work, the difference was not statistically significant ($p=0.0908$). Nor there was significant differences of DELL means between exposed and non-exposed for risk factor C (exposure to glazed ceramic kitchenware, red lead in gates without protective paint coating, batteries removed from cars and pirated-toys, nor risk factor D (smoking) ($p=0.9022$; $p=0.4528$, respectively) (Table 2).

Table 3 shows the association between DELL and the risk factors analyzed. High DELL was significantly associated with residing in contaminated areas or in close proximity and (risk factor A) and someone at home working in ceramic, pigment, battery or paint manufacturing (risk factor B). The adjusted odds ratios for social and familial variables (95% CI) were 4.49 (1.69-11.97) and 3.43 (1.31-9.00) for A and B risk factors, respectively. The odds ratios for DELL vs. risk factor C or risk factor D were not significantly associated with high DELL.

Discussion

This study demonstrated a strong association between high DELL and residing next to or in close proximity (around 2 kilometers) to companies using lead or even living with someone who worked in such places. These findings are not original^{11,12,13,14,15,16}, but their importance lies in that they substantiate that lead exposure is preventable, and that the consequences of lead poisoning are very serious and, furthermore, people continue to be contaminated in the studied area.

Probably, unsuspected sources of lead poisoning are present in the daily routine of millions of people worldwide. Recently, a study concluded that one-fifth of both US-manufactured and Indian-manufactured Ayurvedic medicines purchased via the Internet contained detectable lead, mercury, or arsenic¹⁷. However the principal danger resides in those products destined for children. Toys may contain lead in their paints and small children have the habit of placing objects in their mouth. This can be grave because a child's intestine absorbs lead much faster than an adult's and the developing infant CNS is more vulnerable to toxic agents than the mature CNS, especially in the case of undernourished children. Neural proliferation, differentiation and plasticity are strongly impaired by lead.

The presence of smokers in the familial environment was also identified as a risk factor for higher blood lead levels in children living close to a smelting plant in Sweden¹⁸. In the present study, we did not find an association between high DELL and the adolescent's smoking habit. Dental enamel contamination by lead contained in the cigarettes would show a post-eruptive uptake of lead to dental issue and there is no data that definitely demonstrate that it occurs. From our results, smokers did not present higher DELL than non-smokers; in spite of there are only a few smokers in our sample. The pre-eruptive lead uptake seems to be predominant.

The use of low-quality/pirated-toys, lead-glazed ceramic kitchenware, red lead for iron gates, and/or selling used car batteries was not found to be associated with high DELL. Admittedly, our sample was small and we did not measure the lead content of the toys and glazed ceramic used. This was

partly because the questionnaire asked about early use of the objects in the period when the adolescents were children, which we had not means of measuring. In addition, in Brazil, the “invasion” of imported and low-quality toys is recent. Another interesting fact is that the majority of the people interviewed stated having not lived in houses with iron gates because they lived in shacks, which typically would not have iron gates associated with higher quality housing.

On this point, the socioeconomic status must be stressed. The form in which neurodevelopmental toxicity is expressed depends on factors such as age at exposure, coexposure to other neurotoxicants, nutritional status, genotype and the characteristics of the home environment^{19,20}. Low-socioeconomic status implies residing in risk areas or in areas surrounding contaminated factories, which is aggravated by unpaved streets. These places lack recreation options, and children frequently play in the streets in direct contact with the lead contaminated soil and dust which, then accumulates in their homes. Playing with soil and dust containing lead were indeed found to be risk factors to lead poisoning^{21,22,23,24}. In the studied area, parents related that their children barely had toys, and, the few toys that they had had been bought from street vendors, who sell pirated low-quality toys.

The association found in this study between living with someone working with lead and high DELL has been demonstrated in previous studies^{11,13,25,26,27,28,29}. In the Freitas' study, although the company provided separate bathrooms and lockers, 20% of the studied workers said they use to bring home the clothes they had worked in. Some adolescents studied by us

were residents from the same area or their relatives worked in the same company studied by Freitas et al¹¹.

Lead is long known as a ubiquitous, insidious and devastating neurotoxicant. Lead poisoning is reportedly linked to a high risk of learning disabilities, aggressiveness and criminal offenses^{30,31,32,33,34,35}.

One of the many positive consequences of preventing lead exposure has substantial economic benefits. Grosse et al³⁶ calculated that US preschool children would experience a 2.2-4.7 point IQ increase above what it might have been if leaded gasoline and blood lead had not been reduced. From this, they calculated the IQ-related increase in income and estimated that the economic benefit for each year's cohort of 3.8 million of children aged 2 ranges from \$110 billion and \$319 billion. Landrigan et al³⁷, assuming no threshold for the lead-IQ association, estimated the loss of future earnings for the one-year cohort of children aged 5 in 1997 at \$43.4 billion.

In summary, DELL reflected risk factors more associated with lead exposure among the studied sources. Because lead exposure is preventable, we conclude by calling for public health policies that protect the population of these poisoning risks and avoid individual and national economic losses. Government supported education campaigns should inform the public of the dangers of lead exposure. Such public initiatives for primary prevention already exist in developed countries (www.cdc.gov, www.fda.gov, www.epa.gov); nevertheless, very little has been done in Brazil, where the vast majority of the population is not aware of the dangers of lead poisoning

and it is not known what portion of the population is in danger of lead exposure.

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Competing interests: None declared

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Table 1. Descriptive variables (n) for all subjects and for sample stratified by sex.

Variable	All (n)	Boys (n)	Girls (n)	P value
Mean ± SD of age (years)	15.6±1.3 (179)	15.5±1.3 (103)	15.6 ± 1.2 (75)	0.51
Mean ± SD (years) of adolescent's educational level	8.1±1.8 (111)	8.0±1.9 (81)	8.5±1.7 (30)	0.16
Maternal educational level (mean ± SD and median, years)	1.77±1.46(159) Median= Up to 4 years	1.98±1.45 (90) Median = Up to 4 years	1.51±1.43 (69) Median= Up to 4 years	0.04
Occupation of the head of the household* (Median)	Unskilled work (160)	Unskilled work (90)	Unskilled work (70)	0.60
Mean ± SD of number of children living at home	1.4±1.2 (157)	1.3±1.3 (90)	1.5±1.2 (67)	0.21
Mean ± SD of number of people living at home	5.3±1.6 (158)	5.3±1.7 (89)	5.3±1.6 (69)	0.70

*Hollingshead classification

Table 2. Mean ($\mu\text{g/g}$, $\pm\text{SD}$) (n) of dental enamel lead concentrations for exposed and non-exposed subjects, considering risk factor A (to reside in contaminated area or near of there); risk factor B (to work in paints, pigments, ceramic or batteries manufacturing), risk factor C (to do home use of glazed ceramic, pirate toys, anticorrosive enamel without covering paint in gates, and/or commercializing used car batteries), and risk factor D (smoking).

Risk factor	Exposed				Non-exposed			
	All	Boys	Girls	<i>P</i>	All	Boys	Girls	<i>P</i>
A	222.8 \pm 363.0 (65) Median= 139.5	530.7 \pm 784.4 (11) Median= 248.4	262.1 \pm 287.7 (4) Median= 188.6	0.59	129.9 \pm 189.0 (95) Median= 62.5	147.1 \pm 215.2 (44) Median= 52.3	115.1 \pm 163.8 (51) Median= 62.5	0.40
B	274.3 \pm 500.4 (31) Median= 148.0	356.4 \pm 640.5 (18) Median= 204.8	160.6 \pm 143.7 (13) Median= 129	0.47	142.0 \pm 181.3 (128) Median= 79.3	149.7 \pm 187.0 (72) Median= 78.2	132.2 \pm 175.1 (56) Median= 79.4	0.32
C	178.2 \pm 314.0 (112) Median= 88.7	215.1 \pm 392.6 (62) Median= 88.7	132.4 \pm 166.4 (50) Median= 90.2	0.13	143.1 \pm 155.6 (48) Median= 76.8	137.8 \pm 143.5 (28) Median= 61.5	150.5 \pm 174.7 (20) Median= 99.0	0.94
D	158.2 \pm 134.2 (16) Median= 155.8	155.3 \pm 130.2 (14) Median= 155.8	178.2 \pm 221.1 (2) Median= 178.2	0.75	168.7 \pm 287.9 (144) Median= 84.0	197.6 \pm 362.0 (76) Median= 81.5	136.4 \pm 168.0 (68) Median= 90.2	0.25

Mann-Whitney test (between exposed and non-exposed groups): A - $p=0.0006$; B - $p=0.0908$; C - $p=0.9022$; D - $p=0.4528$

Table 3. Association between dental enamel lead levels and risk factor A (to reside in contaminated area or near of there); risk factor B (to work in paints, pigments, ceramic or batteries manufacturing), risk factor C (to do home use of glazed ceramic, pirate toys, batteries removed from cars, and/or anticorrosive enamel without covering paint in gates), and risk factor D (smoking).

Risk factor	OR (95% CI)	
	Adjusted by biopsy depth	Adjusted by biopsy depth, familial and social variables
A	4.25 (1.63-11.13)	4.49 (1.69-11.97)^a
B	2.30 (0.96-5.49)	3.43 (1.31-9.00)^b
C	1.24 (0.55-2.82)	1.31 (0.56-3.03) ^c
D	2.06 (0.67-6.28)	1.66 (0.52-5.28) ^d

^a adjusted for biopsy depth, number of children at home and head of the family's occupation; ^b adjusted for number of people at home, head of the family's occupation, adolescent's sex and adolescent's age; ^c adjusted for number of people at home, head of the family's occupation and adolescent's sex; ^d adjusted for biopsy depth, mother's schooling and sex.

Methodological alterations of surface dental enamel microbiopsies for lead body burden measurement

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Short title: Surface dental enamel microbiopsies

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ABSTRACT

Albeit lead poisoning is preventable, it continues to be a public health problem in several countries. Measuring lead on the surface dental enamel (SDE) is rapid, safe and painless. However, the methodological protocol for SDE etch-acid microbiopsies must be calibrated to render studies comparable among diverse populations. This study aimed to compare two distinct enamel biopsy protocols. For this, two consecutive enamel layers were removed by each protocol, in the same subject group (n=138). To confirm the *in vivo* results, profilometry tests were performed in bovine enamel blocks. Protocol I consisted of a biopsied site of 4 mm in diameter after application of 10 μ L HCl for 35 s. Protocol II was based on a biopsied site of 1.6 mm in diameter after application of 5 μ L HCl for 20 s. Next, the dental enamel lead levels (DELL) and biopsy depth were compared: a) first and second layers under the same protocol; b) right and left incisors by the same protocol; c) protocols I and II for two removed layers. The adopted significance level was 5%. The biopsy depth, calculated by the cylinder formula, for Protocol II led to misleading results, as confirmed by profilometry tests. This study demonstrated that SDE, analyzed by etch-acid microbiopsy, is a reliable biomarker, but DELL could not be compared when there was any methodological variant among studies. In addition, the cylinder formula does not seem to be adequate for calculating the depth of the removed layer in all biopsy protocols.

Key words: biopsy; dental enamel; environmental health; lead; toxicology; public health

INTRODUCTION

Lead is the most abundant environmental contaminant in industrialized areas worldwide (Cleymaet, 1991). In addition, “silent” human poisoning occurs from unsuspected sources, such as paints covering low-quality and pirated toys, lead-glazed ceramic kitchenware, anticorrosive red lead used on iron gates, to name a few (Olympio et al, 2009a).

For surveillance of the lead levels in the population, blood is the most commonly used biomarker (Rabinowitz, 1995). Although blood is a reliable marker for recent exposures, it is not a perfect biomarker. Recently, an increased interest in lead content of human dental tissues has been clearly demonstrated by the Archaeological Sciences (Budd et al, 1998; Budd et al, 2000; Budd et al, 2004). Unlike bone, dental enamel is not remodeled in later life and the human dental tissues allow for the historic reconstruction of the early exposure (Budd et al, 1998).

Particularly, dental enamel is the most appropriated material to sampling when the aim is to describe the natural history of lead exposure. Dental enamel is highly mineralized and its dense structure makes it resistant to diagenesis in the burial environment. Concomitantly, surface dental enamel acid-etch microbiopsies have been used to measure the body burden lead levels *in vivo* (Almeida et al, 2007; Almeida et al, 2008; Cleymaet, 1991a, 1991b, 1991c, 1991d; Gomes et al, 2004; Olympio et al, 2009b).

Surface dental enamel acid-etch microbiopsies have been carried out by different protocols in which a number of variables are modified. Dental enamel, even though it is not widely utilized, is a promising biomarker

because the surface dental enamel microbiopsies are minimally invasive, easy to operate and inexpensive. However, a trustworthy protocol must be established for comparisons among diverse studies and populations.

During a previous study carried out by our research group (Olympio et al, 2009b), we found notable differences between dental enamel lead levels (DELL) and biopsy depth described in the diverse studies that used surface dental enamel etch-acid biopsy (Almeida et al, 2007; Almeida et al, 2008; Cleymaet, 1991a, 1991b, 1991c, 1991d; Gomes et al, 2004). This finding pointed out for us a research problem – evaluating the accuracy of the formula used to calculate the biopsy depth because DELL is directly dependent on this variable. Lead levels in dental enamel decreases from the outermost superficial layer to the inner layer of dental enamel (Brudevold et al, 1975; Cleymaet et al, 1991). Biopsy depth is commonly calculated by the cylinder formula in surface dental enamel etch-acid biopsies.

Thus, the general objective of this study was to verify whether DELL found in studies that used surface dental enamel etch-acid biopsy with methodological variants in their protocol can be compared. In addition, the specific aims of the present study were: a) to compare DELL and biopsy depths between homologous teeth biopsied by the same protocol; b) to compare DELL and biopsy depths between homologous teeth biopsied by different protocols; and c) to compare DELL and biopsy depths between two consecutive layers biopsied by protocols I and II.

MATERIAL AND METHODS

Subjects. Volunteers were 138 youths aged 14-18, residents of the municipality of Bauru, Southeast São Paulo State, Brazil. Part of this group was composed of adolescents attending in a social project “First Job”, offered by the Irmã Adelaide Center. Some adolescents of this group resided in Tangarás, a neighborhood where a battery recycling plant existed (closed in 2002) and most of the youth lived in Ferradura Mirim, a settlement of shacks, distant around 2 kilometers from the plant. These two areas were considered as “contaminated or close proximity”, respectively. A housing complex, originated from an urbanistic project for resettling shantytown squatters from the city (Fortunato Rocha Lima) was included as a non-contaminated area. This housing complex is located far (11 kilometers) from the battery recycling plant. Of the 138 subjects included in this study, 59 adolescents composed the sample to compare the Protocols I and II and 79 youths composed the sample to compare the Protocol I between homologous teeth. This study was approved by the Institutional Review Board of the Public Health School, University of São Paulo (Proc. No. 244/05).

In vivo human surface dental enamel biopsy. To measure the dental enamel lead levels (DELL), surface enamel acid-etch microbiopsies were performed. The material used during the procedure was cleaned in 10% (v/v) nitric acid to avoid possible prior contamination. The biopsy procedure was performed at the dental clinic. Adolescents were positioned on the dental chair and all the procedures were performed by a dentist (KPKO). The teeth were cleaned with a rotary brush and pumice slurry, washed and dried. For

the protocol I group (n=59), the right maxillary central incisor (11) was isolated with cotton rolls prior to the biopsy and adhesive tape (Magic Tape, 810 Scotch -3M) containing a circular perforation of 4.0 mm in diameter was adhered onto the labial surface of the tooth, delimiting a window for the biopsy site. This window was etched twice according to the following procedure: 10 μ L 1.6 mol/L HCl in 70% (v/v) glycerol were applied to the area for 35 s [Brudevold, 1975]. The biopsy solution was then quantitatively transferred to centrifuge tube (Axygen Scientific, Inc., Union City, USA), containing 200 μ L Millipore Alpha Q water. Afterwards, the surface was rinsed twice for 10 s each with 10 μ L Millipore Alpha Q water which was then transferred to the respective centrifuge tube, making a final volume of 230 μ L. This procedure was repeated in the same window to remove the second sampling (inner layer). For the protocol II group (n=59), the contra lateral tooth (21) was biopsied. For this, the adhesive tape (Magic Tape, 810 Scotch -3M) containing a circular perforation of 1.6 mm in diameter was adhered onto the labial surface of the left maxillary central incisor, delimiting a window for the biopsy site. That window was also etched twice according to the following procedure: 5 μ L 1.6 mol/L HCl in 70% (v/v) glycerol were applied to the area for 20 s [Gomes et al, 2004]. The solution of biopsy was then transferred to centrifuge tube (Axygen Scientific, Inc., Union City, USA), containing 200 μ L Millipore Alpha Q water. Next, the surface was rinsed once for 10 s with 5 μ L Millipore Alpha Q water which was then transferred to the respective centrifuge tube, making a final volume of 210 μ L. For both

protocols (I and II), after the described procedures, the tape was then removed and the teeth were washed with water for 30 s and dried with air jets to receive neutral topical fluoride applications. Biopsies were also performed in various sites at the dental clinic bench or at the centrifuge tube rack to evaluate lead contamination in the environment where the procedures were carried out.

To compare DELs and biopsies depths between homologous teeth by the same biopsy protocol, 79 adolescents had both maxillary central incisors biopsied by protocol I.

Lead and phosphorus determinations. To avoid contamination, all vials and polypropylene flasks used to prepare and store solutions were cleaned with detergent solution, rinsed with 10 % (v/v) HNO₃ overnight, rinsed with deionized water, dried and stored in a closed polypropylene container. High purity water provided by a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used throughout. All reagents used were analytical-grade.

Lead determination was done by graphite furnace atomic absorption spectrometry (GF AAS). For this, a graphite furnace atomic absorption spectrometer, model SIMAA-6000, with a longitudinal Zeeman-effect background correction system, Echelle optical arrangement, solid state detector, end-capped transversal heating graphite tubes (EC-THGA) with integrated pyrolytically coated platforms (Perkin-Elmer, Norwalk, CT) and hollow cathode lamp was used. Solutions were delivered into the graphite

tube by means of an AS-72 autosampler. The instrumental conditions for the spectrometer were 15 mA of current lamp, 0.7 nm of bandpass and 283.3 nm of wavelength. The heating program consisted of 5 steps (temperature/°C, ramp/s, hold/s): 1 (130, 10, 10); 2 (200, 5, 20); 3 (800, 5, 20); 4 (2100, 0, 5); and 5 (2400, 1, 2). Aliquots of 10 µL of samples or analytical solutions were introduced into the graphite furnace with 10 µL of chemical modifier (5 µg Pd + 3 µL Mg). This chemical modifier was prepared using Suprapur solutions of 10 g/L Pd in 15% (v/v) HNO₃ and 10 g/L Mg, prepared from the inorganic salts Pd(NO₃)₂ and Mg(NO₃)₂ (Merck, Darmstadt, Germany). The calibration curve (2 – 40 µg/L) was prepared in the autosampler vials using analytical-grade Tritisol solutions of 1000 mg L⁻¹ of Pb (Pb(NO₃)₃) diluted in 0.06 mol/L HCl in 7% glycerol (v/v). The samples analyses were performed without previous pretreatment. Dilution procedure with high pure water (2-5 times) was done for samples with high concentrations of Pb (>40 µg/L). For each sample, the analytical signals were obtained in triplicate. The accuracy of the GF AAS analytical procedure was checked by analysis of standard reference material of animal bone (H-5, IAEA from Austria).

A Modula ICP optical emission spectrometer (Spectro Analytical Instruments, Kleve, Germany) equipped with radial-viewed plasma torch was used for phosphorus determination. The setting of the instrumental conditions for the analyses is 1400 W of power, cross-flow nebulizer, double pass (Scott-type) spray chamber, 12 L/min of outer gas flow, 1.0 L/min of intermediate and nebulizer gas flow, 1.5 mL/min of sample uptake rate and 213.618 nm of atomic P analytical wavelength. The calibration curve was

obtained using analytical-grade Tritisol solutions of 1000 mg/l of P (KH₂PO₄) from Spex (Spex Sample Preparation, Metuchen, USA) after appropriate dilution (20 times) in water. The analytical range was 0.5-10 mg/L. For each sample, the analytical signals were obtained in triplicate.

Biopsy depth calculation. The calculus, previously described by Cleymaet et al (1991), is based in the information that 17.4% of the enamel weight is phosphorus and the mean density of the dental enamel is 2.95 g/cm³. Biopsy depths are estimated according to the following equation:

$$\text{Biopsy depth} = \text{mass enamel } (\mu\text{g}) / 2.95 \times \text{biopsy area } (\text{mm}^2)$$

Measurement of biopsy depth after the in vitro bovine dental enamel biopsy. Sixteen sound crowns of bovine incisors were embedded in acrylic resin cylinders (Paladur, Heraeus Kulzer, Wehrheim, Germany), and the labial surfaces were ground flat and polished with water-cooled carborundum paper (500, 800, 1200, 2400 and 4000 grit, waterproof silicon carbide paper; Struers, Erkrat, Germany), approximately 200 μm of the outer enamel being removed. Surface hardness of enamel specimens was chosen as the criterion for stratified allocation of the specimens to the protocol I and protocol II groups.

Prior to the experiment, baseline scans were obtained from the specimens with a contact profilometer (Mahr Perthometer, Göttingen, Germany). After preparation, the specimens were stored in water until used in the experiment to avoid dehydration.

In 8 samples, the adhesive tape (Magic Tape, 810 Scotch -3M) containing a circular perforation of 4.0 mm in diameter was adhered on to the labial surface of the tooth, delimiting a window for the biopsy site (Protocol I), while another 8 samples had an adhesive tape containing a circular perforation of 1.6 mm in diameter, delimiting a window for the biopsy site (Protocol II). The biopsies were performed as described earlier. The biopsies were repeated twice in each specimen.

After each etch-acid biopsy, enamel loss was determined by contact profilometry. (Mahr Perthometer, Göttingen, Germany). The reference areas, which remained protected by tape during the biopsy, were marked with a scalpel blade on the outer surface to allow for exact reposition of the tape. Prior to the experiment, five and three equidistant baseline surface scans of each specimen from Protocol I and II were performed, respectively. To determine the enamel loss, the tape was removed and five profiles were recorded at exactly the same sites as the baseline measurement. For this, the enamel specimens were provided with identification marks, which allowed the stylus to be re-positioned accurately at each measurement. The profile scans were performed in the center of each specimen at intervals of 250 μm . Pre- and post-treatment scans were superimposed on graphs and the average depth of the area under curve in the eroded area was calculated with specially designed software (Marh, Göttingen, Germany). The results of the five scans were averaged for each specimen (Magalhães et al., 2008).

Statistical Analysis. For statistical analysis, Graph Pad InStat statistical software was used. DELL and biopsy depth data did not pass normality test (Kolmogorov-Smirnov test) and were then analyzed by Wilcoxon matched-pairs signed ranks tests. To compare the loss of enamel between first and second layers from each protocol paired *t* tests were applied and *t* test was used to compare the protocols I and II. A significance level of 5% was adopted.

RESULTS

The comparison between lead concentrations obtained experimentally (3.08 ± 0.16 mg/kg) and the certified value (3.10 ± 0.18 mg/kg) showed good agreement, considering Student's *t* test at significance level of 95 %.

In the 79 subjects examined, whose both maxillary central incisors were biopsied by protocol I, the biopsy depths ranged between 0.48 and 2.04 μm for the first removed layer, and between 0.65 and 1.55 μm for the second removed layer (n=70). In the 59 subjects whose right maxillary central incisors were biopsied by protocol I, the biopsy depths ranged between 0.26 and 0.99 μm for the first removed layer, and between 0.20 and 0.99 μm for the second layer (n=58). In the same subjects, whose left maxillary central incisors were biopsied by the protocol II, the biopsy depths ranged between 0.08 and 4.99 for the first layer, and 0.13 to 5.64 μm for the second removed layer (n=57). The different numbers sampling between first and second layers are due to losses during the collecting procedures. When we compared the results of biopsy depth and DELLs between homologous teeth,

biopsied by the same protocol (protocol I), there were not significant differences between right and left maxillary central incisors ($p > 0.05$). To calculate the DELL means, we excluded the DELL values that were below the detection limit ($0.5 \mu\text{g/L}$) of the equipment (GFAAS) (Table 1).

Table 2 shows the means \pm SD and medians of DELLs and biopsy depths found by protocols I and II in the biopsy samples. Both protocols were performed in the same adolescent, for the first and second layers in each protocol. As can be observed, there were significant differences in biopsy depths in the first and second layers between both protocols ($p < 0.05$). The biopsy depths were found to be different according to the protocol followed for both layers. Interestingly, the biopsy depths found by the protocol II (dental enamel exposed to acid for a lesser time) were significantly higher than those found by the protocol I, which was contrary to what was to be expected. Thus, we decided to perform an *in vitro* study to confirm these data. The *in vitro* study results will be shown later on.

Albeit the mean biopsy depths were different, mean DELL were not significantly different when the results from protocols I and II were compared ($p > 0.05$). There was a significant difference between DELL in the first and second layers for both protocols (I and II) (Table 2).

Table 3 presents the individual and total means of enamel wear caused by the *in vitro* biopsies carried out in bovine enamel blocks. The enamel wear seen in protocol I was significantly higher than that observed for protocol II, for both layers ($t=5.737$, $p < 0.0001$ and $t=6.239$, $p < 0.0001$, for the first and second layers), respectively. In addition, the enamel wear seen in

the second layer was significantly higher compared to that observed in the first layer, for each protocol ($t=27.463$, $p<0.0001$ and $t=20.980$, $p<0.0001$ for the protocols I and II) respectively.

Figure 1 shows the surface scanning of eroded areas after enamel biopsies performed according to protocols I (A) or II (B). It can be observed that the worn area obtained for A has a shape very similar to a cylinder, while that seen for B has a very different shape.

DISCUSSION

The results of the present study showed that SDE is a reliable biomarker of lead exposure because both biopsy depths and DELs were not different between homologous teeth from the same individual, examined by a single methodology. However, when different methodological protocols were applied in the same individual, biopsy depths were found to be significantly different between homologous teeth. Biopsy depth depends on both pH solution, which was the same for both protocols, as well as exposure time of the SDE to acid etch. When the SDE is exposed to acid for shorter periods, it is expected that the removed layer is smaller. However, when we calculated the biopsy depths (removed by protocols I and II) by the cylinder formula, the layer removed by the acid exposure for 20 s was deeper and DEL was individually higher (although the mean values were not found to be significantly different) than when SDE was exposed to acid for a longer time. This result is not biologically plausible because deeper biopsies contain less lead than more superficial biopsies, since there is a very steep lead gradient

in the surface enamel (Brudevold, 1977). Thus, these results suggested that the protocol II led to an erroneous result for permanent teeth, overestimating DELL, since biopsy depth is critical to calculate it. The profilometry test was then conducted in bovine enamel crowns to help explain these discrepant results. It was observed that when SDE was exposed to acid for a shorter time period, a more superficial layer was removed than when SDE was exposed to acid for a longer period. Then, in fact, DELL was higher because the protocol II biopsy was more superficial, which confirmed the expected results (Brudevold, 1975; Gomes et al., 2004). The profilometry drawings (Figure 1) suggest that the shorter time period of exposure of SDE to acid etch in protocol II was not sufficient to promote homogenous erosion as occurred in protocol I. As shown in Figure 1B, it can be inferred that it is impossible to calculate the biopsy depth by the cylinder formula in this case, because there is no defined height to be considered in the calculations, since the basis of the geometric figure obtained is quite irregular.

It is known that protocol II is suggested to be followed when deciduous teeth are biopsied (Gomes et al., 2004). Deciduous enamel can be considered to be less mineralized than permanent teeth (Wilson and Beynon, 1989), since it has been shown that the superficial microhardness of deciduous teeth (358 KHN) is significantly lower than that found for permanent (368 KHN) (Magalhães et al., 2008). As the crown of deciduous teeth is very small, it was not possible for us to obtain a section with extension sufficient to conduct the profilometry tests using both protocols. Consequently, we decided to use bovine enamel crowns for this purpose. It

must be acknowledged that a definitive conclusion regarding the suitability of the protocol II for deciduous teeth can only be drawn if a study of the worn area conducted in deciduous teeth is done. Considering the area limitation mentioned above, one possible alternative would be the use of atomic force microscopy as a response variable. In this case, it would be possible to evaluate the topography profile of the worn area after the acid biopsy using deciduous enamel and this experiment will be conducted by our research group.

There are many variations among published microbiopsy methodologies. Cleymaet et al (1991) applied a small synthetic fiber pellet to dry the biopsied surface which was added to a tube together with the collected SED sample. This detail is the only difference from protocol I of the present study. Almeida et al (2004) modified the Brudevold's technique to be used in deciduous teeth, as the protocol II in the present study. These authors did not mention how the modifications made to the original technique were evaluated. In relation to the original technique (similar to protocol I), the modified technique (similar to protocol II), the acid etch exposure time of SDE was decreased, as it was the biopsy site diameter, and the volume of the acid applied. Since many variables were modified simultaneously, it could be expected that the geometric figure formed after the acid exposure in protocol II would be different from the expected cylinder, which is formed when the original technique is used. Again, it must be emphasized that the wear results obtained in this study apply to bovine teeth and this profile should be confirmed when using deciduous human enamel.

The biopsy depths found in this study presented lower means than those found in other studies (Cleymaet, 1991 a, b, c, d). Despite this, DELL was almost twice as high in the first layer compared to the second. Observing that the difference between first and second layers is approximately 0.5 μm , this fact highlights the steep lead gradient in SDE and the importance of calculating the biopsy depth with precision. On the other hand, the individual DELL values were very different comparing the results from both protocols applied in the same individual. Nonetheless, the mean DELL was not found to be significantly different. A threshold definition for DELL is a controversial point to discuss. Almeida et al (2008) suggested the use of deeper biopsies is more reliable to compare DELL among different populations because these authors detected a *plateau* (600 $\mu\text{g/g}$) in lead content in the biopsies removed from deciduous teeth in biopsy depths from 3.18-5.9 μm . For this conclusion, the precision of the biopsy depth is crucial.

In summary, this study demonstrated the risk of comparing DELL results between studies that used different methodological protocols. In addition, all alterations performed in analysis techniques should be minutely studied to verify whether the expected results after the modifications would be the same as those obtained by the original method, as in the case in question – the biopsy depth.

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Table 1. Means \pm SD and medians of biopsy depth [μm] and dental enamel lead levels (DELL) [ppm] found in both maxillary central incisors by protocol I (4 mm in diameter; 35 s, 10 μL HCl) in the microbiopsies samples for first and second removed layers.

Layer	Biopsy Depth		DELL	
	Right Incisor	Left Incisor	Right Incisor	Left Incisor
First Layer	1.05 \pm 0.18 (n=79) Median= 1.06	1.04 \pm 0.21 (n=79) Median= 1.09	217.04 \pm 356.88 (n=72) Median= 122.24	219.03 \pm 364.34 (n=72) Median= 111.00
Second Layer	1.12 \pm 0.16 (n=70) Median= 1.11	1.15 \pm 0.19 (n=70) Median= 1.14	110.99 \pm 154.24 (n=61) Median= 76.92	102.03 \pm 153.66 (n=61) Median= 60.00

There were no significant differences between right and left incisors both for depth and DELL, in each layer (Wilcoxon matched-pairs tests, $p > 0.05$).

Table 2. Means \pm SD and medians of dental enamel lead levels (DELL) [ppm] and biopsy depth [μ m] found by protocols I (4 mm in diameter; 35 s, 10 μ L HCl) and II (1.6 mm in diameter, 20 s, 5 μ L HCl) in the microbiopsy samples for the first and second layer in each protocol.

Variable	Protocol I		Protocol II	
	First Layer	Second Layer	First Layer	Second Layer
Biopsy Depth	0.56 \pm 0.13 ^{a,A} (n=59) Median= 0.55	0.61 \pm 0.15 ^{b,A} (n=58) Median= 0.61	1.04 \pm 0.63 ^{a,B} (n=59) Median= 0.98	1.31 \pm 0.76 ^{b,B} (n=57) Median= 1.19
DELL	124.96 \pm 153.15 ^{a,A} (n=59) Median= 49.54	60.99 \pm 84.27 ^{b,A} (n=58) Median= 23.73	126.94 \pm 225.24 ^{a,A} (n=59) Median= 28.81	54.69 \pm 94.73 ^{b,A} (n=57) Median= 18.09

Wilcoxon matched-pairs test: different lowercase letters indicate significant differences ($p < 0.05$) between first and second layers for protocol I and II; different uppercase letters indicate significant differences in the biopsy depth and DELL between protocols I and II for both layers.

Table 3. Individual and total means (μm) of dental enamel wear, measured by profilometry, after *in vitro* surface dental enamel etch-acid microbiopsies in bovine enamel blocks.

Enamel Block	First Layer		Second Layer	
	Protocol I	Protocol II	Protocol I	Protocol II
1	1.909	0.649	3.698	2.252
2	2.674	1.021	4.385	2.898
3	2.182	0.745	3.635	2.439
4	1.771	1.327	3.517	2.791
5	1.665	0.856	3.373	2.276
6	1.905	0.905	3.523	2.730
7	1.969	1.466	3.298	2.824
8	1.403	0.882	3.203	2.208
Mean\pmSD	1.935\pm0.377^{A,a}	0.981\pm0.281^{B,b}	3.579\pm0.366^{A,a}	2.552\pm0.288^{B,b}

Paired *t* test: different uppercase letters indicate significant differences between first and second layers in each protocol; *t* test: different lowercase letters indicate significant differences between protocols I and II for each layer.

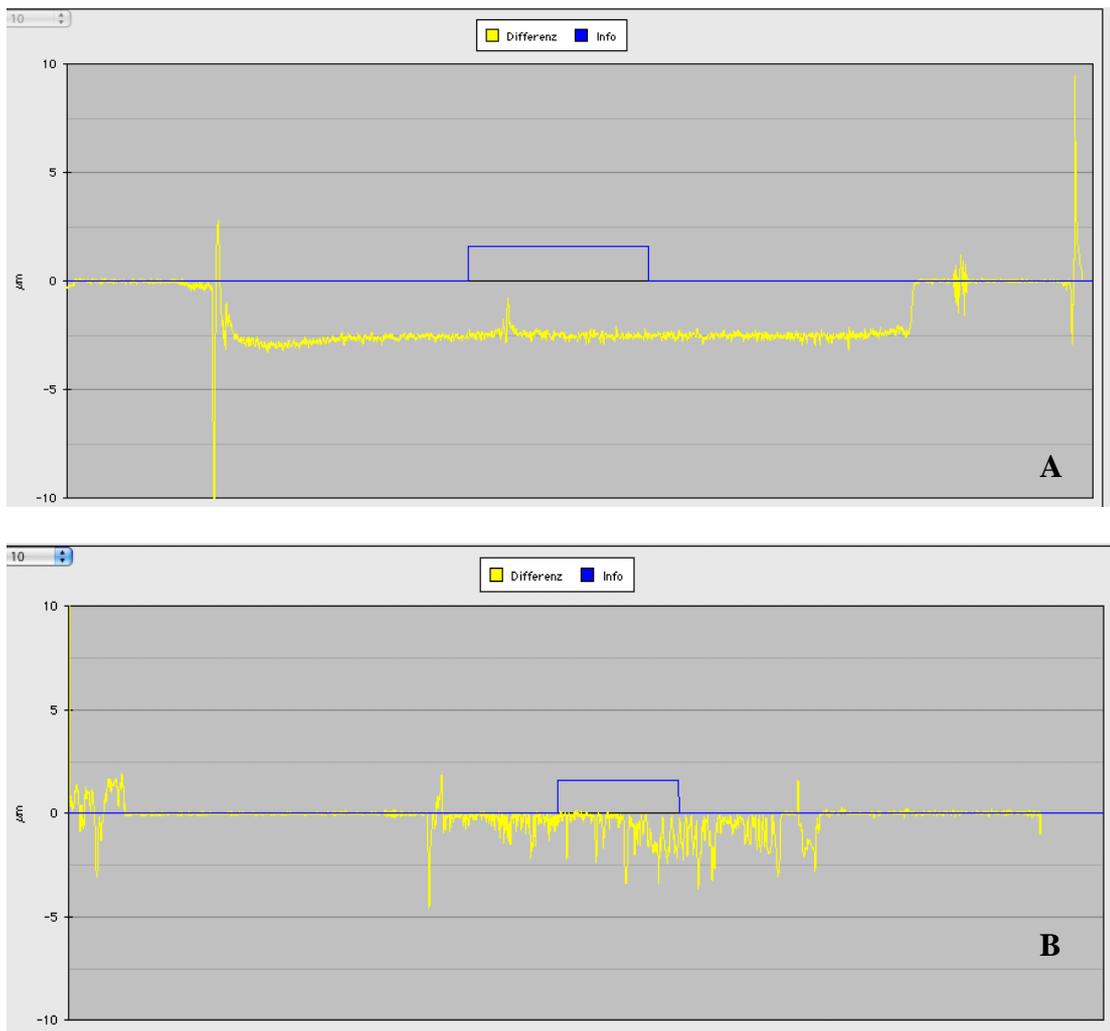


Figure 1 Profilometry drawing (A. Protocol I: 4 mm in diameter, 35 s, 10 μL HCl; B. Protocol II: 1.6 mm in diameter, 20 s, 5 μL HCl). In blue: considered area; In yellow: eroded area.

5 CONCLUSÕES e RECOMENDAÇÕES

1. Mesmo com a presença de tantos problemas familiares e sociais, a exposição ao chumbo foi o fator de risco mais fortemente associado a comportamento anti-social, avaliado pelo CBCL, tendo sido analisado o ponto de vista dos pais ou responsáveis pelos adolescentes brasileiros estudados. Maiores escores alcançados pelo auto-relato de delinqüência não foram associados à maior exposição ao chumbo;
2. A população foi exposta ao chumbo por algumas fontes estudadas. Os fatores de risco domiciliares mais associados à exposição ao chumbo foram residir no entorno ou em região próxima de indústrias que utilizam chumbo em seu processo produtivo e ter alguma pessoa que mora ou morou com o adolescente trabalhando em empresas que utilizam chumbo. Essas empresas devem evitar o arraste externo deste metal, efetuado pelos funcionários por meio das roupas, sapatos e corpos. Sugere-se reforço na vigilância ambiental destas indústrias, visando minimizar a exposição da população circunvizinha e os conseqüentes efeitos à saúde;
3. O esmalte dentário mostrou-se um marcador biológico fidedigno para avaliação da carga corporal de chumbo. No entanto, comparações da concentração de chumbo entre estudos diferentes não deveriam ser realizadas sem cautela, uma vez que variações metodológicas são encontradas entre as diversas pesquisas, com conseqüências de alterações importantes para interpretação dos resultados. Assim, uma padronização metodológica da técnica de microbiópsia ácida de

esmalte dentário superficial faz-se necessária para tornar viável a utilização deste biomarcador como recurso a ser utilizado em Saúde Pública.

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5. Laboratório de Terapia Comportamental do Instituto de Psicologia da Universidade de São Paulo. (Trad.). (2006). Guia para profissionais

**Estas referências são complementares àquelas anteriormente relacionadas nos manuscritos.*

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ANEXO 1



UNIVERSIDADE DE SÃO PAULO

Faculdade de Saúde Pública

DEPARTAMENTO DE SAÚDE AMBIENTAL

Av. Dr. Arnaldo, 715 - CEP: 01246-904 –São Paulo–SP

Fone: 3082-3842/3066-7712 - Fax: 3066-7732

e-mail: hsa@fsp.usp.br

Modelo dirigido aos pais ou responsáveis que responderão ao questionário

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

1ª via

O chumbo está presente em muitos locais como na tinta de casas antigas, pintura de cerâmicas, no solo, água e ar de regiões industrializadas. Existem relatos publicados de que a contaminação pelo chumbo pode causar diversos problemas de comportamento social. Assim, o objetivo desta pesquisa é verificar a concentração de chumbo presente no esmalte do dente e sua relação com desvios de comportamento social. Para isto, será realizada a aplicação de uma gota de ácido clorídrico sobre o dente do adolescente e esta gota será analisada para a verificação da quantidade de chumbo. Além disso, será aplicado um questionário ao adolescente sobre o seu próprio comportamento e um questionário ao pai ou responsável sobre o comportamento de seu filho ou adolescente que esteja sob sua tutela. Todos os adolescentes receberão limpezas nos dentes e aplicação de flúor. Esta pesquisa não trará riscos, desconforto ou gastos financeiros para os participantes. Ao final da pesquisa, os dados obtidos poderão ser publicados em revistas especializadas com fins científicos. As informações obtidas com a pesquisa não identificam o participante, mantendo a privacidade do indivíduo. Os resultados desta pesquisa ajudarão na formulação de políticas públicas de prevenção da contaminação por chumbo pela população e na compreensão dos efeitos da contaminação por chumbo à saúde humana. Esperando contar com o seu apoio, desde já agradecemos. Em caso de dúvida, você poderá entrar em contato com as pesquisadoras pelo e-mail kellypko@usp.br (Kelly), wgunther@usp.br (Profa. Wanda).

Assim, pelo presente instrumento que atende às exigências legais, o Sr. (a) _____, portador (a) da cédula de identidade _____, residente à _____, responsável legal pelo adolescente _____ declara ter tomado conhecimento dos objetivos e procedimentos da pesquisa, devidamente explicada e detalhada pelos pesquisadores, não restando quaisquer dúvidas a respeito do lido e explicado, firma seu **CONSENTIMENTO LIVRE E ESCLARECIDO** para responder ao questionário sobre o comportamento social do adolescente em questão.

Por estarem de acordo, assinam o presente termo.

Local (Bauru ou Lins), ____ de _____ de _____.

Assinatura do participante

Assinatura do autor



UNIVERSIDADE DE SÃO PAULO

Faculdade de Saúde Pública

DEPARTAMENTO DE SAÚDE AMBIENTAL

Av. Dr. Arnaldo, 715 - CEP: 01246-904 –São Paulo–SP

Fone: 3082-3842/3066-7712 - Fax: 3066-7732

e-mail: hsa@fsp.usp.br

Modelo dirigido ao responsável, autorizando a participação do adolescente na pesquisa

1ª via

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

O chumbo está presente em muitos locais como na tinta de casas antigas, pintura de cerâmicas, no solo, água e ar de regiões industrializadas. Existem relatos publicados de que a contaminação pelo chumbo pode causar diversos problemas de comportamento social. Assim, o objetivo desta pesquisa é verificar a concentração de chumbo presente no esmalte do dente e sua relação com desvios de comportamento social. Para isto, será realizada a aplicação de uma gota de ácido clorídrico sobre o dente do adolescente e esta gota será analisada para a verificação da quantidade de chumbo. Além disso, será aplicado um questionário ao adolescente sobre o seu próprio comportamento. Todos os adolescentes receberão limpezas nos dentes e aplicação de flúor. Esta pesquisa não trará riscos, desconforto ou gastos financeiros para os participantes. Ao final da pesquisa, os dados obtidos poderão ser publicados em revistas especializadas com fins científicos. As informações obtidas com a pesquisa não identificam o participante, mantendo a privacidade do indivíduo. Os resultados desta pesquisa ajudarão na formulação de políticas públicas de prevenção da contaminação por chumbo pela população e na compreensão dos efeitos da contaminação por chumbo à saúde humana. Esperando contar com o seu apoio, desde já agradecemos. Em caso de dúvida, você poderá entrar em contato com a pesquisadora pelos e-mails kellypko@usp.br (Kelly), wgunther@usp.br (Profa. Wanda).

Assim, pelo presente instrumento que atende às exigências legais, o Sr. (a) _____, portador (a) da cédula de identidade _____, residente à _____, responsável legal pelo adolescente declara ter tomado conhecimento dos objetivos e procedimentos da pesquisa, devidamente explicada e detalhada pelos pesquisadores, não restando quaisquer dúvidas a respeito do lido e explicado, firma seu **CONSENTIMENTO LIVRE E ESCLARECIDO**, para que o adolescente _____, portador da cédula de identidade _____ possa participar da pesquisa proposta.

Por estarem de acordo, assinam o presente termo.

_____, ____ de _____ de _____.

Assinatura do responsável

Assinatura do autor

Assinatura do adolescente

ANEXO 2

Disponível em <http://www.aseba.org> (Copyright 2001 T.M. Achenbach).

ANEXO 3

Auto-relato de comportamento

Código:

Estas perguntas lidam com seu próprio comportamento. Não se preocupe com as respostas, seja honesto porque assim você estará ajudando a pesquisa. **TODAS AS RESPOSTAS SÃO CONFIDENCIAIS E NÃO SERÃO RELACIONADAS À SUA IDENTIDADE.** Eu vou ler uma série de comportamentos para você. Por favor, fale o número certinho de vezes que você fez cada uma destas coisas nos últimos 6 meses e a idade quando você fez isso pela primeira vez.

Nunca = 0, Uma vez = 1, 2-5 vezes = 2, 6-10 vezes = 3, Mais de 10 vezes = 4

Quantas vezes nos últimos 6 meses você... (repetir esta frase de vez em quando)

- ____ 1. Fugiu de casa. ____ (idade da primeira vez que fez isso)
- ____ 2. Matou aula ou não foi para a escola sem motivo. ____
- ____ 3. Mentiu a sua idade para poder entrar em algum lugar ou comprar alguma coisa, por exemplo para assistir um filme proibido para menores ou para comprar bebida alcoólica? ____
- ____ 4. Andou de carona do lado de fora de ônibus, trens ou carros quando era proibido fazer isto? ____
- ____ 5. Carregou arma escondido? ____
- ____ 6. Gritou, fez bagunça ou não obedeceu as regras de um lugar público e as pessoas reclamaram ou você teve problemas? ____
- ____ 7. Pediu dinheiro ou coisas para estranhos? ____
- ____ 8. Ficou bêbado em lugar público? ____
- ____ 9. Estragou ou destruiu de propósito coisas que não eram suas, por exemplo pixou, quebrou alguma coisa que não era sua? ____
- ____ 10. Colocou fogo em uma casa, prédio, carro ou outra coisa de propósito ou tentou fazer isso? ____
- ____ 11. Tentou não pagar coisas que você comprou ou utilizou como cinema, ônibus e comida? ____
- ____ 12. Entrou ou tentou entrar em um lugar para roubar coisas? ____
- ____ 13. Roubou ou tentou roubar coisas de menos de R\$5,00? ____
- ____ 14. Roubou ou tentou roubar coisas com valor entre R\$5,00 e R\$50,00? ____
- ____ 15. Roubou ou tentou roubar coisas com valor entre R\$50,00 e R\$100,00? ____
- ____ 16. Roubou ou tentou roubar coisas de R\$100,00 ou mais? ____
- ____ 17. Pegou alguma coisa de uma loja sem pagar? ____
- ____ 18. Roubou a carteira ou bolsa de alguém? ____
- ____ 19. Pegou alguma coisa de um carro que não era seu? ____
- ____ 20. Comprou, vendeu ou pegou alguma coisa que você sabia que era roubada ou tentou fazer isso? ____
- ____ 21. Pegou para dirigir um carro ou moto sem que o dono soubesse e tivesse deixado? ____
- ____ 22. Roubou ou tentou roubar uma moto ou carro? ____
- ____ 23. Usou cheque ilegalmente ou dinheiro falso para comprar alguma coisa? ____
- ____ 24. Usou ou tentou usar um cartão de crédito ou do banco sem a permissão do dono? ____
- ____ 25. Tentou enganar alguém, vendendo-lhe alguma coisa que não tinha valor ou que você disse que tinha e não tinha? ____
- ____ 26. Atacou alguém com uma arma com a idéia de machucar ou matar a pessoa de verdade? ____
- ____ 27. Bateu em alguém com a idéia de machucar? ____ (outra situação que você ainda não tenha dito)
- ____ 28. Usou arma ou força para conseguir dinheiro ou coisas das pessoas? ____
- ____ 29. Atirou objetos como pedras ou garrafas nas pessoas? (sem contar o que você já falou) ____
- ____ 30. Envolveu-se em brigas de gangues? ____
- ____ 31. Foi pago (a) para fazer sexo com alguém? ____
- ____ 32. Machucou ou tentou machucar alguém para fazer sexo com você? ____
- ____ 33. Fez sexo ou tentou fazer sexo com alguém contra a vontade da pessoa? ____
- ____ 34. Vendeu maconha ou "haxixe"? ____
- ____ 35. Vendeu drogas pesadas como heroína, cocaína, LSD ou outras drogas pesadas? ____
- ____ 36. Foi levado (a) pela polícia por algum outro motivo, além de uma leve infração de trânsito? ____

ANEXO 4

Questionário de Identificação e fatores de confusão

Endereço: _____

Telefone para contato: _____

Nome do pai, mãe ou responsável que respondeu o questionário:

Há quanto tempo vocês moram neste endereço?

Em que lugares vocês moraram antes? Escrever rua e bairro.

Onde vocês moraram até os 5 anos de idade do adolescente?

Quantas pessoas moram na sua casa? _____ 0 a 12 anos de idade
 _____ 13 anos ou mais

Os pais moram juntos? () sim () não

Até que série o pai estudou?

Até que série a mãe estudou?

() analfabeto

() analfabeto

() primário incompleto

() primário incompleto

() primário completo

() primário completo

() 1º grau incompleto

() 1º grau incompleto

() 1º grau completo

() 1º grau completo

() Ensino médio incompleto

() Ensino médio incompleto

() Ensino médio completo

() Ensino médio completo

() Superior incompleto

() Superior incompleto

() Superior completo

() Superior completo

Você fuma cigarro? (...) Sim

(...) Não

Seu filho fuma cigarro ou masca fumo? (...) Sim

(...) Não

Você trabalha ou trabalhou? Onde?

Alguém que mora com vocês trabalha ou trabalhou em fábrica:

() baterias () pigmentos () tintas () cerâmica

Por quanto tempo?

Na sua casa, você faz uso de:

() Cerâmica vitrificada para alimentos quentes

() Brinquedos importados

() Baterias de carros ao redor ou na sua casa

() Zarcão nos portões da casa

Seguindo a PORT/CPG/03/08



Kelly Polido Kaneshiro Olympio

Possui graduação em Odontologia pela Universidade Estadual Paulista Júlio de Mesquita Filho (2000) e mestrado em Odontologia em Saúde Coletiva pela Universidade de São Paulo (2005). Atualmente é orientadora do Centro de Extensão Universitária de São Paulo, professora de Epidemiologia no Curso de Especialização em Saúde Coletiva da Associação Paulista de Cirurgiões Dentistas (APCD) - Sorocaba e doutoranda em Saúde Pública pela Faculdade de Saúde Pública da Universidade de São Paulo. Publicou 20 artigos em periódicos nacionais e internacionais, 1 livro publicado pela Edusp, 55 resumos em anais de congressos e 4 artigos estão aceitos, aguardando publicação. Tem experiência na área de Saúde Coletiva, com ênfase em Epidemiologia, Odontologia Preventiva e Toxicologia, atuando principalmente nos seguintes temas: fluorose dentária, dentífricos fluoretados, levantamentos em saúde bucal, esmalte dentário como biomarcador e efeitos da exposição ao chumbo à saúde humana.

(Texto informado pelo autor)

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Dados Pessoais

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Formação Acadêmica/Titulação

- 2005** Doutorado em Saúde Pública.
Universidade de São Paulo, USP, Sao Paulo, Brasil
Título: Correlação entre exposição a chumbo e comportamento anti-social em adolescentes
Orientador: Wanda Maria Risso Günther
Bolsista do(a): Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
- 2003 - 2005** Mestrado em Odontologia Em Saúde Coletiva.
Universidade de São Paulo, USP, Sao Paulo, Brasil
Título: Análise da incorporação de flúor no esmalte dentário in vivo após o uso de dentífricos com concentrações reduzidas de flúor e sua relação com a biodisponibilidade de flúor nestes dentífricos em comparação com outros comercialmente disponíveis, Ano de obtenção: 2005
Orientador: Marília Afonso Rabelo Buzalaf
- 2001 - 2002** Especialização em Odontologia Em Saúde Coletiva.
Associação Paulista de Cirurgiões Dentistas, APCD, Brasil
Título: Cárie e doença periodontal causadas por tratamento ortodôntico na ausência de um programa educativo-preventivo.
Orientador: Prof Dr. José Roberto de Magalhães Bastos
- 1995 - 2000** Graduação em Odontologia.
Universidade Estadual Paulista Júlio de Mesquita Filho, UNESP, Sao Paulo, Brasil
- 1999 - 1999** Aperfeiçoamento em Dentística Restauradora.
Universidade Estadual Paulista Júlio de Mesquita Filho, UNESP, Sao Paulo, Brasil
Orientador: Jorge Komatsu e equipe

Formação complementar

- 1995 - 1995** Curso de curta duração em Tratamento Endodôntico de Dentes Com Lesão Peri...
Universidade Estadual Paulista Júlio de Mesquita Filho, UNESP, Sao Paulo, Brasil