Ministério da Saúde
FIOCRUZ
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ESCOLA NACIONAL DE SAÚDE PÚBLICA
SERGIO AROUCA
ENSP

"Níveis elevados de manganês e déficit cognitivo em crianças residentes nas proximidades de uma metalúrgica ferro-manganês na Região Metropolitana de Salvador, Bahia"

por

José Antonio Menezes Filho

Tese apresentada com vistas à obtenção do título de Doutor em Ciências na área de Saúde Pública e Meio Ambiente.

Orientadora principal: Prof.ª Dr.ª Paula de Novaes Sarcinelli
Segundo orientador: Prof. Dr. Josino Costa Moreira

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Esta tese, intitulada

"Níveis elevados de manganês e déficit cognitivo em crianças residentes nas proximidades de uma metalúrgica ferro-manganês na Região Metropolitana de Salvador, Bahia"

apresentada por

José Antonio Menezes Filho

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Resumo

As crianças, sobretudo aquelas socialmente vulneráveis, são mais susceptíveis aos efeitos tóxicos da exposição ambiental aos agentes químicos. No processo de desenvolvimento, o sistema nervoso imaturo apresenta grande oportunidade de ação de contaminantes ambientais como o mercúrio (Hg), chumbo (Pb) e o manganês (Mn). Os objetivos desta investigação foram quantificar o grau de exposição ao Mn em crianças residentes nas proximidades de uma planta metalúrgica de ligas ferro-manganês e avaliar a associação entre os níveis deste metal no sangue e no cabelo e efeitos na função cognitiva. Para tal fim, foi realizada uma revisão da literatura científica sobre exposição de crianças ao Mn e efeitos neuropsicológicos, a qual originou o primeiro artigo. A avaliação da exposição ao Mn foi realizada na Vila Cotegipe, no município de Simões Filho, Bahia. Na primeira etapa do trabalho foram obtidas das crianças de 1 a 10 anos amostras de cabelo para determinação do Mn, sangue para hemograma e amostras para parasitológico de fezes. Foram também coletadas amostras ambientais como: água bruta e tratada, material particulado na fração respirável ($PM_{2.5}$) e poeira domiciliar. Para fins de comparação, crianças de uma comunidade distante 7,5 km da metalúrgica, e a favor dos ventos, foram incluídas como grupo controle Na segunda etapa da avaliação, foram incluídas somente as crianças de 6 a 11 anos e 11 meses matriculadas na escola municipal local. Novas amostras de cabelo e sangue foram coletadas para análise de Mn, chumbo (Pb) e ferro sérico, sendo solicitada a mãe ou responsável a doação da amostra de cabelo. Nessa etapa foi realizada a avaliação cognitiva, através dos instrumentos WISC-III (Wechesler Intelligence Scale for Children), matriz progressiva de Raven para medir a cognição materna e inventário HOME adaptado para estimar o ambiente familiar. Os resultados das amostras ambientais mostraram que os teores de Mn na água estavam dentro dos padrões aceitáveis; a concentração de Mn no ar ($PM_{2.5}$) estava em média três vezes superior a concentração referência da EPA (RfC 0,05 $\mu g/m^3$) e os níveis de Mn na poeira domiciliar estavam aproximadamente 20 vezes mais elevados do que os níveis deste na poeira em residências do grupo controle. Das 165 crianças elegíveis os pais de 147 delas concordaram com a participação no estudo e 109 (66,1%) aceitaram doar amostras biológicas. Os níveis de Mn no sangue estavam na maioria (97%) dentro dos valores normais (4-

14 µg/L); porém, tanto em 2007 como em 2008, os níveis de Mn no cabelo tiveram mediana de 9,70 µg/g (1,10-95,50) µg/g e 6,51 µg/g (0,10-76,78 µg/g), respectivamente, superando em muitas vezes a mediana encontrada na população controle 1,09 µg/g (0,30-5,58 µg/g). Os níveis de Mn no cabelo materno encontravam-se igualmente elevados 4,04 µg/g (0,10-77,75 µg/g). Foi observada uma associação significativa entre os níveis de Mn no cabelo da criança e decréscimo no QI na Escala Total, subescala Verbal e fatorial Compreensão, após ajuste pela escolaridade materna e índice nutricional. Foi possível demonstrar pela primeira vez que o Mn também interfere na cognição de adultos, pois as mães ou responsáveis apresentavam um significativo decréscimo de acordo com a concentração de Mn no cabelo, ajustado pela idade, renda familiar e grau de escolaridade. Nossos resultados comprovam que as crianças desta comunidade estão sujeitas a uma exposição excessiva ao Mn oriundas das emissões da metalúrgica, com possíveis conseqüências negativas no desenvolvimento intelectual. Devido aos efeitos observados nas mães, se pode pensar que essas crianças sejam duplamente afetadas pela exposição ao Mn, tanto de forma direta, resultante do efeito do Mn nos seus sistemas nervoso e outra indireta, devido ao seu efeito no intelecto materno, conduzindo a uma menor estimulação neuropiscológica da criança.

Palavras-chave: Manganês, crianças, cognição, cabelo, planta metalúrgica

Abstract

Children, especially those socially vulnerable, are more susceptible to toxic effects resulting from environmental exposure to chemical agents. The developing nervous system has great opportunities to the action of environmental contaminants like mercury (Hg), lead (Pb) and manganese (Mn). The objectives of this research were to evaluate the Mn exposure levels in children living in the vicinity of a ferro-manganese alloy plant and investigate the association between Mn levels in blood and hair with the effects on the cognitive function. Initially, we carried out an intensive literature review on the association between children's exposure to Mn and neuropsychological effects, which led to the first article. The field work started with the populational pool and registration of all families within the limits of the Cotegipe Village, Simões Filho town, Bahia, Brazil. After obtaining the informed consent, we collected socio-demographic data among the volunteers. To assess Mn exposure level, we performed the first sampling campaign with children aged 1 to 10 years: hair samples for Mn determination, blood sample for haemogram and stool for intestinal parasites analyses. At this phase we collected environmental samples: water pre a post treatment, particulate matter from respirable fraction ($PM_{2.5}$) and house dust. In the second exposure assessment campaign we included only children aged 6 to 11 years and 11 months, enrolled in the local public school, who provided hair and blood samples for Mn, lead and serum iron determination. Mothers or caregivers were asked to provide hair sample. This happened concomitantly with the cognitive evaluation, which was assessed using WISC-III (Wechesler Intelligence Scale for Children), Raven's Progressive Matrices for measuring maternal cognition and the adapted HOME to estimate the family environment stimulation. The results of the environmental assessment showed that water Mn levels were within the acceptable standards, Mn concentrations in the air ($PM_{2.5}$) were on average three times higher than the USEPA reference concentration (RfC 0.05 $\mu g/m^3$) and Mn levels in house dust were approximately 20 times higher than levels of the house dust in residences distant 7.5 km (control). Of the 165 children enrolled, the parents of 147 agreed to participate in the study and 109 children (66.1%) consented to donate biological samples. Blood Mn levels were in the majority (97%) within the normal range (4-14 µg/L), however in the two campaigns conducted in 2007 and 2008, hair Mn level

medians were 9.70 µg/g (1.10-95.50 µg/g) and 6.51 µg/g (0.1-76.78 µg/g), respectively. These levels were much higher than the median level observed in the control group 1.09 µg/g (0.30-5.58 µg/g). Maternal Mn hair levels were also elevated 4.04 µg/g (0.1-77.75 µg/g). We observed a significant association between Mn hair levels and a decreament in Full-Scale, Verbal and factorial Comprehension IQ scores, after adjusting for maternal education and nutritional index. It was possible to demonstrate for the first time that Mn interferes with maternal cognition as well. Cognitive function of mothers and caregivers presented a significant decrease with increasing Mn concentrations in hair, adjusted for age, family income and years of schooling. Our results show that children of this community are subjected to excessive Mn exposure from emissions arising from the industrial plant, with a consequent measurable negative effect on the intellectual development. Based on these findings we could hypothesize that these children are doubly affected, directly due to the Mn effect on their own brains and indirectly as a result of the effect on their mothers' cognition, which would tend to provide a poorer neuropsychological stimulation of their children.

Key-words: Manganese, children, conition, hair, alloy-plant

Índice

Introdução

A Organização Mundial da Saúde (OMS) estima que no mundo mais de 30% das doenças nas crianças podem ser atribuídas a fatores ambientais. A Academia de Ciências dos Estados Unidos da América estimou que a exposição ambiental a substâncias tóxicas contribui para a causa de mais de 28% dos distúrbios neurocomportamentais nas crianças americanas. As crianças são altamente vulneráveis às substâncias tóxicas (Landrigan, 2000). O autor pontua que a exposição infantil é desproporcionalmente mais elevada, tomando como base a massa corpórea, pois as crianças bebem mais água, ingerem mais alimentos e têm uma taxa respiratória mais elevada que os adultos. A OMS chama a atenção que elas têm variações na susceptibilidade durante os diferentes estágios da vida, devido aos processos de desenvolvimento, assim como às diferenças fisiológicas, metabólicas e comportamentais (WHO, 2006). Da concepção até a adolescência ocorrem rápidos processos de crescimento e desenvolvimento que podem ser perturbados pela exposição ambiental a agentes químicos. As vias e os padrões de exposição podem também ser diferentes nas diversas fases da infância: exposições podem ocorrer através da transferência materna, tanto na fase uterina quanto durante a amamentação; através da dieta, da água (ingerida ou na recreação) ou decorrente do hábito da criança de levar a mão à boca, de engatinhar e brincar no chão (WHO, 2006).

O sistema nervoso (SN) em desenvolvimento tem sido demonstrado ser o órgão alvo primário dos efeitos adversos de inúmeros agentes químicos (Landrigan et al, 2005; Weiss e Landrigan, 2000; Rice e Barone 2000; Bellinger

2009). Os níveis de exposição que produzem muito pouco ou nenhum efeito no SN maduro podem impor sérios riscos ao cérebro em desenvolvimento (Faustman et al, 2000). Ocorrem inúmeros períodos de susceptibilidade desde o início do desenvolvimento do SN no período embrionário, que continua durante a fase fetal e pós natal (Barone et al, 2000).

Dentre as neurotoxinas de origem ambiental mais estudadas estão o mercúrio (Hg) e o chumbo (Pb). Grande parte do conhecimento da toxicidade clínica do metil-mercúrio ambiental está relacionada aos desastres ambientais ocorridos há mais de 40 anos em Minamata e Niigata, Japão, como também no Iraque. Os altos níveis de exposição ao metil-Hg causaram a morte e danos neurológicos em milhares de pessoas expostas direta ou intra-uterinamente (Harada 1978; Amin-Zaki et al, 1974). Quanto aos seus efeitos neuromotores e neurocomportamentais muitos avanços têm originado de estudos de coortes prospectivas nas Ilhas Faroé, Dinamarca (Grandjean et al, 1997), Ilhas Seychelles (van Wijngaarden et a, 2006) e nas investigações das populações ribeirinhas do Amazonas (Malm et al, 1995; Passos et al, 2008). No que concerne ao Pb, os efeitos neurológicos e neuropsicológicos resultantes da exposição ambiental em crianças têm sido exaustivamente estudados (Kordas et al, 2004; Lanphear et al, 2005; Jusko et al, 2008), sobretudo dada a sua alta correlação com nível sócio-econômico (Bellinger, 2009), as crianças menos favorecidas tem maior grau de exposição e maior risco de sofrer os efeitos deletérios nas esferas da memória, atenção e cognição.

É relativamente recente a preocupação com os efeitos neuropsicológicos resultantes da exposição ambiental de crianças ao manganês (Mn). Muito do conhecimento sobre ação neurotóxica deste metal teve origem nas altas

exposições ocupacionais. O quadro neurológico foi primeiro descrito por Couper em 1837 em trabalhadores escoceses que processavam minério de Mn em uma fábrica de produtos químicos (Iregren, 1999). A evolução da doença depende da intensidade e da duração da exposição, assim como da susceptibilidade individual. Mergler et al, (1994) sugere que esta disfunção neurológica desenvolve-se de modo contínuo e segue basicamente três etapas: a primeira é caracterizada por efeitos não específicos como astenia, anorexia, apatia, cefaléia, hiperosmia, espasmos, dormência nas pernas, astralgias e irritabilidade. Na fase intermediária ocorrem alterações psicológicas e psicomotoras. Na terceira fase ocorre uma síndrome clinicamente similar ao Mal de Parkinson (Parkisonismo mangânico), que se caracteriza por rigidez muscular, com tremores e inabilidade de andar para trás. Os efeitos neuropsicológicos nas crianças decorrentes da exposição crônica em níveis ambientais, diversas ordens de magnitude inferiores à exposição ocupacional, têm sido associados ao déficit cognitivo (He et al, 1998, Wright at al. 2006, Wasserman et al, 2006; Kim et al, 2009), hiperatividade e déficit de atenção (Barlow, 1983; Bouchard et al, 2007 e Erikson et al, 2007).

A toxicologia do Mn é complexa porque diferente do Hg e Pb que são xenobióticos, o Mn é um oligo-elemento essencial. O Mn é fundamental no metabolismo de macromoléculas, tendo papel importante na resposta imune, homeostase da glicemia, regulação da síntese de ATP (Adenosina trifosfato), reprodução, digestão e no crescimento ósseo (Aschener & Aschener 2005 apud Burton e Guillarte, 2009). É cofator de metaloenzimas tais como Mn-superóxido dismutase, arginase, fosfoenol-piruvato descarboxilase e glutamina sintetase (Aschener e Aschener, 2005). Nos níveis dietéticos normais, a

homeostase sistêmica do Mn é mantida tanto pela taxa de transporte através dos enterócitos da parede intestinal e pela sua eficiente remoção no fígado (Papavasiliou et al, 1966). Ainda, o Mn, devido a sua semelhança química com o ferro (Fe), observada através dos números de oxidação em sistemas biológicos (2+ e 3+) e raio iônico semelhantes, “mimetiza” o Fe e é transportado ativamente através das membranas, utilizando as mesmas proteínas carreadoras deste (Roth, 2006).

Na população em geral o alimento é a fonte primária de Mn. A principal fonte deste micronutriente na dieta são os cereais, embora todos os alimentos contenham vestígios de manganês. A WHO (1981) relatou que a ingestão diária normal de Mn na dieta em países europeus e americanos varia de 2 a 5 mg. A Food Standards Agency (EVM, 2003) avaliou a ingestão média diária de Mn na dieta de adultos no Reino Unido em 4,9 mg (aproximadamente 0,07 mg/kg de peso corporal). Esse valor tem pequena variação nos países europeus, por exemplo: 2,5 mg na França (Biego et al, 1998), 2,19 mg, na Espanha (Llobet et al, 1998) e 3,5 mg na Alemanha (Becker et al, 1998 *apud in* EVM 2003). Nos países onde cereais e arroz são consumidos mais rotineiramente a ingestão diária pode chegar a 5 a 9 mg (cerca de 0,07-0,13 mg/kg) a exemplo dos países asiáticos. O consumo médio diário para crianças foi relatado em cerca de 0,06-0,08 mg/kg de peso corporal e para crianças alimentadas com leite materno ou com fórmulas infantis apenas de 0,002-0,004 mg/kg (WHO, 1981).

A via de absorção pulmonar ou respiratória é mais importante nas exposições ocupacionais. Porém, tem sido relatado ser também significante para populações residentes em áreas próximas às atividades industriais e de

mineração (Mergler et al, 1999; Santos-Burgoa et al, 2001 e Lucchini et al, 2007). Sessenta a 70% das partículas grandes de óxidos metálicos insolúveis, presentes nos aerossóis e poeiras produzidas por essas atividades, são transferidas para a área naso-faríngea, após remoção do trato respiratório por mecanismos muco-ciliares, sendo eventualmente deglutidas (Davis et al, 1993). A absorção pulmonar da fração respirável, que é depositada nos alvéolos, é muito limitada e dependente normalmente da solubilidade dos compostos do manganês (Dorman et al, 2006). Por exemplo, sulfatos de Mn são mais solúveis que os óxidos (Roels et al, 1997). Os primeiros são gerados por veículos abastecidos com MMT (Metilciclopentadienil tricarbonil manganês), aditivo antidetonante da gasolina, enquanto os óxidos são gerados na metalurgia do minério de Mn (Boudia et al, 2006). Nanopartículas de Mn podem ser transportadas diretamente para o cérebro através dos nervos olfatórios, como foi demonstrado com estudos com roedores e primatas (Brenneman et al, 2000; Dorman et al, 2006).

Aproximadamente 1-5% do Mn ingerido é absorvido via trato gastrointesinal (TGI) (Davis et al, 1993). A maior parte do Mn^{2+} na circulação portal, aquele absorvido do TGI, está ligada a α-micro-globulina e é eficientemente removida do sangue pelo fígado, enquanto que na circulação geral, o Mn está predominantemente dentro dos eritrócitos, e boa parte complexada a porfirina. (Gibbons et al, 1976). No soro, uma pequena proporção de Mn^{3+} é transportada ligada a β_1-globulina (Mn transferrina) (ATSDR, 2000). A depuração do sangue é rápida, o metal primeiro se concentra principalmente no fígado, cuja capacidade é de 1 mg/kg peso úmido. Tem um tropismo por tecidos ricos em melanina como o cabelo, principalmente

os escuros, retina, conjuntiva pigmentada, pele escura e o cérebro, armazenando-se intracelularmente nas mitocôndrias (Aschner, 1999). Este metal atravessa ativamente as barreiras hemato-encefálica e placentária, assim como é secretado no leite materno (Ruoff et al, 1995).

Em humanos, o Mn absorvido é removido do sangue através do fígado onde é conjugado aos sais biliares e excretado no intestino (Davis et al,1993). Parte deste pode ser reabsorvida no intestino através da circulação enterohepática. A sua concentração biliar excede a plasmática numa razão de quase 10 vezes, sugerindo um mecanismo de transporte ativo (Klaassen 1974). Entre 92 a 99% do total de Mn excretado são encontrados nas fezes, uma pequena fração (0,1 a 1,3%) da dose diária absorvida são eliminados na urina (Davis et al, 1993). O cabelo pode ser considerado também uma via de excreção em pessoas expostas (ATSDR, 2000). Este metal tem uma meia-vida curta, em média 36 dias, que depende da ingestão e da carga corpórea de manganês, enquanto que no cérebro a meia-vida é consideravelmente mais longa do que no corpo como um todo (Newland et al, 1987). Portanto, numa situação de absorção excessiva, o Mn pode acumular no cérebro (Aschner, 1990).

Os efeitos do Mn, sobretudo aqueles relacionados à exposição ocupacional, estão relacionados a danos neuro-motores. A acumulação excessiva do Mn nas regiões do *striatum* e *globus pallidus* do cérebro humano ocorre quando a absorção do Mn excede a taxa de eliminação por um período muito longo. Este desequilíbrio pode acontecer pela alta dosagem através das vias digestiva, respiratória ou mesmo por via parenteral ou em conseqüência da redução do clearance hepatobiliar deste metal (Aschner et al, 2005). O

aumento da concentração de Mn no cérebro pode dar início a danos neuronais, resultando em diminuição no número de neurônios dopaminérgicos (Dorman et al, 2006).

Exposições crônicas a baixas concentrações, como observadas em comunidades em áreas contaminadas, estão mais relacionadas com efeitos neuro-psicológicos. Estudos recentes com primatas têm demonstrado que o efeito cognitivo e na memória é decorrente da ação do Mn no córtex cerebral (Schneider et al, 2006, Guilarte et al, 2006b). Macacos expostos cronicamente ao Mn através de injeção inatravenosa ou por inalação apresentaram significativos decréscimos na função cognitiva e alterações comportamentais compatíveis com transtorno compulsivo. Numa revisão recente dos seus estudos com primatas, Burton e Guilarte (2009) sugerem que ocorrência da expressão de genes (ex. Supressor de tumor p53 e da proteína precursora beta amilóide (1APLP1)) e surgimento de marcadores de neurodegeneração do córtex frontal (desregulação da homeostase do cobre e acumulação do peptídeo tóxico beta amilóide (Aβ)) podem explicar os repentinos déficits cognitivos e outras manifestações precoces neurotóxicas do Mn em humanos. Estas condições são normalmente observadas em cérebros envelhecidos e estão relacionadas com perda da memória funcional e comportamentos psicóticos.

Estudos com populações expostas

Um dos primeiros estudos que relacionou Mn e desenvolvimento intelectual através da avaliação do desempenho acadêmico foi realizado por pesquisadores canadenses (Pihl e Parkes, 1977). Os autores compararam

crianças que tiveram diagnóstico de déficit cognitivo com crianças sem tal diagnóstico. Foram determinadas as concentrações de catorze metais pesados e elementos traços no cabelo. O Mn foi um dos metais que tinham níveis significativamente mais elevados, porém estes autores não o correlacionaram ao efeito. No entanto, Collipp et al, (1983) percebendo o fato, investigou a elevada concentração de Mn nas fórmulas infantis. Observaram a relação entre este metal e hiperatividade em crianças de até 10 anos, as quais foram classificadas se foram amamentadas ou se usaram fórmulas infantis a base de leite de soja. Compararam-se os níveis de Mn no cabelo de crianças de 7 a 10 anos com diagnóstico de hiperatividade com crianças sem o diagnóstico. As crianças diagnosticadas com hiperatividade tiveram níveis mais elevados de Mn (0,434 µg/g) quando comparadas aos controles (0,268 µg/g) pareando por idade ($p<0,05$). Estudo semelhante, associando hiperatividade e níveis elevados de Mn no cabelo foi realizado com crianças inglesas (Barlow, 1983). Foi detectada maior concentração de Mn nas crianças hiperativas (0,84±0,64 µg/g) do que em crianças sem tal diagnóstico (0,68±0,45 µg/g), embora com baixo grau de significância ($p=0,10$).

A exposição das crianças ao Mn através da água potável torna-se um problema mundialmente estudado. Na China He et al, (1994) e Zhang et al, (1995) investigaram a contaminação de uma comunidade rural através da água de irrigação da lavoura. As crianças do Vilarejo Wu consumiram água com concentrações de Mn entre 0,244 e 0,255 mg/L por muitos anos. Foi observado que a concentração média de Mn no cabelo das crianças deste vilarejo foi significativamente maior do que a média nas crianças de uma localidade sem tal contaminação: 1,252±0,720 µg/g e 0,961±0,418 µg/g, respectivamente. Nos

12 escores dos seis testes neurocomportamentais as crianças expostas aos altos níveis de Mn tiveram desempenho mais baixo ($p<0,01$) do que as crianças no grupo controle.

As águas subterrâneas podem ter níveis de Mn naturalmente elevados, devido à composição das rochas, ou decorrentes da atividade humana. Wasserman et al, (2006) investigaram o efeito em crianças de Bangladesh, que obtem toda água potável de poços artesianos. A função intelectual foi avaliada com a escala WISC-III. Após o ajuste pelas covariáveis (escolaridade da mãe, qualidade da habitação, acesso a TV e circunferência craniana), os níveis de Mn nas águas dos poços apresentaram associação significativa com decréscimo nos escores das escalas Verbal, Execução e da escala Total. Wright et al, (2006) observaram a interação da coexposição ao Mn e As em crianças residentes próximas a sítios contaminados com resíduo de mineração. O principal achado desta pesquisa foi que os altos níveis de Mn e de As, particularmente quando em conjunto, estavam significativamente associados à diminuição nos escores de função intelectual e nos testes de memória verbal.

A exposição da população infantil através da via atmosférica tem sido pouco estudada. Um recente estudo com crianças coreanas expostas a concentrações ambientais de manganês e chumbo foi realizado por Kim et al, (2009). Neste estudo não foi relatado as fontes nem as vias de exposição. Os autores observaram um efeito de interação entre os níveis sanguíneos desses metais e a função cognitiva. Uma associação estatisticamente significativa entre os níveis de chumbo no sangue e déficit cognitivo no QI nas escalas total e verbal foi observada naquelas crianças com níveis elevados de Mn no

sangue (>14 µg/L), sugerindo, portanto uma modificação de efeito entre o Pb e Mn.

O problema na Bahia

A vila de Cotegipe com população em torno de 719 habitantes faz parte do município de Simões Filho, cuja população estimada é de 116.662 habitantes (IBGE, 2009). Está situada às margens da rodovia federal BR-324, a 30 km de Salvador, Bahia.

A comunidade de Cotegipe teve origem dos escravos dos engenhos de açúcar do Barão de Cotegipe, João Maurício Wanderley, senador do Império e contrário a abolição dos escravos. A ocupação da área data do século XVI. A estação de Cotegipe da Linha Férrea Centro-Atlântica, que foi criada para escoar a produção açucareira do recôncavo baiano, foi inaugurada em 1860 e tirou a comunidade do isolamento. Em 1973 é definida como distrito censitário do Município de Simões Filho da Região Metropolitana de Salvador, sendo interligada por estrada asfaltada somente em 2002. Esta comunidade foi caracterizada em estudo prévio: "Dados Preliminares para Avaliação de Estudo Epidemiológico" (Fundação Crê, 2002), encomendado pelo Ministério Público, Promotoria de Justiça de Simões Filho, segundo o termo de ajuste de conduta (TAC).

A média de idade da população é 25,2 anos (54,1% do sexo masculino e 45,9% do sexo feminino). Existia um excesso de 52 homens (8.2%), que é uma situação rara frente ao censo da população brasileira. Fato que é chamado atenção neste relatório, é que a distribuição por sexo de crianças de menos de um ano é quase 2:1 (62,9% meninos vs. 37,5% meninas). A distribuição por

idade é típica das comunidades de baixo nível sócio-econômico: 33,2% <15 anos; 40,8% entre 15–35 anos, 20,5% entre 36-65 anos e 4,5% maiores de 65 anos, sendo oito não respondentes. Como era de se esperar em comunidades rurais, há um grande número de analfabetos, mas quase todas as crianças em idade escolar freqüentavam a escola regularmente. Foi constatado que mais de 50% da população habitava Cotegipe por mais de 10 anos. Subsistiam da plantação de mandioca e banana, fabricando a farinha e a puba com a mesma tecnologia da época de escravidão. Toda produção é comercilaizada no mercado de Simões Filho e Salvador, sendo esta a principal fonte de renda. Neste inquérito foram cadastradas 182 casas no povoado, 61% recebia água encanada da represa após tratamento precário, 37 delas (20,3%) coletavam água de poços artesianos, e 28 (15,4%) das residências captavam água de córregos. Das queixas de saúde, as mais comuns eram as respiratórias (falta de ar e alergias), fadiga e afecções da pele. As doenças infecciosas eram as causas mais predominantes para atendimento no posto de saúde localizado a 2 km, no povoado de Mapele (dengue 59,9%, diarréia 12% e infecções parasitárias 5.2%).

A Siderúrgica do Brasil (SIBRA), atual Rio Doce Manganês (RDM) do grupo Vale, com incentivo econômico da SUDENE e do governo da Bahia, deu início às suas atividades em 1965. O governo brasileiro foi o maior acionista até 1988 quando foi privatizada. O minério é originado das minas de Carajás no estado do Pará (15.800 ton/mês), de Urucum no Mato Grosso do Sul (8.500 ton/mês) e de quatro minas na Bahia 15.600 (ton/mês). Produz ligas de ferro-manganês e ferro-silício através de processo termodinâmico de redução de óxidos de manganês, silício e ferro. A produção é destinada às grandes

siderúrgicas do aço no Brasil e também para exportação. Durante seu funcionamento teve como característica principal, sob o aspecto de seu passivo ambiental, a emissão de poluentes atmosféricos (material particulado e poluentes gasosos), sobretudo fumos de óxidos de metais pesados, que foram priorizados para o estudo de exposição populacional incluídos no termo de ajuste de conduta (TAC), determinado pelo Ministério Público de Simões Filho (1999). Das negociações resultaram acordados 59 itens que compõem a cláusula das obrigações da empresa, incluindo cronograma de execução e forma de fiscalização. Dentre estes, estava o estudo epidemiológico do impacto na saúde.

Um relatório do estudo epidemiológico realizado foi apresentado em audiência pública: “Relatório Final do Estudo Epidemiológico para Verificação de Efeitos Sobre a Saúde da População Circunvizinha à Empresa RDM” (VEEP, 2005) em maio de 2006. Embora o TAC exigisse que o estudo fosse feito de forma censitária, a avaliação da contaminação humana foi realizada em uma amostra de 10% da população adulta e 10% da população infantil, sem apresentar o cálculo do tamanho amostral e técnica de amostragem. Dentre os principais resultados destacam-se: manganês em cabelo (MnC) em maiores de 14 anos, cujos valores médio foram 66,38 e 177,43 a µg/g, nos habitantes que residiam mais distantes e mais próximos, respectivamente. Enquanto que entre os indivíduos tomados como referência, a média de MnC foi de 16,56 µg/g. Mesmo a população usada como controle, Comunidade de Caboto, distante 10,5 km e na direção dos ventos predominantes na região, que segundo o modelo de dispersão de partículas é predominantemente norte-noroeste (Relatório de Monitoramento de Chaminés, RDM), os níveis de MnC

são, aproximadamente, 16 vezes mais elevados do que os valores de referência na população Brasileira 0,15 a 1,15 µg/g (Carneiro et al, 2002). Nas crianças foi determinada somente a concentração de chumbo sanguíneo. Quanto à poluição ambiental, o relatório citou o informe de um centro de pesquisa da Bahia (CETIND/LBESP), no qual consta que a concentração de Mn na água bruta do vertedouro da represa foi de 2,3 mg/L, e de 0,17 mg/L na água tratada que é servida a comunidade e a concentração deste metal no ar na fração do material particulado inalável (PM_{10}) foi de 1,25 mg/m^3**.**

Objetivos

Geral

Investigar a intensidade da exposição ambiental ao Mn às emanações de uma metalúrgica de ligas ferro-manganês e avaliar se os níveis deste metal estão associados ao desempenho intelectual em crianças da comunidade de Cotegipe.

Específicos

1. Fazer uma revisão sistemática da literatura que associa níveis elevados de Mn e efeitos neuropsicológicos (cognição, memória, déficit de atenção, hiperatividade, impulsividade, etc.)
2. Estudar a relação entre a exposição ambiental (Mn na poeira doméstica) e níveis biológicos de manganês (sangue e cabelo).
3. Analisar como contaminação por Mn distribui-se no entorno da planta a partir dos indicadores ambientais (níveis de Mn na poeira domiciliar) e biológicos (sangue e cabelo).
4. Verificar a relação entre a concentração de Mn no sangue e a concentração deste metal no cabelo.
5. Mensurar as concentrações basais de Mn em sangue e cabelo em uma comunidade sem exposição pontual a este metal (grupo controle).
6. Medir o desenvolvimento intelectual através do quociente de inteligência, com o propósito de identificar se existe associação entre a exposição excessiva ao Mn e déficit cognitivo.

Abordagem do Problema e Considerações Éticas

Abordagem do Problema – Metodologia Geral

A presente investigação é de natureza epidemiológica e teve um desenho transversal. O estudo foi divido em duas fases.

Na fase I, realizada a partir de abril 2007, nos propusemos a avaliar a exposição ao Mn de todas as crianças na faixa etária de 1 a 10 anos. Para tanto, realizamos um censo populacional em toda vila Cotegipe, incluindo a Rua Germínio Dame, a qual fica situada no lado noroeste da planta metalúrgica, exatamente na direção dos ventos predominantes.

Após o cadastramento de todas as residências e seus moradores, fizemos reuniões com a comunidade na Escola Municipal de Cotegipe para explicarmos os objetivos da pesquisa e convidar os pais e as crianças a participarem como voluntários.

Entrevistadores, acadêmicos dos cursos de Farmácia, Enfermagem e Nutrição, alunos da Turma de ACC FAR457 Diagnóstico de Anemias, após treinamento, visitaram cada residência onde habitavam as crianças na faixa etária desejada, explicaram mais uma vez o caráter da pesquisa aos pais ou responsáveis e leram o TCLE (Anexo 1). Após a obtenção da assinatura no termo, aplicaram os questionários para levantamento de dados sócio-econômicos e hábitos gerais (Anexo 3) e outro questionário sobre aspectos particulares de cada criança (Anexo 4).

Realizamos uma campanha de coleta de material biológico para medida de exposição ao Mn (sangue e cabelo), assim como para estimar a prevalência de anemias (hemograma e ferro sérico) e de parasitoses intestinais. Logo após essa campanha, iniciamos a coleta de poeira domiciliar, assim como coleta de material particulado em suspensão ($PM_{2.5}$) e água de consumo humano. Todas as residências (N=55) onde habitavam as crianças que participaram da coleta de material biológico foram incluídas nessa etapa.

Uma comunidade localizada a 7,5 km a sudeste e a favor dos ventos relativos a planta metalúrgica, de composição étnica e nível sócio-econômico aparentemente similares, foi selecionada como área controle. Igual

procedimento foi realizado nesta comunidade. Após censo populacional, apresentamos o projeto e convidamos a participar como voluntários, sendo assinado o TCLE (Anexo 2). Coletamos amostras de sangue e cabelo das crianças voluntárias e que compareceram no dia da campanha de coleta, assim como amostra de poeira domiciliar de nove residências e $PM_{2.5}$.

Na fase II, realizada em Julho de 2008, executamos a avaliação do desempenho intelectual, usando a escala WISC III, sendo incluídas somente as crianças de 6 a 11 anos e 11 meses que frequentavam regularmente a Escola Municipal de Cotegipe e as cinco crianças na mesma faixa etária da Rua Germínio Dame, totalizando 83 crianças. Foram coletadas novas amostras de sangue e cabelo para medir os níveis de Mn no organismo concomitante a avaliação neuro-psicológica. Um questionário para obtenção de dados referentes ao desenvolvimento infantil foi aplicado aos pais ou responsáveis (Anexo 5), além do Inventário HOME de estimulação doméstica (Anexo 6). O desempenho intelectual materno ou dos responsáveis foi avaliado através da Matriz Progressiva de Raven. Solicitamos às mães para doar uma amostra de cabelo da região occipital para a determinação de Mn.

O banco de dados gerado foi analisado no pacote estatístico SPSS v.13. Os resultados das análises descritivas dos dados foram apresentados em média aritmética ou geométrica, desvio padrão, mediana, mínimo e máximo para as variáveis contínuas ou em percentual para as variáveis categóricas. Foram utilizados testes estatísticos mais adequados aos diferentes tipos de variáveis, com significância p=0,05. Análise correlação de Pearson ou Spearman e regressão linear múltipla foram utilizadas para definir os preditores com significância estatística para os diferentes desfechos avaliados.

Considerações de Ordem Ética

Esta investigação seguiu a resolução 196/1996 do Conselho Nacional de Saúde de 10 de outubro de 1996, sendo o projeto avaliado pelos Comitês de Ética em Pesquisa (CEP) da ENSP e Universidade Federal da Bahia.

Resultados

Os objetivos propostos deram origem a quatro artigos. O artigo de revisão "*Manganese exposure and neuro-psychological effect on children and adolescents: A review*", que analisa sistematicamente os estudos que de alguma forma associam a exposição ao Mn com efeitos neuro-psicológicos. O manuscrito foi aceito para publicação em 28.07.2009 pelo *Journal of Public Health/Revista Pan-americana de Saúde Pública* (artigo 1).

A primeira avaliação da exposição ao Mn realizada em Junho de 2007 resultou no artigo "*High levels of hair manganese in children living in the vicinity of a ferro-manganese alloy production plant*" (artigo 2), publicado na *Neurotoxicology.* Neste trabalho descrevemos o grau de contaminação ambiental e humana por este metal e discutimos os fatores de riscos associados aos altos níveis de Mn detectados nas crianças de Cotegipe.

O terceiro artigo "*Blood and air manganese levels in children living in the vicinity of a ferro-manganese alloy plant and their correlations with house dust Mn levels*" (artigo 3) apresenta os resultados da segunda avaliação da exposição realizada em Julho de 2008. Discutimos as correlações entre o indicador ambiental de contaminação (Mn na poeira domiciliar) com os indicadores biológicos de exposição, além de comparar os níveis de Mn com os valores observados na comunidade tomada como referência. Avalia também as correlações entre as concentrações deste metal no sangue e no cabelo, assim como faz uma análise temporal da intensidade de contaminação nos dois anos consecutivos.

O quarto e último artigo analisa os resultados da avaliação do desempenho intelectual das crianças de Cotegipe. O título deste artigo "*Elevated manganese affects mothers' and children's cognition*" (artigo 4) antecipa que fomos capazes de demonstrar que os altos níveis de Mn observados nesta comunidade além de estarem associados ao déficit cognitivo nas crianças, também estão correlacionados com efeito na cognição materna.

Artigo 1

Systematic Review

Manganese exposure and the neuropsychological effect on children and adolescents: a review*

Running head: Manganese exposure and effects in children

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- **ABSTRACT**

Objectives. Manganese (Mn) is an essential element, but overexposure can have neurotoxic effects.

Methods. In this article, we review and summarize studies on exposure to Mn and nervous system impairments in children.

Results. We identified 12 original articles published between 1977 and 2007. Overexposure to Mn was suspected to occur through diverse sources: infant milk formula, drinking water, industrial pollution, and mining wastes. The most common bioindicator of exposure to Mn was hair Mn content, but some studies measured Mn in blood, urine, or dentin; one study on prenatal exposure measured Mn content in cord blood. Most studies indicate that higher postnatal exposure to Mn is associated with poorer cognitive functions and hyperactive behavior.

Conclusions. The limitations of the existing studies are numerous: most were cross-sectional, had a modest sample size, and lacked adjustment for important confounders. Future investigations should be performed on a larger sample size and include a more detailed exposure assessment, addressing multiple sources of exposure such as food, water, and airborne particulates.

Key words Manganese, neurobehavioral manifestations, cognition, neurotoxicity.

INTRODUCTION

Manganese (Mn) is very common in the environment, being the 5th most abundant metal and the 12th most abundant element on earth (1). It is an essential nutrient, with an adequate daily intake level of 1.8–2.6 milligrams (mg) for adults (2), but also has the potential to produce neurotoxic effects when, depending on the route and dose of exposure, it accumulates in an organism, especially in the brain (3). The vast majority of studies on neurotoxic effects of Mn were conducted in occupational settings where exposure occurs mainly though inhalation of airborne particulates. Few studies have investigated possible overexposure of children to Mn. It is generally accepted that children are at greater risk than adults exposed to the same contaminants from the environment (4). Although data are sparse, there is increasing concern for possible harmful effects of environmental exposures to Mn, including the economic implications of intellectual deficits due to Mn toxicity (5).

Mn is an essential element, necessary for bone mineralization, energy and protein metabolism, regulation of cell metabolism, and protection against oxidative stress (6). With normal dietary consumption, systemic homeostasis of Mn is maintained by both its rate of transport across enterocytes lining the intestinal wall and its efficient removal in the liver (7). Ingestion of Mn is subjected to homeostatic mechanisms that regulate its concentration in the body, but exposure through inhalation bypasses most of these protective mechanisms. Therefore, inhaled Mn poses greater risks of toxicity, as seem to be supported by the preponderance of occupational reports of toxicity in environments with airborne exposure.

Mn shares several characteristics with iron (Fe); both are transition metals with valences of 2^+ and 3^+ in physiological conditions and proximate ionic radius. In addition, as Mn and Fe both strongly bind to transferrin and accumulate in the mitochondria, low iron stores are associated with increased Mn uptake and retention in the blood (8).

Neurotoxic effects resulting from excessive Mn exposure were first described by Couper in 1837 in Scottish labors grinding Mn black oxide in the chemical industry (9). Neurological symptoms of manganism include decreased memory and concentration, fatigue, headache, vertigo, equilibrium loss,

insomnia, tinnitus, trembling of fingers, muscle cramp, rigidity, alteration of libido, and sweating (10). Many reports of neurotoxic effects in Mn-exposed workers were later published (for a review, see Zoni et al., (11)), and the definition of Mn intoxication has evolved to include subclinical signs of intoxication indicated by alterations of neurobehavioral functions (12).

Mn can accumulate in the central nervous system, particularly the basal ganglia, but also the cortex (13). Exposure to Mn was shown to interfere with several neurotransmitter systems, especially in the dopaminergic system in areas of the brain responsible for motor coordination, attention, and cognition (14, 15). Mn is a potent dopamine oxidant, which could explain the toxic lesions in certain dopaminergic brain regions (16). Excessive exposure could result in dopamine receptor loss or inactivation through damage to the membrane mediated by free radicals or cytotoxic quinones generated by the Mn catalyzing effect on autooxidation of this neurotransmitter (17). One hypothesis for the toxic mechanism of Mn is the production of excess free radicals in the nerve cell, potentiating lipid peroxidation and resulting in tissue destruction (18).

Several factors could predispose children to Mn overexposure and subsequent toxic effects. Exposure to Mn by ingestion or inhalation could have different consequences in children than in adults and through different mechanisms. First, the observed intestinal absorption rate of ingested Mn in children was higher than in adults (19). Second, high demand for iron linked to growth could further enhance the absorption of ingested Mn (20). Third, a low excretion rate was observed in infants due to the poorly developed biliary excretion mechanism in neonatal animals (21).

Data are lacking on the overall retention of ingested Mn in infants and children, but in mice, rats, and kittens, there is almost a complete absence of biliary Mn excretion during the neonatal period (22). With inhaled Mn, the ratio of inhaled air/weight is much higher in children, leading to a higher dose of exposure for almost any contaminant in air. Thus, exposure during this period may result in increased delivery of Mn to the brain and other tissues.

Mn neurotoxicity has been extensively studied and a lot has been learned about its mechanism of action at the cellular and molecular levels and the detection of subclinical effects at low exposures, although there is a long way to go until its toxicology can be fully understood. Recently, several literature

reviews have been published on aspects such as neurotoxic effects on exposed laborers (15, 23), the application of magnetic resonance imaging (24), neuropsychological testing for the assessment of Mn neurotoxicity (11), Mn neurotoxicity focused on neonates (25), and neurotoxicology of chronic Mn exposure in nonhuman primates (26).

As far as we know, no study has addressed the issue of children's exposure and the effect on behavior and cognition. Therefore, we propose here to review and summarize the scientific literature on the associations between Mn exposure and effects on children's neuropsychological functions.

METHODOLOGY

A systematic scientific literature search was carried out on the electronic database Medline (National Library of Medicine, Bethesda, Maryland, United States) for 1997–2007, using the keywords manganese, child, children, infant, childhood, adolescents, neurotoxicity, neuropsychological effects, behavior, motor, cognition, cognitive, intellectual functioning, hyperactivity, ADHD, and hyperactive behaviors. Upon reviewing a large number of article abstracts yielded by these search terms, we selected studies for this review only if information was presented that pertained specifically to assessment of the neuropsychological effects on children exposed to Mn from all possible sources. We found six articles on exposed populations in the United States, Canada, Spain, Bangladesh, and Malaysia. In the reference list of those articles, we selected six more investigation reports. Only full-length research articles were reviewed.

RESULTS

Table 1 shows a schematic summary of the studies with their general characteristics such as country of origin, type of design, sample size, source of exposure, and outcome investigated. In Table 2, we summarize the main findings of the studies. The first published article exploring a possible adverse neurobehavioral effect of exposure to Mn was a case-control study conducted in Canada on learning-disabled children (27). The authors postulated that nutritional deficiencies and exposure to toxic substances, using hair mineral content as an indicator of body burden, would be associated with learning

disabilities in children. A cohort of third- and fourth-grade students was evaluated on a series of cognitive scales. The children diagnosed with a learning disability were compared with children without such a diagnosis and were matched by frequency for school attendance, grades, and gender; the groups had similar socioeconomic status. Children with learning disabilities had a significantly higher concentration of hair Mn as well as six other elements (sodium, cadmium, copper, lead, chromium, and lithium), which makes the findings difficult to interpret.

The high Mn concentration in infant milk formula drew the attention of researchers (28), who conducted a two-part study in the United States. First, hair Mn concentration was measured in children fed infant formula and in children exclusively breast-fed. Hair Mn levels were found to increase significantly from 0.19 microgram per gram (μg/g) at birth to 0.69 μg/g at 4 months in the infant formula group; no significant increase was observed in the breast-fed group. Second, the metal levels in the hair of children with hyperactivity were compared with those in age-matched children without this disorder. Significantly higher levels of Mn were observed in the hair of hyperactive children (0.43 μg/g) than in controls (0.27 μg/g).

Barlow (29) investigated the association of Mn exposure and hyperactivity in the United Kingdom. A hair sample was collected by the family and sent to the researchers by mail. Slightly higher Mn concentrations were detected in children with hyperactivity (0.84 ± 0.64 μg/g) than in controls (0.68 ± 0.45 μg/g), although this difference did not reach a significance level ($p = 0.10$). The author identified several limitations—including the subjectivity of the diagnosis made by different psychiatrists, psychologists, and social workers—and the risk of contamination of hair samples. In addition, the control group was inappropriate, coming from a different area of residence and socioeconomic status was not controlled.

A research group in China published two articles (30, 31) on the investigation of a rural community with Mn-contaminated drinking water following the use of high-Mn sewage water for irrigation. The first publication addressed Mn levels in drinking water and children's hair and the associated neurobehavioral effects on children. The second article addressed the

association of Mn hair and blood levels with learning deficiencies and the level of neurotransmitters measured in peripheral blood.

The exposed group was composed of 92 students from a village where the concentration of Mn in drinking water was between 0.24 and 0.35 mg per liter (L) for many years. They were compared with children in another rural town with low Mn in water (< 0.03 mg/L).

The children in both groups were evaluated by neurobehavioral tests that assessed emotional state, motor coordination, visual memory, and time of reaction. Children from the exposed village had a mean hair Mn concentration significantly higher than control children (1.25 ± 0.72 µg/g versus 0.96 ± 0.42 µg/g); the same difference was observed with Mn blood levels (33.9 µg/L versus 22.6 µg/L). Children from the exposed village had lower performance ($p < 0.01$) than controls on 5 of 12 neurobehavioral tests: digit span, Santa Ana manual dexterity, digit symbol, Benton visual retention test, and pursuit aiming test. In addition, exposed children had significantly lower school performance than the control children. Multiple regression analysis showed that school grade for language and mathematics was correlated to serum levels of the neurotransmitters 5-hydroxytryptamine, norepinephrine, and dopamine and to the activity of acetylcholinesterase.

Woolf and associates (32) reported a case of suspected Mn intoxication in a 10-year-old child from a suburb of Boston, Massachusetts (United States). For more than 5 years, the family had used water from a tube well with a very high Mn concentration (1.21 mg/L). The child had high Mn levels in serum (9.00 µg/L, reference normal < 0.27 µg/L), whole blood (38.2 µg/L, reference normal <14.0 µg/L), and hair (3.09 µg/g, reference normal < 1.20 µg/g). However, magnetic resonance imaging showed no changes in the globus pallidus and basal ganglia that indicated Mn accumulation. The results of a battery of neuropsychological tests (Table 2) showed that the child had a normal intelligence quotient (IQ) but unexpectedly poor verbal and visual memory as well as the ability to coordinate alternating movements. The child had no medical history that explained these findings. The detailed neurological evaluation was otherwise normal, with no tremors, normal gait and muscle tone, and no cog wheeling, nystagmus, or fixed faces. The patient's balance was good, fine motor skills and reflexes were normal, and the sensory examination

was unremarkable. The mother reported that teachers had noticed the inattentiveness of the child. The family discontinued all use of well water and the residence was connected to the municipal water system. Eighteen months later, teachers continued to observe his difficulty in carrying out certain tasks due to attention deficit. It is noteworthy that the 16-year-old brother of this child had normal psychometric evaluation, with elevated Mn in hair but not in blood.

Takser and associates (33) conducted a prospective epidemiologic study in 247 healthy pregnant women and their babies to investigate the long-term effect of in utero Mn levels on their children's psychomotor development. The population was recruited from a maternity hospital in Paris (France). Mn levels were measured in the mother's blood and hair at delivery, in umbilical cord blood, and in placental tissue; children's hair and monoamine (dopamine and serotonin) metabolite concentrations were assessed in umbilical cord plasma. Children's development was assessed at 9 months with the Brunet-Lézine scales, and at 3 and 6 years of age with the McCarthy scales. The results showed significant negative correlations between Mn levels in cord blood and nonverbal scales (attention, nonverbal memory) and boys' manual ability at 3 years, after adjusting for potential confounders (sex and mother's educational level). However, no relation between Mn and development subscales was observed at the 6-year follow-up. The authors concluded that the exposure to high in utero Mn levels can affect children's psychomotor development but that sociocultural factors might have masked Mn effects in older children. Finally, it was emphasized that fetal life can be regarded as a period of great vulnerability to Mn toxicity at low environmental levels.

A cross-sectional study was performed in Spain (34) with the objective of investigating correlations between hair metal concentrations and cognitive functions of adolescents living in areas with contrasting levels of industrial contamination. One hundred adolescents (12–14 years old) were selected from schools in urban areas and in the vicinity of the industrial complex in the region of Tarragona, Catalonia. Hair samples were collected for analysis of cadmium, chromium, mercury, lead, Mn, nickel, and tin by inductively coupled plasma mass spectroscopy. The attention span test was used as an indicator of attention, and tests of visuospatial abilities and abstract reasoning were used as a general intelligence index. No significant correlations were found between

chromium, Mn, nickel, and tin levels and cognitive measures. A negative correlation was found between hair lead level and the ability to concentrate, after adjustment for socioeconomic status, and a positive correlation was observed between mercury and visuospatial ability. The authors suggested that the mercury might come from consumption of fish, also associated with a high intake of fatty acids with a beneficial effect on brain function.

Wasserman and associates (35) investigated the association between Mn exposure from well water and children's IQ in Bangladesh. In a previous study, Mn levels were found to be associated with arsenic levels, and intellectual function was negatively related to both metals. However, the association between Mn and intellectual function was no longer significant when the level of arsenic in water was included in the regression model. Therefore, a study (36) was designed to test the hypothesis that Mn had an independent effect on cognitive function. In the new study, 54 children were added who lived in houses supplied by water with very low arsenic levels (< 10 µg/L). All children received a complete clinical examination and provided urine samples for determination of arsenic and a blood sample for Mn, lead, and arsenic determination. Intellectual function was evaluated by the Wechsler Intelligence Scale for Children version III (WISC-III), providing three IQ scores: verbal, performance, and full scale. After adjusting for covariables (mother's schooling, quality of housing, access to television, and cranial circumference), increasing Mn water levels were associated with lower IQ on all three scales. Mn levels in water were not associated with Mn in whole blood, and the latter were not associated with WISC-III scores.

In the study conducted by Wright and collaborators (37), the interaction of children with coexposure to Mn and arsenic was also evaluated. Hair was used as a biomarker, psychometric scales were used to assess cognitive functioning, and a series of other scales were administered for behavioral evaluation (see Table 2). The main finding of this study was that high levels of both Mn and arsenic were significantly associated with lower intellectual function and verbal memory scores.

Bouchard and collaborators (38) evaluated children from a community in Quebec, Canada, which was served by municipal water supplied from two wells with different Mn concentrations (0.61 mg/L versus 0.16 mg/L). Forty-six

children participated in the study (median age 11 years, range 6–15 years). Mn levels were measured in children's hair, and parents and teachers completed the Conner's Rating Scale on hyperactive/attention deficit behaviors. The results showed that (1) children living in houses supplied with water at higher Mn concentrations had significantly higher levels of Mn in hair, and (2) hair Mn concentrations were associated with higher scores for hyperactive and oppositional behavior in the classroom after adjusting for age, gender, and family income. In addition, all children with high scores on these subscales, based on clinical cut-off provided by the test manual, had hair Mn levels higher than the upper limit of the reference range (> 3.0 μg/g). An intriguing result of this study is that 90% of the children did not drink water from the tap, but elevated Mn levels were found in hair of a large proportion of them; use of tap water in food preparation or in showering might contribute to the dose of exposure. This pilot study was used to design a larger epidemiologic study on the effects of exposure to Mn in tap water and food.

In another study in the same year, Ericson and associates (39) evaluated neurobehavioral effects with scales that measure the degree of disinhibition. Unlike previous investigations, the enamel of shedding teeth was used as a matrix for Mn determination, with the objective of measuring the previous exposure during the intrauterine stage when these tissues were formed. The children of this study were randomly selected from a cohort of normal newborns from a prospective developmental study started in 1991 in the United States. Three psychometric tests had been directly applied to children in two phases of development: at 36 and 54 months. Scales that assessed behavior were applied to parents and teachers when children were in the first and third grades. The authors concluded that, even after adjusting for levels of lead in the tooth enamel, measured at the same time as Mn, children with high levels of this metal in the uterine phase had higher scores on all scales of disinhibitory behavior: more children played with forbidden toys at 36 months of age, committed more errors by impulsiveness at 54 months, and were more often evaluated by their mothers and teachers as having externalizing and attention problems.

DISCUSSION

The literature on possible adverse effects of exposure to Mn on children's health is relatively sparse, despite the fact that Mn is acknowledged to be a neurotoxin. There are some indications in the literature that Mn exposure might be related to cognitive deficits and hyperactive behaviors.

Five studies reported indications of adverse effects of exposure to Mn in water on the central nervous system of children (29, 31, 32, 36, 38). Given that high levels of Mn in well water is not uncommon, this situation could pose a significant public health risk. Mn levels corresponding to the level found in the Chinese study to have a significant effect on children (around 0.3 mg/L) are found in 6% of household wells in the United States (40).

Two studies (33, 39) addressed possible adverse neurobehavioral effects from in utero exposure to Mn, although sources of exposure were not specified. It is well documented that Mn levels rise in the mother's blood, where Mn has an important role as a cofactor of several enzymes that regulate metabolism and bone growth (40, 41). Although newborns' exposure to Mn through infant milk formula is a cause for concern (see the review by Ericson et al., (39)), only one investigation addressed this possible source of exposure (29), despite the fact that Mn levels in baby formula, especially those based on soy bean, have been shown to have considerably high Mn levels (42, 43).

Children's exposure to Mn resulting from environmental contamination was addressed in Spain (34), and no significant association was found between Mn levels and cognitive deficits, and in the United States (37) where significant associations with cognitive deficits have been observed in children living near a mining waste site.

Another study (29) evaluated children from the general population with no known exposure and reported associations based on the level of Mn in different biological tissues. The lack of detailed exposure assessment to identify Mn sources precludes the proper determination of the risk factors and therefore does not allow proposing solutions to reduce exposure.

Studies published so far have several serious limitations, including sample size, research design, adjustment for potential confounding variables, and control of coexposure to other neurotoxicants. All reviewed studies except that of Takser and colleagues (33) were cross-sectional and had a modest sample size. Cross-sectional studies provide less convincing evidence than

cohort studies in showing a potentially harmful effect. Most studies attempted to control for confounders by design, selecting a reference group matched on some important variables (i.e., socioeconomic status, age, gender) or controlling confounders by restriction, as in the study of Wasserman et al., (36), which selected households supplied with water containing low levels of arsenic.

The early studies had greater limitations in this respect. For instance, in the study by Pihl and Parkes (27) the only controlled variable was age. The significant difference observed in the levels of Mn in hair in children with and without a learning disability could have been associated with metal exposure from other sources—for example, due to airborne particulate matter exposure from vehicular traffic and soil or dust around residences or different levels of exposure to metals from the diet. Although it is known that maternal educational level has a large influence on children's intellectual development, only three of the reviewed studies controlled for this factor (33, 36, 37).

Studies conducted in the occupational setting reported relationships between neurological outcomes and concentration of airborne Mn. However, the levels of exposure in the general environment are several orders of magnitude lower than in most occupational settings where Mn is present in the process and therefore are more difficult to measure. For example, a concentration of 80.2 µg of Mn per cubic meter (m^3) was reported in the air of a ferromanganese plant (44), a Mn range of 1.5 to 450 mg/m^3 was reported in mines in the United States (45), but the level in the air nonindustrialized regions is expected to be at most 0.15 $\mu g/m^3$ (46). In general, studies that measure exposure from environmental sources will always have weaker exposure levels and consequently the possibility of an association will be overshadowed by confounding variables.

One difficulty in the study of Mn exposure is the lack of a well-recognized bioindicator of exposure. Most studies reviewed here used hair as an indicator of Mn exposure, except for the Wasserman et al., study (36), which measured Mn in blood, and the Ericson et al., study (39), which used Mn in the dentin of deciduous teeth as a biomarker of in utero exposure. Use of hair has several advantages over other biomarkers. Hair averages off the variations of Mn levels found in blood or plasma, as it grows an average of 11 millimeters/month, thus representing a time-weighted average over the duration of exposure. In

addition, the sample collection procedure is simple, can be performed by minimally trained staff, and is not invasive. A major drawback to the use of hair as a marker of internal dose of exposure is exogenous deposition, which is particularly problematic in the context of exposure to airborne Mn particulates. Washing can minimize the problem of external contamination, but using a very abrasive reagent can erode the capillary structure, leading to loss of endogenous elements. Research would benefit from a standardized washing procedure.

Others have hypothesized the possibility of overexposure to Mn through ingestion of infant milk formula (47), showering in water with a high Mn level (48), and adding methylcyclopentadienyl manganese tricarbonyl (MMT) to gasoline (49). Whether these exposures represent a significant toxic risk remains to be investigated, but some exposure data suggest that might be the case. In a study in South Africa, levels of Mn in deposited dust and in blood of children were compared in Johannesburg, where the gasoline additive MMT had been used for 1 year, and in Cape Town, where MMT is not used. The mean level of Mn in dust and blood was significantly higher in Johannesburg than in Cape Town (blood Mn 9.8 ± 3.6 µg/L versus 6.7 ± 3.5 µg/L). In addition, a significant association was found between Mn in classroom dust and children's Mn blood levels (50).

Although limited by poor study design and difficulties in exposure assessment, the evidence of adverse effects from Mn environmental exposure on children is compelling enough to warrant further research. Future investigations based on a prospective design will shed more light on the relation of Mn exposure and neuropsychological effects on environmentally exposed children. Finally, it is of paramount importance that epidemiologic studies include a comprehensive environmental assessment in order not only to better understand the exposure pathways but also to provide reliable data for risk assessment, which can be used later to design efficient interventions to abate exposure.

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Table 1. Characteristics of the studies on the association between children Mn exposure and neuro-psychological effects, published between 1977-2007

Study	Country	Design	Sub-Population	N Exp./Ref.	Exposure source	Effect sought
Pihl and Parkes 1977	Canada	Case-control	Children 3rd e 4th grades (±10 years)	31/22	Not reported	Learning deficit
Collipp et al., 1983	USA	Cross-sectional	Neonates & Children ≤ 10 years	16/44	Baby formula	Hyperactivity Learning deficit
Barlow PJ 1983	United Kingdom	Cross-sectional	Children & adolescents ≤ 16 years	68/65	Unknown	Hyperactivity
He et al., 1984	China	Cross-sectional	11-13 years	92/92	Drinking water	Neuro-behavioral
Zhang et al., 1995	China	Cross-sectional	11-13 years	92/92	Drinking water	Learning deficit
Woolf et al., 2002	USA	Case report	Child 10 years	1	Well water	General neuroeffects
Takser et al., 2003	France	Prospective	Neonates	247 60% loss	Mother's blood	Psychomotor development
Torrente et al., 2005	Spain	Cross-sectional	Adolescents 12-14 years	54/45	Industrial Pollution	Cognitive deficit
Wasserman et al., 2006	Bangladesh	Cross-sectional	Children 10 ±0,5 years	142	Well water	Cognitive deficit (IQ)
Wright et al., 2006	USA	Cross-sectional	Adolescent 11-13 years	32	Mining waste	Neuro-psychological & neurobehavioral
Ericson et al., 2007	USA	Cross-sectional in a follow-up cohort	Adolescents 11-13 years	27	Mother's blood	Behavioral disinhibition
Bouchard et al., 2007	Canada	Cross-sectional	Children & adolescents 6-15 years	28/18	Well water	Hyperactive behaviors

Notes: N = one studied group (No controls); EXP (Exposed), Ref. (Referents).

TABLE 2. Results of studies on the association between children's exposure to manganese and neuropsychological effects, published between 1977 and 2007

Study	Exposure index	Analytical method	Mean	Main findings
Pihl and Parkes 1977 (27)	Mn[a] in hair	AA-Flame[b]	Case 0.83 μg/g[c] Control 0.58 μg/g	Children with learning disability had significantly higher Mn levels in hair than controls ($p < 0.001$). Adjusted for sex, age, school, class, and language.
Collipp et al., 1983 (28)	Mn in hair	GFAAS[d]	Case 0.43 μg/g Control 0.27 μg/g	Children with learning disability had significantly higher Mn levels in hair than controls ($p < 0.05$). Adjusted for age.
Barlow 1983 (29)	Mn in hair	GFAAS	Exp.[e] 0.84 μg/g Ref.[f] 0.68 μg/g	Higher Mn level in hair in hyperactive children ($p < 0.10$).
He et al., 1994 (30)	Mn in hair	AA-Flame	Exp. 1.25 μg/g Ref. 0.96 μg/g	Differences in Mn in hair ($p < 0.001$) exposed/referents. Poorer neurobehavioral performance ($p < 0.01$) in exposed than referents. Significant negative relation between Mn in hair and visuomotor coordination, executive function, and memory ($r = –0.213$ to $r = –0.319$).
Zhang et al., 1995 (31)	Mn in hair and Mn in blood	AA-Flame	Exp. 1.25 μg/g/Ref. 0.96 μg/g Exp. 33.9 μg/L[g]/Ref. 22.6 μg/L	Higher Mn in hair and Mn in blood levels ($p = 0.001$) in exposed children compared with referents. Exposed had lower grades for math and language.
Woolf et al., 2002 (32)	Mn in hair Mn in blood Mn in urine	ICP-MS[h]	3.09 μg/g 38.2 μg/L 8.5 μg/L	Case report on a child with high Mn exposure who had normal cognition, except for impaired memory.
Takser et al., 2003 (33)	Mn in hair Mn in umbilical cord blood Mn in placenta	GFAAS	Initial, at 6 years 0.75, 0.77μg/g (GM)[i] 38.5, 38.6 μg/L (GM) 0.1 μg/g (GM)	Higher Mn in cord blood significantly associated with poorer psychomotor indices ($r = –0.33$, $p < 0.001$) at 3 years. No correlation observed for postnatal Mn blood levels and later psychomotor development (assessed up to 6 years of age). Adjusted for child's gender and maternal education.
Torrente et al., 2005 (34)	Mn in hair	ICP-MS	Exp. 0.18 μg/g Ref. 0.26 μg/g	No significant difference ($p > 0.05$) in Mn levels in hair in exposed and controls. Attention negatively correlated ($r = –225$, $p = 0.023$) with lead in hair. No correlation observed with Mn in hair.
Wasserman et al., 2006 (36)	Mn in blood Mn in water	ICP-MS	12.8 μg/L 795 μg/L	Mn in water negatively associated with intelligence quotient (IQ) full scale (β=-4.56) and IQ performance ($\beta = –3.82$). Adjusted for water arsenic levels and blood lead levels.
Wright et al., 2006 (37)	Mn in hair	ICP-MS	0.47 μg/g	Mn in hair inversely associated with full-scale ($\beta = –0.01$, p =,0.07) and verbal ($\beta = –0.12$, $p = 0.002$) IQ. Adjusted for sex and maternal education.
Ericson et al., 2007 (39)	Mn in dentin	IMS[j]	Not reported	Mn in dentin (formed during 20th gestation week) was correlated with attention deficit hyperactivity disorder ($r = 0.47$, $p = 0.001$) and disruptive disorder ($r = 0.41$, $p = 0.001$) at school age. Adjusted for lead levels.
Bouchard et al., 2007 (38)	Mn in hair	ICP-MS	Exp. 6.2 μg/g Ref. 3.3 μg/g	Mn in hair levels significantly higher ($p = 0.025$) in the more exposed group than in the less exposed group.

							Mn in hair was significantly associated with more problems with oppositional behaviors (p = 0.031) and hyperactive behaviors (p = 0.008).

[a] Mn = manganese.
[b] AA-Flame = atomic absorption spectroscopy, flame mode.
[c] μg/g = micrograms per gram.
[d] GFAAS = graphite furnace atomic absorption spectrometry.
[e] Exp. = exposed.
[f] Ref. = referents.
[g] μg/L = micrograms per liter.
[h] ICP-MS = inductively coupled plasma mass spectrometry.
[i] GM = geometric mean.
[j] IMS = ion mass spectrometry.

Artigo 2

High Levels of Hair Manganese in Children Living in the Vicinity of a Ferro-Manganese Alloy Production Plant*

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Abstract

Manganese (Mn) is an essential element, but an effective toxic at high concentrations. While there is an extensive literature on occupational exposure, few studies have examined adults and children living near important sources of airborne Mn. The objective of this study was to analyze hair Mn of children living in the vicinity of a ferro-manganese alloy production plant in the Great Salvador region, State of Bahia, Brazil and examine factors that influence this bioindicator of exposure. We examined 109 children in the age range of 1 to 10 years, living near the plant. Four separate housing areas were identified a priori on the bases of proximity to the emission sources and downwind location. A non-exposed group (n=43) of similar socio-economic status was also evaluated. Mn hair (MnH) concentration was measured by graphite furnace atomic absorption spectrometry (GFAAS). Possible confounding hematological parameters were also assessed. Mean MnH concentration was 15.20 µg/g (1.10-95.50 µg/g) for the exposed children and 1.37 µg/g (0.39-5.58 µg/g) for the non-exposed. For the former, MnH concentrations were 7.95±1.40 µg/g (farthest from the plant), 11.81±1.11 µg/g (mid-region), 34.43±8.66 µg/g (closest to the plant) and 34.22±9.15 µg/g (directly downwind). Multiple regression analysis on log

transformed MnH concentrations for the exposed children derived a model that explained 36.8% of the variability. In order of importance, area of children's residence, gender (girls > boys) and time of mother's residence in the area at the birth of the child, were significantly associated with MnH. *Post hoc* analyses indicated 2 groupings for exposure areas, with those living closest to and downwind of the plant displaying higher MnH concentrations compared to the others. The contribution of the time the mother lived in the community prior to the child's birth to the children's current MnH suggests that *in utero* exposure may play a role. A study of neurobehavioral performance with respect to Mn exposure in these children is currently underway.

Keywords: Manganese, Children, Hair, Environmental contamination, Ferro alloy plant

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1. Introduction

Manganese (Mn) is an essential element, necessary for bone mineralization, energy and protein metabolism, cell metabolism regulation, and protection against oxidative stress (Keen et al., 2000). With normal dietary consumption, systemic homeostasis of Mn is maintained by both its rate of transport across enterocytes lining the intestinal wall and by its efficient removal within the liver (Papavasiliou et al., 1966). Exposure through inhalation is more effective than ingestion because Mn bypasses some of the homeostatic mechanisms that normally regulate its concentration in the body. In addition, animal studies have shown that inhaled Mn compounds can be taken up by the olfactory nerve and through axonal transport reach the olfactory bulb and other parts of brain (Brenneman et al., 2000; Dorman et al., 2006). Neurotoxic effects resulting from excessive Mn exposure were first described by Couper in 1837 in Scottish laborers grinding Mn black oxide in a chemical industry (cited in Iregren, 1999).

Neurological symptoms of manganism include decreased memory and concentration, fatigue, headache, vertigo, loss of equilibrium, insomnia, tinnitus, trembling of fingers, muscle cramp, rigidity, alteration of libido and sweating (Tanaka, 1988). Studies of active workers with Mn exposure show diminished motor and cognitive functions, with changes in affect (for review see: Zoni et al., 2007). At even lower levels of exposure, studies of communities living in proximity to airborne Mn from mining and transformation activities have likewise reported neurobehavioral deficits (Mergler, 1999; Rodriguez-Agudelo et al., 2006), as well as changes in prolactin levels (Montes et al., 2008), associated with biomarkers of Mn exposure. Lucchini et al. (2007) showed a higher prevalence of Parkinsonian disturbances in a region of an Italian province with several ferro-manganese production plants, compared to other areas of the province.

Few studies have examined children exposed to airborne Mn. Blood Mn increases during pregnancy and Mn is actively transported across the placental barrier (Krachler et al., 1999). Smargiassi et al. (2002), who compared Mn concentration in cord blood from Montreal, where MMT was used as a gasoline additive, and Paris, with no MMT, reported that although there was no overall difference, there was a higher prevalence of highMnin cord blood (defined as

the 95th percentile of the concentration in Paris: _6.8 mg/L), in Montreal. Takser et al., (2004) found higher bloodMn in pregnant women who reported that pesticides were used within a less than 1 km from their home.

Children's hair Mn has been shown to increase with Mn intake from baby formula (Collip et al., 1983) and drinking water (He et al., 1994; Woolf et al., 2002; Agusa et al., 2006; Bouchard et al., 2007). This biomarker of Mn exposure has been associated with poor performance on neurobehavioral tests (He et al., 1994; Wright et al., 2006; Woolf et al., 2002) and with behavioral problems (Bouchard et al., 2007) for children exposed through drinking water or living near toxic waste sites. In Spain, Torrente et al. (2005) did not find neurobehavioral deficits associated with hair Mn, but levels were lower than those reported in the other studies.

Mn shares several characteristics with iron (Fe), both are transition metals, with valences of 2+ and 3+ in physiological conditions, relatively similar ionic radius. In addition, sinceMnand Fe both strongly bind to transferrin and accumulate in the mitochondria, low Fe stores are associated to increased Mn uptake and retention in the blood (Roth, 2006). In a community study of adults, Baldwin et al. (1999) reported an inverse relation between serum Fe and whole blood Mn. It has been shown that Fe deficient state (sideropenic anemia) is related to increased levels of Mn in blood (Mena et al., 1969).

The purpose of the present study was to determine hair Mn levels of children living in the vicinity of a ferro-manganese alloy production plant and identify factors that influence these concentrations.

2. Materials and methods

2.1. Study design and population

The present study used a cross-sectional design in which we sought to compare children, aged 1–10 years, living in different areas around a ferro-manganese alloy production plant and a non-exposed group of children of similar age. Because anemia and Fe status may affect Mn concentrations (Mena et al., 1969; Baldwin et al., 1999; Kim et al., 2005), these hematological parameters were determined. The study protocol and consent procedure were

evaluated by the National School of Public Health–Oswaldo Cruz Foundation Ethical Committee.

The plant, inaugurated in 1970 has an annual production of SiMn and FeMn alloys of 280,000 tonnes. It is located in the metropolitan area of Salvador, capital of the State of Bahia, Brazil (see Fig. 1) in the Cotegipe village (total population 620 inhabitants), a district of Simões Filho with 109,269 inhabitants (IBGE, 2007). The area is separated from the urban area by the BR-324 highway. The Centro Atlaˆntica Railway, which brings in raw material to the plant and transports the ferro-manganese lingots to the Aratu harbor, passes through the village. The plant's geographical coordinates are 1284701800S and 3882404100W.

Air Mn in PM2.5 was measured over 24 h period during 7 days in August 2007. The air sampler was installed on the roof of one house (geographical coordinates 1284702300S and 3882502400W) located 1.3 km from the plant and about 70 m from the school and the daycare center. Sampling was performed using a Cyclone URG (URG, 2000) coupled to a vacuum pump adjusted to 10 L/min flow rate. The 47 mm diameter quartz membranes (SKC1) were extracted according to the EPA procedure (Compendium Method IO-2.1. EPA). Field and reagent blanks along with spiked samples were analyzed. The average and median Mn concentrations during this rainy period were 0.151 $\mu g/m^3$ and 0.114 $\mu g/m^3$, respectively (range 0.011–0.439 $\mu g/m^3$).

In July 2006 we carried out a census in which we listed and georeferenced 154 houses regularly inhabited by 165 children from 1 to 10 years of age. Information obtained from a community leader indicated that the village was spread along the road and divided into three sectors. We decided to include the residents of Virgınio Dame Street, an unpaved 2-km road on the north-west side of the industry, who complained of heavier dust fall-out on their residences. This is an estuarine area mostly plains with small hills; yearly wind prevalence is from south-east to north-west (VEEP, 2005). Spatial stratification, based on the distance and geographical position on relation to the plant's chimneys, was used to identify a priori distinct exposure areas (Fig. 1).

- Area A: Houses along a road located on the edge of the southwest side of the plant fence, at an average distance of 0.6 km. A total of 16 children (9.7%) live in this area.

- Area B: The village center located at an average distance of 1.5 Km west of the plant. It is more densely inhabited. Daycare and elementary schools are located here. We identified 108 (65.5%) children living in this area.
- Area C: This area is located approximately 1.6 km from the plant towards the Southwest on a plateau, approximately 80 m high. We identified 19 (11.5%) children in this area.
- Area D: This is an isolated community living downwind along a west-bound road ranging from 0.9 km to 1.7 km from the plant. Twenty-two children (13.3%) children were identified in this area.

The non-exposed children lived in the community of Capiarara, in the municipality of Lauro de Freitas town, located 7.5 km southeast from the plant in an upwind direction. A census of the whole community, performed in March 2008, identified a total of 379 inhabitants (103 children in desired age range).

In the exposed community, the local economy is based on the cultivation of cassava and rudimental processing and commercialization of manioc flour. In the reference community, the main income is from informal jobs in the nearby wholesale vegetable and fruit market.

2.2. Recruitment

In April 2007, a meeting was held with the community living near the plant. The objectives and procedures of the study were explained. Written informed consent was obtained from the parents of the 145 (87.9%) children who were still living in the region. In June 2007, a total of 109 (75.1%) children within the selected age range provided hair and blood samples.

Children in the reference community were recruited following a census. We visited each household and invited the parents or the caregiver (when not a parent) to participate in the research. Parents of 76 children agreed to participate in the study, providing written consent. On the day of biological sampling 49 children (64.5%) were available to provide blood and hair samples. Due to the fact that 6 boys had their hair shaved to the scalp, we could not collect samples, final sample size consisted of 43 children.

2.3. Data collection

2.3.1. Hair sampling and analysis

A tuft of hair of approximate 0.5 cm diameter was cut off with a surgical stainless steel scissor as close as possible to the scalp in the occipital region, after tying with a Teflon string at the proximal end. For boys with short hair (less than 2 cm in length), an equivalent amount was trimmed directly into the sterile sampling plastic bag. After identification with the proper child code, the sampling bags were stored at room temperature until analysis. In the laboratory, hair samples were washed according to the procedure described by Wright et al., (2006). Briefly, the first centimeter or the amount available was washed for 15 min in 10 ml of 1% Triton X-100 solution in a 50-ml beaker in ultrasonic bath. Rinsing was performed several times with Type I pure water (Milli-Q, Millipore). Hair samples were dried wrapped in Whatman N#1 filter paper in a drying oven at 70ºC overnight. Approximately 10 mg of hair was weighed in 50-ml beaker and digested with 2 ml of spectroscopic grade concentrated HNO_3 acid for 2h on an 80ºC hotplate. The digest was then diluted to 10 ml with Type I pure water in a polypropylene centrifuge tube (Corning®).

Acid digested samples and reference material were analyzed using electrothermal atomic spectroscopy with Zeeman background correction (GTA-120, Varian Inc.). All glassware and plastic ware were thoroughly decontaminated by soaking for 24 h in 3% neutral detergent (Extran1, Merck), followed by soaking overnight in 10% HNO_3 and finally rinsed with Type I pure water. Reagent blanks were analyzed along with samples in every batch. The detection limit was 0.1 µg/L. Routine checks of accuracy and precision were accomplished using human hair reference material from the International Atomic Energy Agency (IAEA-085). The intra-batch and batch-to batch precisions were 4.4% and 5.1%, respectively. Accuracy in the concentration range of 8.3–9.3 µg/g was 103.2%. All samples and SRM were determined in duplicates and a difference lower than 10% was considered acceptable.

2.3.2. Anemia

Blood samples were drawn by venipuncture into two different vacutainer tubes, one with EDTA for determination of hemoglobin (Hb) and cell counting by automated equipment (Hematology Analyzer Pentra 80, ABX) and the other with no additive for serum Fe determination by colorimetric method using a commercial kit (Roche Hitachi 747, Roche1). We applied the WHO criteria for

anemia: For children under 6 years of age, we used Hb levels ≤11.5 g/dL and for the older children ≤12.0 g/dL (WHO, 1994).

2.3.3. Socio-demographic information

Parents or caregivers responded to a socio-demographic questionnaire, administered by trained interviewers. One questionnaire included information on socio-demographic characteristics of the family (housing structure, educational level, time living in the community, etc.), general habits (consumption of water and vegetables grown locally). A second questionnaire focused specifically on the child and included general information on development, education, health status and recreational activities.

2.4. Data analysis

Each child was coded with respect to area of residence and house number. Descriptive statistics were used to determine the distribution of socio-demographic information, hair Mn and
hematological parameters.

Frequency distributions were compared using Fisher's exact test. Normally distributed continuous variables were compared using the Student's t-test, while for variables that were not normally distributed, Mann–Whitney (MW) or Kruskal–Wallis were used depending on the number of categories.

Since the distribution of hair Mn was skewed, data were log 10 transformed for further analyses. Backward stepwise regression models were used to identify variables that were potentially associated with hair Mn (0.100 to enter; >0.05 to exclude). These variables were then included in a linear regression model. A Tukey post hoc test was used to determine inter-area differences. A significance level ($p = 0.05$) was used. All statistical analyses were performed using SPSS version 13 software.

3. Results

3.1. Population characteristics

Table 1 presents a summary of the study populations' main characteristics. Both communities are ethnically comprised of a majority of Afro-Brazilians. The large majority has dark hair. In the
exposed community, for 70.3% hair color is brown to dark brown; 28.7% are black haired and one child is blond. In the reference community, all have dark

brown to black hair. They are low income families, with an average monthly stipend of US$ 150. All families receive a federal government stipend per child enrolled at school (Bolsa família program). The majority of those who responded to the questionnaire was the biological mother (85% in the exposed versus 76% in the referents); 7% and 14% were fathers and 8% and 10% caregivers (grandmothers or godmothers), respectively. Parents and caregivers reported a low number of years of formal
schooling (mean < 3.0 years).

For the children, gender proportions were similar in the exposed (48.6% boys) and reference (42.9% boys) communities. Children from both communities did not differ in age, hemoglobin and serum iron levels. In the exposed group, 21 of the 106 children (19.9%) were classified as anemic; the prevalence of anemia in the reference community was 18.8% (9/48). This difference is not statistically significant (p = 0.533).

3.2. Children's Mn hair levels

Box plots of MnH data according to area of residence, clustered by gender are in Fig. 2. For the reference group, geometric mean and median MnH concentrations were 1.13 µg/g and 1.19 µg/g, respectively, ranging between 0.39µ g/g and 5.58 µg/g. A total of 7% (3 children) surpassed 3.0 µg/g. Geometric mean and median MnH for the children living in the vicinity of the plant were 9.96 µg/g and 9.70 µg/g, ranging from 1.10 µg/g to 95.50 µg/g; the large majority (91.7%) of MnH levels was above 3.0 µg/g. Girls MnH levels were significantly higher than boys. For the reference group, median levels were: 1.59 µg/g versus 0.95 µg/g, respectively (MW, p = 0.023), while in the exposed group girls presented a median concentration of 13.78 µg/g and boys: 6.56 µg/g (MW, p < 0.001). Among those who lived near the plant, 88.1% were born in the community and no difference was observed in MnH levels between the children who were born there and those who were not (boys born in the community 10.70 µg/g; others 13.47 µg/g; girls born in the community 19.21 µg/g; others 18.88 µg/g).

No correlation was observed between MnH with age or with hemoglobin or serum iron levels. For those with anemia median MnH for the reference group was 1.35 µg/g versus 1.15 µg/g for those who did not present anemia. For the

exposed group, these median concentrations were 9.20 µg/g and 9.70 µg/g, respectively.

Table 2 shows MnH levels with respect to the four residence areas. The highest levels were observed in Areas A and D, which correspond to residences closest to the plant and those directly downwind. Gender differences (girls > boys) were present at every location. No differences were observed in children's age between areas of residence (ANOVA, $p > 0.05$).

Table 3a presents the results of the multiple regression model for log MnH with only the exposed children. Gender and area of residence enter significantly into the model, explaining 26.9% of the variance. Post hoc tests showed that residence area could be grouped into two with those residing next to the plant or downwind with the higher MnH concentrations (Areas A and D) and those in the centre of the village and the outskirts displayed lower levels (Areas B and C). Both are significantly different from the control group.

We explored the influence of time of mothers' residence in the area at childbirth as a possible surrogate of in utero exposure. For the exposed group, maternal exposure duration before child's birth was obtained by subtracting the age of the child in years from the time the mother reported living in the area near the ferromanganese alloy production plant. The average time of mothers' residency in the area was 8.0 years and ranged from 0 (for those children who were not born there) to 29 years. When mothers' time of residency in the area was included in the above model (Table 3b), the t-values for gender and area of residence were basically unchanged and the model explained 36.8% of the variance of log MnH. The model respected the linear regression assumptions and the standardized residues displayed normal distribution
(mean = 0 and S.D. = 0.986). Fig. 3 shows a scatter graph of the residual plot of log transformed MnH level with respect to years of maternal exposure time previous to child's birth, the partial correlation coefficient shows that this variable alone explained 12.1% MnH variation.

4. Discussion

The levels of MnH found in the exposed children in this study are on average 10 times higher than those in the non-exposed children. MnH reference value for

Brazilian adults (0.15–1.2 µg/g) has been previously determined using inhabitants of Rio de Janeiro in a sample of 1091 men and women (Miekeley et al., 1998).

In Cotegipe village, Mn concentrations in raw and drinking water were 74.4±8.63 µg/L and 27.7±15.02 µg/L, respectively (data not published). These values are relatively low compared to the WHO guidelines of 400 µg/L (WHO, 2006). In the region surrounding the manganese alloy production plant, air Mn appears to be the major determinant of children's hair Mn. Air Mn concentrations in ultrafine particles ranged from 0.011 µg/m^3 to 0.439 µg/m^3 at the sampling site, located in the core of the community, 1.3 km from the plant. Lucchini et al., (2007) measured Mn in the respirable fraction, using a similar technique, in six locations within 2 km from a manganese alloy plant with similar processes to the one here. They reported a geometric mean of 0.69 µg Mn/m^3 (range 0.2–1.8 µg Mn/m^3). The authors indicated that in the metropolitan area of Brescia, about 50 km downwind from the alloy plant, Mn concentrations were 0.08 µg Mn/m^3 and ranged 0.050–0.30 µg Mn/m^3. In the present study, we were unable to perform air environmental monitoring in all four areas due to equipment availability and limited budget.

The findings of the present study suggest that the main source of airborne Mn exposure is the fumes from the alloy plant chimneys. A clear pattern was observed when MnH levels were analyzed spatially, with the highest concentrations in those children who lived closest to the plant or in the downwind direction.

MnH levels observed in this study are the highest concentrations reported in children environmentally exposed to manganese (He et al., 1994; Woolf et al., 2002; Wright et al., 2006; Bouchard et al., 2007). However, measurement techniques may differ from one study to the next. A panel convened by ATSDR (2001) to provide guidance for agency health assessors on the use and interpretation of hair analysis data emphasizes that although the technology exists for assessing substances in hair, variations in sample collection, preparation, and analytical methods can drive what will be measured in the final analysis. It has also been suggested that Mn may be more readily found in darker colored hair (Lyden et al., 1984; Sturaro et al., 1994). The exact mechanism of Mn uptake in hair follicle has not been fully elucidated, but it is

well known that Mn has a high affinity for all types of melanins encountered in hair, skin, iris and in the CNS (Lyden et al., 1984). A report of MnH concentration among adults in Cotegipe village indicated average concentrations between 66.38 µg/g and 177.43 µg/g, depending on the area of residence (VEEP, 2005), but hair sample collection protocol, washing procedure and analytical methods were not indicated. In the present study, since Mn exposure is mainly through airborne route, it could be argued that the high Mn level observed in this study could be due to external deposition. It should be noted that extra care was taken in the hair sample treatment. The procedure described by Wright et al., (2006) was adopted to wash hair samples, because it applies a mild detergent in ultra-sound bath for 15 min. This procedure yields a thorough hair wash without destroying hair structure.

In this study, girls had significantly higher Mn hair levels than boys. Similar results were reported by Bouchard et al., (2007) for boys and girls exposed to high levels of Mn in well water in Québec, Canada. Wright et al., (2006) evaluated 31 children living near a waste site in Oklahoma, USA and found no significant difference between hair Mn levels in boys and girls, but hair Mn concentrations were considerably lower (mean 0.47 µg/g) than those observed in the present study or in the study by Bouchard et al., (2007). In a community-based study of adults, Baldwin et al., (1999) observed that women presented higher Mn blood levels than men.

The authors suggest that men and women differ in Mn metabolism, which may be related to Fe status.

Due to its electrochemical similarity to Fe, Mn competes for the same transport mechanisms for intestinal Fe absorption, where both bind to the divalent metal transporter-1 (DMT1), Thus Mn may be absorbed more efficiently when there is a depletion of Fe stores (Garrick and Dolan, 2003; Roth, 2006). Although, anemia was present in approximately 19% of the children, there was no difference in MnH with anemia status, nor any correlation of hair Mn content with the biomarker of Fe status. In adults, Montes et al., (2008) observed a negative correlation between blood manganese and hemoglobin levels in persons exposed to a mining and processing plant in Mexico and Baldwin et al., (1999) reported an inverse relation between blood Mn and serum Fe. To our knowledge, these relations have not been reported in children. Wasserman et

al., (2004, 2006) assessed Hb in their study of neurobehavioral effects of manganese exposure through well water in Bangladeshi children, but they do not report its relation with the biomarkers of Mn.

The relation between mothers' length of residence at childbirth and children's MnH several years later suggests that in utero exposure may contribute to higher Mn concentrations in children. Elevated in utero exposure may also influence future neurodevelopment. Ericson et al., (2007) measured Mn in the enamel of deciduous teeth, whose formation begins during fetal life. In a prospective study, the authors reported that after adjusting for levels of Pb, children with higher Mn in the uterine phase had higher scores on all scales of disinhibitory behavior. Takser et al., (2003) observed, after adjusting for potential confounders (sex and mother's educational level), negative correlations between Mn levels in the umbilical cord blood and various psycho-motor subscales at 3 years (attention, non-verbal memory and manual ability), even though these effects were not observed at 6 years of age.

The findings of this study indicate that children living the vicinity of this Mn alloy production plant have elevated hair Mn levels, which vary with respect to their geo-spatial location of residence. The major sources of Mn are probably fumes expelled by the alloy plant chimneys, dust re-suspension by traffic and possibly dust from the train passing through the village carrying mineral ore and the transformed product. A study of neurobehavioral effects of Mn exposure in these children is currently underway.

Conflict of interest

None.

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Anexes

Table 1. Summary of socio-demographic characteristics.

	Exposed n (%)					Reference n (%)					P value
Gender											
Boys	53 (48.6%)					19 (44.2%)					n.s.
Girls	56 (51.4%)					24 (55.8%)					n.s.
Ethnicity											
African-Brazilian	79 (72.7%)					35 (79.5%)					n.s.
Non African-Brazilian	30 (27.3%)					8 (20.5%)					n.s
	Mean	**Median**	**SD**	**Min.**	**Max.**	**Mean**	**Median**	**SD**	**Min.**	**Max.**	
Age (months)	78.5	84.9	32.85	10.1	136.5	81.4	83.3	33.73	14.1	132.8	n.s.
Hb (g/dL)	12.3	12.4	1.08	9.7	15.0	12.2	12.1	1.06	9.0	15.0	n.s.
FeS (μg/dL)	57.6	55.0	24.9	10.0	133. 0	52.9	51.5	22.6	14.0	106.0	n.s.
Parents self-reported years of school	2.82	3.0	0.11	1.5	3.6	2.86	3.0	1.0	0.22	4.0	n.s.
Number of years mother lived in the community at the child's birth	10.06	8.0	9.04	0	32.0	8.85	5.0	9.83	0	37.0	n.s.

n.s: not significant for Chi-square or Student´s t tests.

Table 2. Hair manganese (μg/g) in children according to area of residency.

Area of residency	n	Geometric mean	Median	SD	Min.	Max.
Reference	43	1.37	1.19	0.95	0.39	8.58
Exposed						
Area A	8	27.37	31.30	24.50	8.81	86.23
Area B	75	9.61	9.68	9.61	1.10	46.23
Area C	15	6.36	6.90	5.42	1.36	19.92
Area D	11	21.33	28.96	30.38	2.05	95.50

Table 3a. Results of the multiple regression model for log MnH with the dependent variables: age, gender and area of residence.

Variable	Unstandardized Coefficients	t Stat.	P value
Intercept	1.506	16.439	<0.001
Age (months)	-0.0004	-0.241	0.810
Gender	-0.255	-3.838	<0.001
Area of residence	-0.206	-4.790	<0.001

logMnH (n=109; r^2=0.268; F=19.420, p<0.001)

Table 3b. Results of the multiple regression model for log MnH with the dependent variables: age, gender, area of residence and maternal exposure time before child's birth (years).

Variable	Unstandardized Coefficients	t Stat.	P value
Intercept	1.915	12.458	<0.001
Age (months)	0.0004	0.210	0.834
Gender	-0.219	-3.508	0.001
Area of residence	-0.502	-6.142	<0.001
Maternal exposure time before child's birth (y)	0.013	3.802	<0.001

logMnH (n=109; r^2=0.368; F=20.378, p<0.001)

Figures

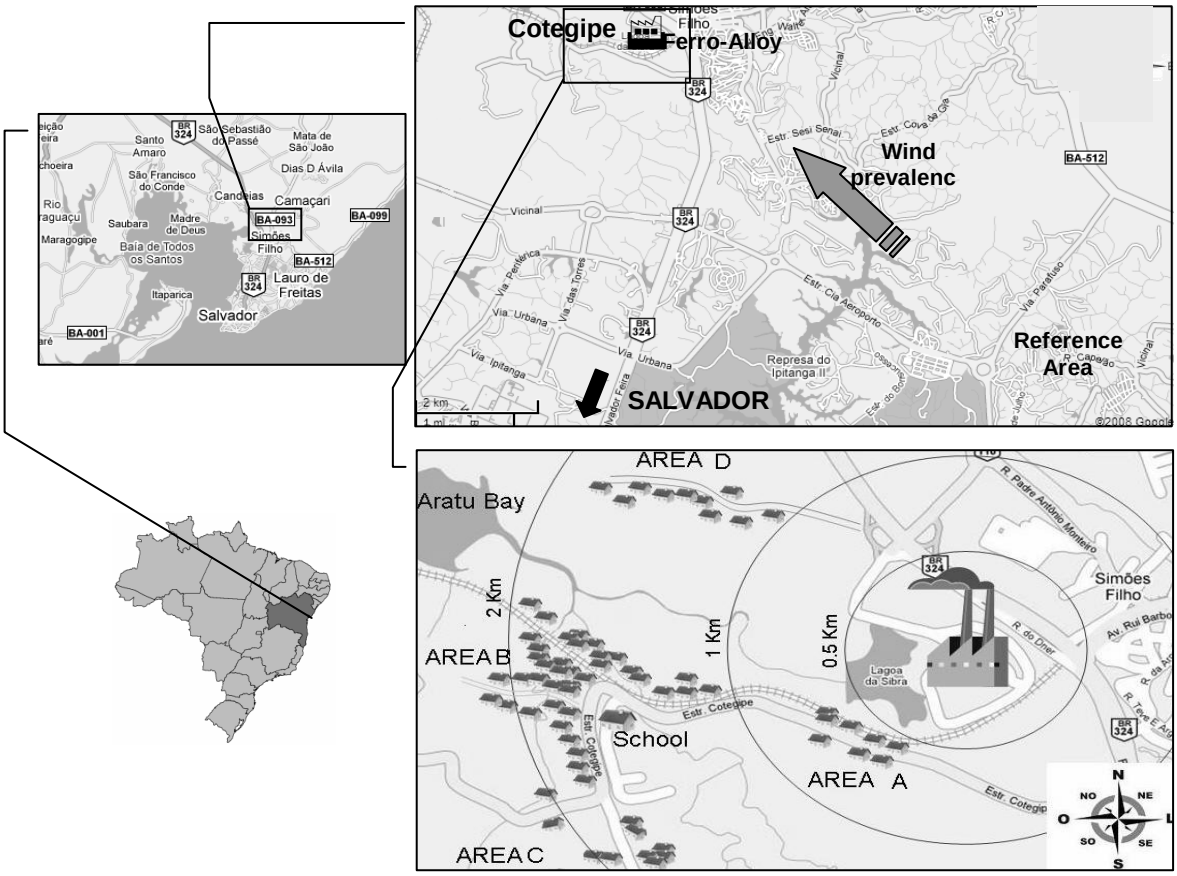

Figure 1

Figure 1. Schematic map of the Cotegipe Village (exposed community) in Simões Filho town and reference community (Capiarara, Lauro de Freitas), in the Metropolitan Area of Salvador, Bahia, Brazil (top); showing the four residential areas with radial distances from the plant (bottom).

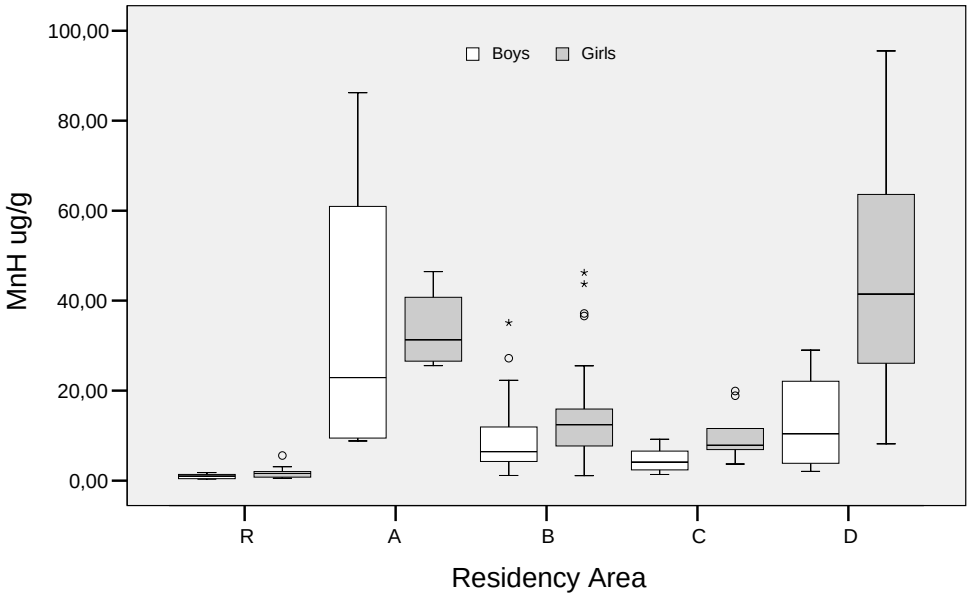

Figure 2

Figure 2. Box plot of MnH data according to area of residence, clustered by gender. R is for referents and A thru D the four residential areas in the exposed community.

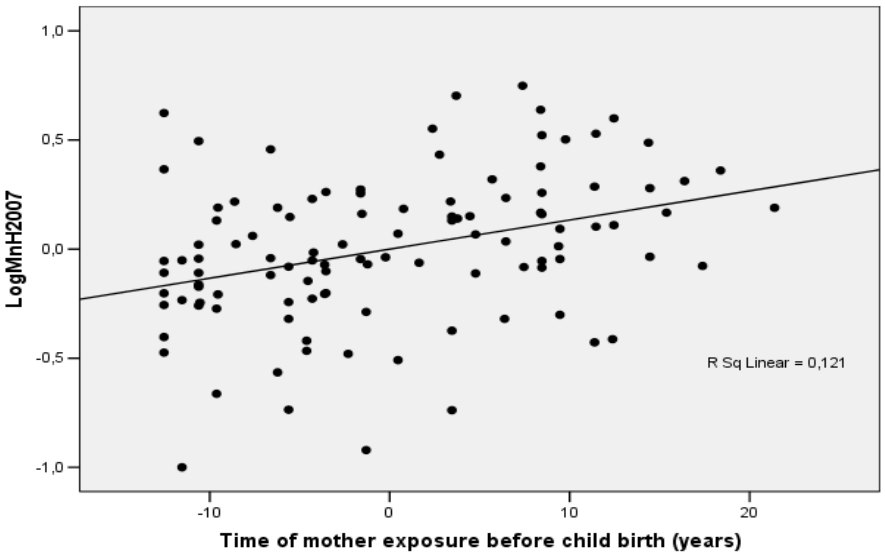

Figure 3

Figure 3. Residual plot of log transformed Mn concentration in hair *versus* years of maternal exposure time previous to child birth.

Artigo 3

Blood and hair manganese levels in children living in the vicinity of a ferro-manganese alloy plant and their correlations with house dust Mn levels*

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Summary

Background: For over thirty years a ferro-manganese alloy plant has been polluting the Cotegipe village in the metropolitan area of Salvador, Brazil. This report is part of an ongoing investigation on Mn exposure and effect on cognitive function of children living in the vicinity of that plant. In our previous report we observed a gradient of exposure according to the distance and location relative to wind direction from the plant, when we measured hair Mn levels. *Objectives*: Now, we aim to evaluate children exposure to Mn comparing two Mn bioindicators and investigate their correlations with Mn levels in settled house dust and other co-variables. *Methods*: The present study examined Mn exposure in children (n=83), in the age range of 6 to 10 years, living in the vicinity of the plant (0.5 to 2 Km radius) and comparing with children (n=26) living 7.5 km upwind of the point source emission. Airborne Mn concentration, measured in the respirable fraction ($PM_{2.5}$) during 24h-sampling period; settled house dust (MnD), collected by aspiration onto cellulose ester filters; blood (MnB) and hair (MnH) were analyzed by electrothermal atomic absorption spectrometry (ETAS) with Zeeman background correction. Serum iron status was also assessed by automated colorimetric technique. Hair (PbH) and blood lead (PbB) levels were also measured by ETAS in order to control for this ubiquitous neurotoxin. *Results*: Manganese mean concentration in the air of the Cotegipe village was 0.15 µg/m^3 (range 0.01 – 0.44 µg/m^3) versus 0.004 µg/m^3 (range 0.002-0.004 µg/m^3) in the reference area. A gradient of exposure with respect to proximity to the emission source and the downwind location was observed with Mn levels in house dust (MnD): 2,643 µg/g; 2,295 µg/g and 129 µg/g, respectively. Blood Mn levels were 7.22 µg/L, 7.99 µg/L and 4.78 µg/L, respectively. Mnb as an exposure biomarker was only able to discriminate

differences between exposed and reference groups; on the other hand, MnH levels as a biomarker was able to detect differences in exposure levels among the three groups: 24.82 µg/g, 4.87 µg/g and 1.09 µg/g, high, moderate and reference, respectively. No correlation was observed between MnH with MnB levels, but hair lead concentration was significantly related to MnB, MnH and PbB levels. *Conclusion*: The data suggest that MnH is the best surrogate of Mn exposure and the major sources of Mn body burden in these children are originated from airborne particles and the settled house dust may represent a source of recontamination.

Key-words: Manganese, hair, blood, house dust, children

***** A ser submetido para a Environmental Reaserch.

Introduction

Manganese (Mn) is an essential element, necessary for bone mineralization, energy and protein metabolism, cell metabolism regulation, and protection against oxidative stress (Keen et al., 2000). With normal dietary consumption, systemic homeostasis of Mn is maintained by both its rate of transport across enterocytes lining the intestinal wall and by its efficient removal within the liver (Papavasiliou et al., 1966). Exposure through inhalation is more effective than ingestion because Mn bypasses some of the homeostatic mechanisms that normally regulate its concentration in the body. In addition, animal studies have shown that inhaled Mn compounds can be taken up by the olfactory nerve and through axonal transport reach the olfactory bulb and other parts of brain (Brenneman et al. 2000; Dorman et al. 2006).

Neurotoxic effects resulting from excessive Mn exposure were first described by Couper in 1837 in Scottish laborers grinding Mn black oxide in a chemical industry (cited in Iregren, 1999). Neurological symptoms of manganism include decreased memory and concentration, fatigue, headache, vertigo, loss of equilibrium, insomnia, tinnitus, trembling of fingers, muscle cramp, rigidity, alteration of libido and sweating (Tanaka 1998). Studies of active workers with Mn exposure show diminished motor and cognitive functions, with changes in affect (for review see: Zoni et al., 2007). At even lower levels of exposure, studies of communities living in proximity to airborne Mn from mining and transformation activities have likewise reported neurobehavioral deficits (Mergler et al., 1999; Rodríguez-Agudelo et al., 2004), as well as changes in prolactin levels (Montes et al, 2008), associated with biomarkers of Mn exposure. Lucchini and associates (2007) showed a higher prevalence of Parkinsonian disturbances in a region of an Italian province with several ferro-manganese production plants, compared to other areas of the province.

Epidemiological studies have demonstrated a robust association between exposure to elevated concentration of fine particulate matter ($PM_{2.5}$) and the increase of morbi-mortality rates (Chen et al., 2008, Ulirsch et al., 2007). Metals associated with particulate matter have also been responsible for effects on the

lung and cardio-circulatory systems (Chattopadhyay 2007 & Mills, 2007). Metal contents in dust have been a concern of several investigators, because most of them can be very toxic at low levels, for example lead (Pb), arsenic (As), cadmium (Cd), mercury (Hg) and even manganese (Mn) that is also a micronutrient (Sai et al., 2002). Dust frequently has high concentrations of several toxic compounds, which has a direct impact on environmental quality and on human health, especially on children and elderly (Dominici, 2007).

According to the WHO (2006) children have different susceptibilities during different life stages owing to their dynamic growth and developmental processes as well as physiological, metabolic, and behavioral differences. In this publication it is stated that from conception through adolescence, rapid growth and developmental processes occur that can be disrupted by exposures to environmental chemicals. These include anatomical, physiological, metabolic, functional, toxicokinetic, and toxicodynamic processes. It is emphasized that exposure may also be different in different stages of childhood. Exposure can occur in utero through transplacental transfer of environmental agents from mother to fetus or in nursing infants via breast milk. Children consume more food and beverages per kilogram of body weight than do adults, and their dietary patterns are different and often less variable during different developmental stages. They have a higher inhalation rate and a higher body surface area to body weight ratio, which may lead to increased exposures. Finally, it concludes that children's normal behaviors, such as crawling on the ground and putting their hands in their mouths, could result in exposures not faced by adults.

Children contamination by lead via dust exposure has been extensively studied by several investigators (Sayre et al., 1974, Lanphear et al., 1998, 2002, Gaitens et al., 2009). To our knowledge very few studies evaluated children exposure to Mn in house dust. Buchet et al., (1980) measured Mn and other metals in groups of school-age children living around a lead smelter. They measured metals in blood, urine, hand rinsing, dust and dirt from school playground. Rölling et al., (2005) evaluated 814 elementary school children of two South Africa cities. They found that MnB levels were 9.8±3.59 µg/L (Johannesburg) and 6.74±3.47 µg/L (Cape Town). They observed that MnB levels were significantly associated with concentrations of manganese in

classroom dust at schools. In Sydney, Australia, Gulson et al., (2006) found that dust sweepings were a significant predictor of Mn in handwipes, which in turn was the only significant predictor for MnB [1.8 to 45 µg/L (GM 11.6) (n=254)].

In our previous study (Menezes-Filho et al., 2009) we observed that children living in the vicinity of a ferro-manganese alloy plant presented very high MnH levels (GM 9.96 µg/g, ranging 1.10-95.5 µg/g); and some factors like: gender (girls>boys), time of mothers exposure previous to the child's birth and area of residence could explain 36.8% of the variance in MnH levels. In this present study we aim to evaluate children exposure to Mn comparing two biomarkers (MnB and MnH) and investigate their correlation with Mn levels in settled house dust and other co-variables.

Material and Methods

Study design

This is a cross-sectional study with a cohort of children from Cotegipe village, district of Simões Filho located in the metropolitan area of Salvador, capital of the State of Bahia, Brazil (Figure 1). Previously, in June 2007, we evaluated exposure of 109 children living near the alloy plant, age range of 1 to 10 years. In April thru July 2008, when we established a cognitive function evaluation protocol, we were able to collect new hair and blood samples of all children (n=83) in the age range 6 to 10 years, who were enrolled in the local elementary school. For comparison purpose, we evaluated children (n=43), but only 26 were in the same age range, enrolled in the elementary school of Capiarara community, in the municipality of Lauro de Freitas town, located 7.5 Km southeast from the plant in an upwind direction. Written informed consent was obtained from all parents or guardians. The study protocol and consent procedure were approved by the National School of Public Health – Oswaldo Cruz Foundation Ethical Committee.

Data Collection

House dust vacuum sampling method

Along the months of August thru September 2007, and November 2008, settled house dust samples were collected in 53 houses of the exposed community and 9 houses in the reference area, respectively. We followed the procedure described by Quitério et al., (2004), which consisted of vacuuming internal surface area with personal sampling pump. Each residence unit was sampled in three replicates, generally in the living room, in the children's room and a third one on surface out of reach, like on top of cupboard or wardrobe when available. Each sampling site was delimitated by a 30 cm x 30cm flexible polyethylene template. Personal air samplers (SKC 224-PCXR model – Eighty Four, Pennsylvania, USA) were calibrated at 2.5 liters/minute against a primary standard with a mixed cellulose ester filter (0.8 µm pore size) in line (SKC, MEC 225-5 – Omega Specialty Division). The sampling train consisted of a piece of ¼" I.D. Tygon tubing attached to the pump and a 37 mm filter cassette. Acid washed piece of ¼" I.D. Tygon tubing measuring 2 cm in length was attached to the inlet of the cassette. The open end of the Tygon tubing was cut at about 45^{o} angle, the surface was vacuumed with contiguous, non-overlapping left-to-right strokes by pressing the end of the tubing on the surface and drawing it across the sampling area. The vacuuming process was then repeated in a top to bottom motion. On average it took 4 minutes per sampling area.

Quantitative analysis

Gravimetric determination of filter masses (pre and pos sampling) was performed at the Laboratory of Toxicology of Jorge Duprat Foundation (Fundacentro – Bahia) in a temperature and humidity controlled room using a five decimal analytical balance (Metler H54AR, Mettler Instruments AG, New York, USA) following a standardized procedure NHO 03 (Fundacentro). All dust samples were partially digested using a 3 mL of ultra-pure hydrochloric acid and nitric acid (3:1 v/v) mixture on a hotplate adjusted to 90^{o}C for four hours. Field blanks, spiked samples and estuarine sediment reference material (STDS-4, Canadian Certified Reference Materials Project) were analyzed along with real samples. Manganese was analyzed by electrothermal atomic absorption spectroscopy (ETAS) with Zeeman background correction (GTA-120, Varian

Inc.) and results were expressed in load (*MnL*, µg Mn/m^2) and in concentration (*MnD*, µg Mn/g of dust).

Air sampling

Air particulate matter in respirable fraction ($PM_{2,5}$) were sampled during seven consecutive days, in August 2007, in the exposed area and during three days, in November 2008, in the reference community. Sampling was performed using a Cyclone URG (URG, 2000) coupled to a vacuum pump calibrated to a flow rate of 10 L/min. Sampler was set up on the roofs of houses in the center of each community. The 47 mm diameter quartz membranes (SKC1) were extracted according to the EPA procedure (Compendium Method IO-2.1. EPA). Field and reagent blanks along with spiked samples were analyzed concomitantly the samples. Manganese concentration was determined by the same technique mentioned above.

Manganese in Blood, MnB

Venous blood samples were collected from participating children into sodium–EDTA vacuum tubes proper for metal analysis (Vacutainer, Bencton & Dickson, USA). We adapted the method described by Montes et al., (2002). Blood sample was diluted 1:5 with matrix modifier (1% ammonium-dihydrogenphosphate in 0.1% Triton X-100 solution). After homogenization in a vortex, it was centrifuged for 10 minutes at 14,000 rpm. Quality control of blood Mn analysis was assured by measuring human blood reference materials QMEQAS07B-03 and QMEQAS07B-06 (Centre de Toxicologie/INSPQ, Canada). Samples were measured in duplicate; every measurement consisted of two injections into graphite furnace, in all cases standard deviation was lower than 10%; if otherwise, sample was reanalyzed.

Manganese in Hair, MnH

A tuft of hair of approximate 0.5 cm diameter was cut off with a surgical stainless steel scissor as close as possible to the scalp in the occipital region, after tying with a Teflon string at the proximal end. For boys with short hair

(less than 2 cm in length), an equivalent amount was trimmed directly into the sterile sampling plastic bag. We followed the procedure described by Wright et al., (2006) to clean up hair. Human hair reference material from the International Atomic Energy Agency (IAEA-085) was analyzed along with hair samples for quality control purpose. Detailed information on hair sample treatment and analysis can be found in Menezes-Filho et al (2009).

Manganese Determination

In every samples Mn was analyzed by electrothermal atomic absorption spectroscopy with Zeeman background correction (GTA-120, Varian Inc.). The furnace temperatures were optimized using SRM procedure, which indicated the ashing and atomization temperatures of 700ºC e 2400ºC, respectively. The analyses were performed with the wavelength 279.5 nm, slit width of 0.2 nm. Calibration was accomplished using the auto-mix procedure with standard Mn solution in 0.2% nitric acid made freshly from a stock solution 1 mg/mL (AccuStandard, New Haven, USA, traceable to NIST). All glassware and plasticware were thoroughly decontaminated by soaking for 24 hours in 3% neutral detergent (Extran□, Merck), followed by soaking overnight in 10% HNO_3 and finally rinsed with Type I pure water (Milli-Q, Millipore). Reagent blanks were analyzed along with samples in every batch. The detection limit was 0.1 µg/L. Routine checks of accuracy and precision were accomplished using specific reference material samples described above.

Serum Iron, FeS

Additional blood sample, collected into no additive vacuum tube (Vacuntainer, BD) was obtained for serum iron determination. After blood clogging, tubes were centrifuged 9000 rpm for twenty minutes. Serum iron was determined by automated method using a commercial kit (Roche Hitachi 747, Roche®).

Hair (PbH) and Blood Lead (PbB) Determination

As lead is an ubiquitous contaminant and recognized neurotoxin, associated with effect on cognition and behavior in children at low blood lead levels, we also measured PbH and PbB levels by electrothermal atomic

absorption spectroscopy with Zeeman background correction (GTA-120, Varian Inc.). Detailed procedures are described elsewhere (data to be published: Paes e Menezes-Filho, 2010).

Data Analysis

Each child was coded with respect to area of residence and house number. Descriptive statistics were used to determine the distribution of socio-demographic information, hair and blood Mn, serum iron, blood and lead levels.

Frequency distributions were compared using Fisher´s exact test. Normally distributed continuous variables were compared using the Student t test, while for variables that were not normally distributed, Mann-Whitney (MW) or Kruskal-Wallis were used depending on the number of categories.

Paired t-test was applied to compare the differences in MnH levels in children's hair collected in two consecutives years. A non-parametric correlation method (Spearman Rho correlation coefficient) was applied in order to evaluate the relation of Mn environmental levels with Mn bioindicators and co-variables. Since the distribution of MnH and MnD or MnL levels were skewed, data were log10 transformed for further analyses. Analysis of variance one way (ANOVA) with Tukey´s or Dunnett's *post hoc* test was used to compare similarities between groups of exposure levels.

A significance level (p=0.05) was used. All statistical analyses were performed using SPSS version 13 software.

Results

Population Characteristics

Table 1 presents a summary of the study populations' main characteristics. Both communities are ethnically comprised of a majority of Afro-Brazilians. The large majority has dark hair. In the exposed community, for 70.3% hair color is brown to dark brown; 28.7% are black haired and one child is blonde. In the reference community all twenty-six children included have dark brown to black hair. They are low income families, with an average monthly stipend of U$ 150.

The large majority of the families in both communities receive a federal government stipend based on child enrollment at school (*Bolsa família* program). The majority of those who responded to the questionnaire was the biological mother (85% in the exposed *versus* 76% in the referents); 7% and 14% were fathers and 8% and 10% care-givers (grandmothers or godmothers), respectively. For the children, gender proportions were similar in the exposed (51.8% boys) and reference (50.0% boys) communities. Exposed and referents children did not differ in age (108±20.9 months *vs.* 104.5±16.8 months), blood lead levels (2.0±1.5 µg/dL *vs.* 1.8±1.1 µg/dL) and hair lead levels (2.28±3.67µg/g *vs.* 2.26±1.58 67µg/g), respectively. However, it was observed a very significant difference ($p<0.001$) in serum iron levels: exposed (106.6±30.9 µg/dL.) and referents (62.1±16.4 µg/dL)

Environmental Monitoring

The mean air ($PM_{2.5}$) Mn concentration in Cotegipe village of seven sampling days was 0.151±0.1445 µg/m^3 (range 0.011-0.439 µg/m^3). In the reference community, the mean Mn concentration of three sampling days was 0.004±0.0014 µg/m^3 (range (0.002-0.005 µg/m^3)

Manganese levels in the settled house dust were expressed in concentration (µg/g) and in area load (µg/m^2). In Cotegipe village, we collected in 2007 settled dust in all the 53 houses where children lived. As we sampled each house unit in three spots and one of each represented a testimonial of long last deposition, the intra house variability was very high. In order to cope with it, we deleted the outlier replicate to shrink the variance. In the reference community, we collected settled house dust in nine houses of the 26 children in 2008, which represented more than one-third of the homes. Table 2 shows Mn levels in house dust in concentration (MnD) and in load (MnL) in both communities. Data distribution did not follow a normal pattern (KS $p<0.001$). The Mn geometric mean (GM) concentrations were 2,344 µg/g and 129 µg/g, exposed vs. reference, respectively. The GM Mn load levels were 35.3 µg/m^2 and 0.35 µg/m^2, respectively.

Biological Monitoring

Of the 83 children in the exposed area, we were able to obtain 71 blood

samples (86%), the remaining refused to provide blood specimen. Table 2 shows MnB levels in children from the exposed and from the reference areas, categorized in exposure level. The data distribution followed a normal curve (K-S, p=0.274). The average and the geometric means were 8.46 µg/L and 7.89 µg/L in the exposed children and 5.23 and 4.78 µg/L, respectively. Student t-test showed that the difference in MnB levels is statistically significant (p<0.001). In the same way, we compared MnB levels between boys (8.32 µg/L) and girls (8.59 µg/L) in the exposed area and no statistical difference was observed. The same with boys (4.89 µg/L) and girls (5.85 µg/L) from the reference area.

Children's hair was sampled in two consecutive years for those living near the plant. In 2007 during the first phase of this investigation, 109 children provided hair samples. MnH hair medians were 6.56 µg/g and 13.78 µg/g for boys and girls, respectively. In 2008, eighty-two children in the exposed community and 26 children of the reference community were willing to donate hair samples. As we observed previously (Menezes-Filho et al., 2009), MnH levels did not follow a normal distribution pattern (K-S, p<0.001). We performed a log transformation of this variable for further analyses (logMnH, K-S p=0.489). MnH geometric mean and median of children living near the plant were 5.83 µg/g and 6.60 µg/g, respectively. Among those in the reference area were 1.09 µg/g and 1.09 µg/g, accordingly. We observed a strong and significant correlation (r=0.884, p<0.001) between MnH levels sampled in these two years (Figure 2). When we compared the change in hair levels along that period, the difference was not significant (p=0.061) for the whole group. However, for girls we observed a significant (p=0.004) decrease in MnH levels, mean difference -2.22 µg/g. For boys, there was a slightly increase, but this change was not statistically significant. No statistical difference was observed in median MnH levels for boys (7.14 µg/g) and for girls (5.67 µg/g) of the exposed community, but significant (p=0.011) for boys (0.90 µg/g) and for girls (1.65 µg/g) of the reference area.

Correlations between environmental and biological monitoring – Individual basis

Spearman matrix correlation coefficients of environmental and biological Mn indices and other important co-variables exclusively with data on the

exposed children are presented in Table 3. Children's age did not show statistical significant relation with any of the biomarkers measured (MnH, MnB, FeS, PbH and PbB). As far as Mn biomarkers are concerned, we observed that MnB levels was not related to MnH nor with FeS, but a weak positive significant correlation was observed with PbH (rho 0.246, p=0.04). Hair Mn levels were mildly positive correlated with PbH (rho=0.371, p=0.001) and weakly positive correlated with PbB (rho=0.278. p=0.021). None of the house dust Mn indices used was correlated with Mn bio-indicators, even though, Mn level in house dust per squared area (load) was positively correlated (rho=0.429, p=0.029) with PbH levels.

Environmental and biological monitoring comparisons – Group basis

Previously, we reported that the four residential areas of the exposed community (Cotegipe village) could be grouped into two with those residing next to the plant or downwind with the higher MnH concentrations and those in the centre of the village and the outskirts displayed lower levels. Here we categorized them as high exposure and moderate exposure areas, respectively. ANOVA analyses show that there is a significant difference ($p<0.05$) in MnD, MnH and MnB levels in the three exposure groups (reference included). Dunnett *post hoc* test (for equal variance not assumed) showed that Mn in dust levels in the reference group differentiate from the moderate and from the high exposure levels. As far as MnB is concerned, Dunnett test showed that the high and moderate exposure areas are not different from each other but both are different from the reference group. On the other hand, it was observed a significant difference in MnH levels among the three exposure levels. These results are illustrated in Figure 3, which shows box-plots of house dust levels in concentration (a) and load (b), MnH (c) and MnB (d) stratified by exposure level.

Discussion

In this investigation we measured children exposure to manganese, assessing biological samples (hair and blood) and environmental samples (air

and house dust). All parameters indicated a high level of exposure of the Cotegipe village children, who live in the surroundings of a ferro-manganese alloy plant.

Mn concentrations in ultrafine particles ranged from 0.011 µg/m^3 to 0.439 µg/m^3 at the sampling site, located in the core of the community, 1.3 km downwind from the plant. These levels are in average 35 times higher than air Mn concentration in the reference community, which is located about 7 km upwind from the plant. Lucchini et al., (2007) measured Mn in the respirable fraction, using a similar technique, in six locations within 2 km from a manganese alloy plant with similar processes to the one here. They reported a geometric mean of 0.69 µg Mn/m^3 (range 0.2–1.8 µg Mn/m^3). The authors indicated that in the metropolitan area of Brescia, about 50 km downwind from the alloy plant, Mn concentrations were 0.08 µg Mn/m^3 and ranged 0.050–0.30 µg Mn/m^3.

House dust Mn levels expressed as load (µg/m^2) or concentration (µg/g) displayed a large variability in the exposed area. The Mn geometric mean (GM) levels were: concentration 2344 (range 120-26,983) µg/g and load 35.3 (1.9-906.8) µg/m^2. As can be observed there is a high spatial variability in Mn dust levels within the exposed community. The intra house variance was also too wide, probably due to the fact that we measured in three replicate, one of each was generally collected in a spot of difficult access, representing a testimonial of long last settling period. Figure 3a shows that median MnD levels have a similar gradient pattern similar to MnH levels; ANOVA analysis identified significant difference between the groups. The levels found here are in average 18 times higher than the Mn levels observed in the control area, which is only 7.5 km a part. Even though, dust samples were collected by a different technique, MnD concentrations observed in schools of two South Africa cities were 72.5±22.6 µg/g (Cape Town) and 404±342 µg/g (Johannesburg) (Rollin et al., 2005). The MnD levels observed in our reference area (129±117µg/g) fits well within the levels observed above for the South Africa cities. Several metals, including Mn, were measured across the city of Syracuse NY, USA by sweeping floors of kitchens. Mn concentrations varied 48 to 476 µg/g. The authors concluded that the relatively large variability in floor dust metal concentration over a small spatial scale suggests that the geographic distribution of metal sources is also

an important exposure factor (Johnson et al., 2005).

Blood Mn levels in Cotegipe children are in the majority in the normal range (4-14 µg/L) specified by the Agency for Toxic Substances Disease Registry (ATSDR, 2000). Only three children (3.1%) had MnB above this range. Despite that, MnB levels are approximately twice as much as the levels observed in children of similar age, ethnical and socio-economical background in the reference community. In a study with children of two South Africa cities MnB levels were 9.8±3.59 µg/L (Johannesburg) and 6.74±3.47 µg/L (Cape Town). This higher level in the former town was related to the use of methylcyclopentadienyl manganese tricarbonyl (MMT) as gasoline additive (Röllin et al., 2005). Wasserman et al., 2006 measured MnB (12.8±3.2) µg/Land other metals in blood (As and Pb) and urine (As, Cr) of children from Bangladesh. MnB levels were not correlated with Mn levels in the exposure sources (tube well water) nor associated with cognitive function endpoints. Blood Mn as a bioindicador was sensitive to detect a difference between exposed and not exposed groups, but not good enough to detect difference between levels of exposure.

We observed an excellent correlation between hair Mn levels in samples collected in two consecutive years and in general a significant decrease in MnH levels, even though more pronounced in girls. It is probably related to improvements in the emission rates after the plant introduced a major technology modernization, which has been perceived by the community, when they reported that the dust fall out had diminished considerably. However, MnH levels are in average six times higher than the levels observed in the reference community and than those in the general Brazilian population (0,25 -1,15 µg/g) (Miekeley et al., 1998). As in our previous report MnH levels as marker of exposure was able to identify a gradient of exposure, as can be seen in Figure 3c, which is in accordance with ANOVA analysis. The great majority of MnH levels (76.8%) are above the 3.0 µg/g, which is the upper limit of MnH concentration used by Bouchard et al., (2006). In that investigation they observed that all children of a small town in the province of Quebec, whose MnH levels were above this cutoff value had higher scores of hyperactive and oppositional behavior in classroom. MnH levels found in our study is approximately one order of magnitude higher that those observed in children

living near a waste site in the USA (mean 0.47, range 0.89 – 2.15 µg/L), which was found to be significantly related, inversely, to children's general intelligence scores, particularly verbal IQ scores (Wright et al., 2006).

In an individual basis we were not able to detect a significant correlation between dust Mn levels and Mn biological indicators (hair or blood). However, in a group basis house dust Mn levels has a similar trend of the Mn biological levels, particularly hair Mn concentrations. Another interesting associations identified here are the positive correlations of lead in hair with Mn levels in blood and in hair, and even significantly correlated with Mn levels in house dust. Montes et al., (2008) evaluated adults from a community living near a mining site in Mexico observed negative correlations between MnB and age (Sp. rho -0.164, P=0.007), and PbB (Sp. rho -0.119, p=0.037). In another study with workers of ferroalloy plant in China, Cowan et al., (2009) observed very significant ($p<0.01$), positive, partial correlations between MnH with Mn in saliva ($r=0.35$), erythrocytes ($r=0.26$), in urine ($r=0.29$) and they reported that the airborne Mn levels were significantly associated with Mn/Fe ratio (MIR) of erythrocytes (eMIR) ($r=0.77$, $p<0.01$). The authors, in other publication (Cowan et al., 2009b), showed that eMIR is negatively associated with several neurobehavioral alterations. To our knowledge no study has tried to investigate the correlation of Mn blood level with its levels in hair of children.

The major limitation of this study we credited to the environmental sampling technique we opted to apply. The settled house dust as a surrogate of environmental contamination is prone to several variables like the kind of house floor and sealing, frequency of cleaning, number of people in the dwelling, proximity to the road and so on. Another fact is that we did not keep track of the replicate sample that was collected in a place of difficult access, which would represent a long period of dust deposition. Some houses were so poor that we could not find a place like that, and some had floor made of dirt, but we did not find any association with his factor.

The major conclusion of this study is that environmental and biological parameters measured here show that Cotegipe children are highly exposed to the ferro-alloy plant emissions, even though it is suggestive of a decrease in Mn levels in biological samples, which needs to be confirmed in a follow-up study.

Acknowledgments

The authors would like to express their gratitude to the Cotegipe and Capiarara Village communities, especially to the children and their parents, who kindly participated in the study. We are also thankful to Fabricio Souza and Suzivânia Silva trainees of the Fundacentro, Bahia, who helped performing house dust sampling and gravimetric analysis. Menezes-Filho, JA has a scholarship from CAPES (Brazil) and is an Irving J. Selikoff International Scholar of the Mount Sinai School of Medicine. JCM would like to thank CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico). This work was supported in part by Award Number D43TW00640 from the Fogarty International Center. The content is solely the responsibility of the authors and does not necessarily represent the official views of the Fogarty International Center or the National Institutes of Health

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Annexes

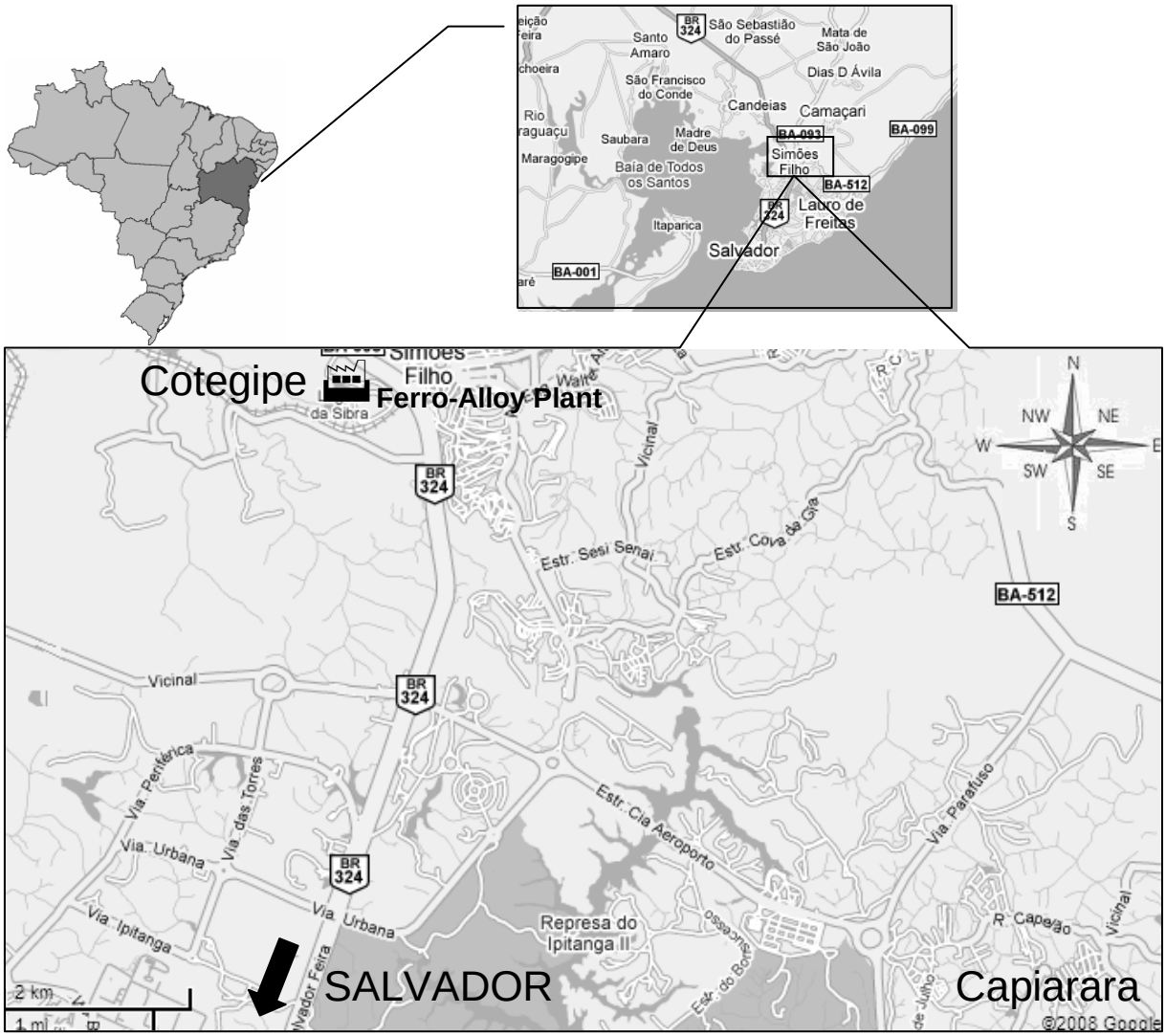

Figure 1. Schematic map of the Cotegipe Village (exposed community) in Simões Filho town and reference community (Capiarara, Lauro de Freitas), in the Metropolitan Area of Salvador, Bahia, Brazil.

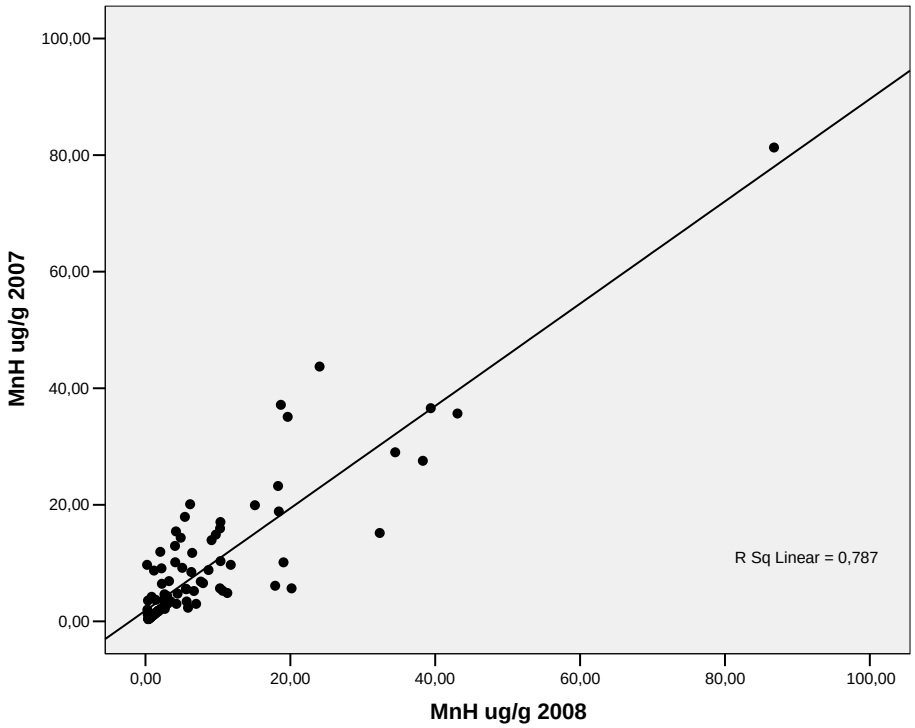

Figure 2. Correlation of MnH levels sampled in two consecutive years.

Table 1. Summary of socio-demographic characteristics and important biological markers of exposed and referent children.

	Exposed n=83					Reference n=26					P value
Gender											
Boys	43 (51.8%)					13 (50%)					n.s.
Ethnicity											
African-Brazilian	61 (72.7%)					21 (80.0%)					n.s.
Non African-Brazilian	22 (27.3%)					5 (20.0%)					n.s
	Mean	**Median**	**SD**	**Min.**	**Max.**	**Mean**	**Median**	**SD**	**Min.**	**Max.**	
Age (months)	108.0	108.1	20.9	73.1	150.0	104.5	105.6	16.8	72.8	132.8	n.s.
FeS (μg/dL)	106.6	107.0	30.9	54.0	166.0	62.1	55.5	16.4	39.0	98.0	<.001
PbB (μg/dL)	2.0	1.7	1.5	0.5	6.7	1.8	2.1	1.1	0.5	4.3	n.s
PbH (μg/g)	2.28	1.19	3.67	0.10	24.23	2.26	1.93	1.58	0.53	6.35	n.s

n.s: not significant for Chi-square or Student´s t tests.

Table 2. Mn levels in house dust and biological markers in children living in the vicinity of the alloy plant and in those from the reference community.

Exposure Level		MnD (µg/g)	MnL (µg/m^2)	MnB (µg/L)	MnH (µg/g)
High	N	8	8	9	10
	GM	2,643	34.0	7.22	24.82*
	SD	8,819	323.1	3.73	22.84
	Median	3,333	48.9	8.54	25.03
	Minimum	237	1.9	2.96	8.71
	Maximum	26,983	906.6	13.41	86.78
Moderate	N	45	45	62	72
	GM	2,295	37.2	7.99	4.77*
	SD	4,714	220.2	3.32	6.79
	Median	2,124	31.0	7.83	6.02
	Minimum	120	2.3	4.23	0.10
	Maximum	19,494	869.3	23.38	39.39
Reference	N	9	9	26	26
	GM	129*	0.4*	4.78*	1.09*
	SD	117	0.8	2.43	1.09
	Median	96	0.5	4.53	1.09
	Minimum	54	0.1	2.32	0.39
	Maximum	423	2.7	11.68	5.58

* ANOVA (Dunnets) $p<0.05$, N = house units or number of children where applicable.

Table 3. Spearman`s correlation coefficients of environmental and biological Mn indices and other important co-variables in the exposed children (Rho coefficient, p-value and n, respectively).

	Age	FeS	PbH	PbB	MnH 2007	MnH 2008	MnB	MnD	MnL
Age	1.000	.098	.021	.191	-.021	.073	.044	.145	.079
		.478	.872	.162	.830	.577	.745	.302	.573
		55	61	55	109	61	56	53	53
FeS		1.000	.057	-.023	.013	-.105	-.087	.140	.333
			.642	.848	.924	.392	.472	.536	.130
			69	69	55	69	70	22	22
PbH			1.000	.306*	.340**	.371**	.246*	.171	.429*
				.011	.007	.001	.040	.404	.029
				69	61	81	70	26	26
PbB				1.000	.145	.278*	.060	.196	.145
					.289	.021	.623	.394	.530
					55	69	70	21	21
MnH - 2007					1.000	.643**	.006	.075	-.061
						.000	.965	.595	.663
						61	56	53	53
MnH -2008						1.000	-.013	.106	.184
							.912	.605	.369
							70	26	26
MnB							1.000	-.302	-.075
								.172	.740
								22	22
MnD)								1.000	.516**
									.000
									53
MnL									1.000

* Correlation is significant at the 0.05 level (2-tailed), ** Correlation is significant at the 0.01 level (2-tailed).

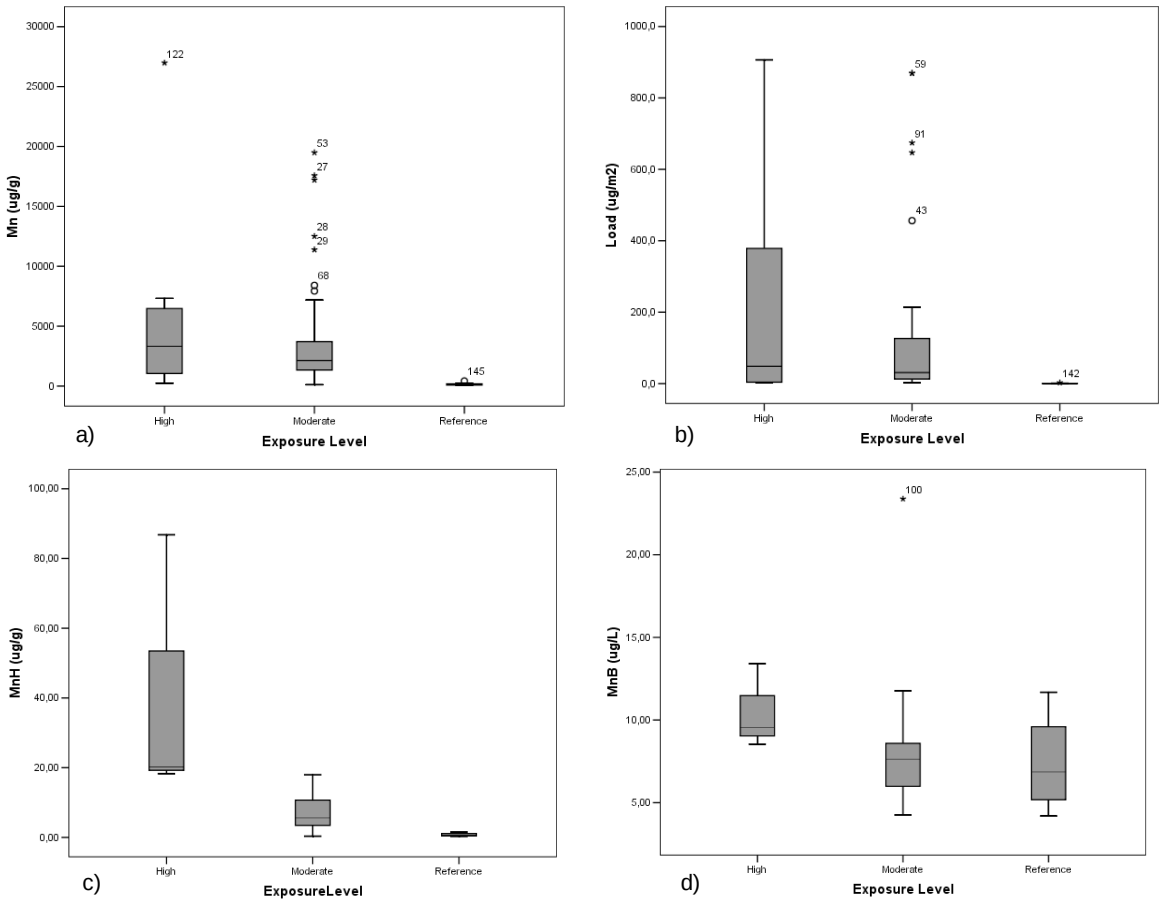

Figure 3. Box plots of Mn levels in settled house dust in concentration (a) and in load (b) according to exposure areas. Figures 3c and 3d are box plots of Mn levels in hair and in blood showing the same pattern of distribution according to exposure areas.

Artigo 4

Elevated manganese affects mothers' and children's cognition*

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Abstract

Background: It has been evidenced that environmental exposure to manganese (Mn) is associated with neurobehavioral impairments. Populational and experimental studies with rodents and non-human primates have demonstrated that high Mn exposure causes brain accumulation and decrements in cognitive, attention and motor functions.
Objectives: Our goals were to investigate the association between airborne Mn exposure and effects on children's intellectual function, and describe the risk factors associated with it.
Methods: A cross-sectional evaluation of Mn exposure (blood and hair levels) along with blood lead levels (BLL), and cognitive performance (Intelligence Quotient, IQ) were determined for 83 children aged 6–11 years and 11 months. Maternal exposure and cognition were also measured.
Results: The mean blood and hair Mn concentrations were 8.2 µg/L (range = 2.7 - 23.4) and 5.83 µg/g (range = 0.1 - 86.68 µg/g), respectively. BLL presented a mean of 1.43 µg/dL (range = 0.2 - 10.35 µg/dL). Maternal MnH levels had mean of 3.50 µg/g (range = 0.10 - 77.45), which were significantly correlated (rho = 0.294, p = 0.010) with children's MnH levels. Children's MnH concentrations were significantly, negatively, related with Full-Scale and Verbal IQ. After adjusting for maternal education, nutritional status the unadjusted coefficients for Mn were β = -5.782, p = 0.033 and β = -6.723, p = 0.019, FS and Verbal IQs, respectively. MnH levels explained 5.6% and 6.8 % of the variances

of the respective IQ scores. Maternal MnH levels were negatively associated with their intellectual performances (β = -2.689, p=0.055), after adjusting for education years, family income and age (r^2 = 43.2% and partial Mn r^2 = 5.6%). *Conclusions*: The present study confirms that high MnH levels in children are associated with cognitive deficits, especially in the verbal domain. Additionally, we also demonstrate that maternal intellectual function is equally affected. We thus hypothesize that these children's cognition may have been doubly affected.

Key-words: Manganese, intelligence, children, neurobehavioral, alloy plant.

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Introduction

There is growing interest in environmental manganese exposure in children. Recent studies suggest that excess Mn may interfere with developing brain functions. In studies in Bangladesh, Wasserman and associates (2004, 2006) observed a negative effect of high Mn levels in tube well water on children's cognitive capacities. In Quebec, Bouchard et al., (2007) reported a significant association between hair manganese (MnH) levels and hyperactive and oppositional behavior in children exposed to Mn through municipal well water. Decrements in IQ scores in Korean children were associated with elevated blood Mn levels in a population-based study, but Mn sources were not identified (Kim et al., 2009). Zoni et al., (2007), who reviewed the recent studies on manganese exposure in adults and children, suggest that in children cognitive functions may be particularly vulnerable to manganese.

The developing nervous system has been shown to be a prime target for the disrupting effects of toxic chemicals (Landrigan et al., 2005; Weiss and Landrigan, 2000; Rice and Barone 2000; Bellinger 2009). Levels of exposure that produce few, or no, obvious effects on the mature nervous system, can pose a serious risk to the developing nervous system (Faustman et al., 2000). There are numerous periods of susceptibility since CNS development begins during the embryonic period and continues during the fetal period and post-natally (Barone et al., 2000). Cord blood Mn was negatively associated with non-verbal scales (attention, non-verbal memory) and boys' manual ability at 3 years, after adjusting mother's educational level in a birth cohort study carried out in France (Takser et al., 2003). Mn levels in the enamel of shedding teeth, tissue formed during the intra-uterine phase, were significantly associated with

disinhibitory behavior evaluated at 36 and 54 months of development (Ericson et al., 2007).

Among the many factors that influence children's neurodevelopment, mothers' education and/or IQ have consistently been shown to be a major determinant (To et al., 2004; Takser et al., 2003; Wright et al., 2006; Wasserman et al., 2006). In an exposure situation, parents may also be exposed and suffer some of the toxic effects of the polluting agent (Bellinger, 2009). In a non-occupational population living in the vicinity of a ferro- and silico- manganese alloy plant, Mergler et al., (1999) reported a decrease in memory and learning, as well as poor performance on motor tests among persons with elevated blood Mn levels. Two studies were carried out with communities exposed to dust from Mn mines and transformation plants in Mexico (Santos-Burgoa et al., 2001; Sollis et al., 2009). In a pilot study, Santos-Burgoa et al., (2001) detected an increased risk of deficient cognitive performance in persons with elevated MnB levels, while Sollis et al (2009) reported an association between air Mn concentrations, but not MnB levels, and attention impairments. Bellinger in his studies on lead exposure raises the issue of a possible double toxic effect from lead-related reduced mothers' IQ that further contributes to reduced IQ in children exposed to lead.

Children's exposure has been investigated primarily with respect to ingested Mn through diet: baby formulas (Collip et al., 1983), water (He et al., 1994; Zhang et al., 1995; Wasserman et al., 2006 and Bouchard et al., 2007) and for childen with elevated Mn from total parenteral nutirtition (Alves et al., 1997). We recently reported elevated hair Mn in children living in the vicinity of a ferro-manganese alloy plant (Menezes-Filho et al., 2009). There was no

evidence of waterborne Mn and MnH concentrations were significantly associated to the distance and position of their houses relative to the wind direction. Mean air Mn concentration in the respirable fraction ($PM_{2.5}$) sampled during the raining season in the center of the community was, on average, three times higher than the US EPA reference concentration (RfC 0.05 µg/m^3) (US EPA, 1993).

The objectives of the present study were to investigate the associations between (i) biomarkers of Mn exposure (hair and blood) and neurobehavioral performance in children living in the vicinity of a ferro-maganese alloy plant; (ii) mothers' hair Mn concentrations and performance on a test of intelligence.

Material and Methods

Exposure context

Manganese exposure of children from the Cotegipe village, a small community of 620 people in the municipality of Simões Filho, 30 km from the city of Salvador, State of Bahia, Brazil (Figure 1) was characterized in 2007 (Menezes-Filho et al., 2009). The community resides within a 2-km radius from a ferro-manganese alloy plant and mostly in a downwind direction. The plant was inaugurated in 1970 and after two expansions, three ovens are currently in operation, with an annual production of SiMn and FeMn alloys of 280,000 tons. In 1999 it was taken over by a major multinational Brazilian mining and metallurgical company, with branches all over the world; annual production is around 2.3 million tons of manganese ore and 500,000 tons of manganese alloys (Vale, 2009).

For the children living in this area, there is a gradient of exposure in relation to the child's house distance and position with respect to the the plant (Menezes-Filio et al, 2009).

Study design

A cross-sectional study design was used. Children in the age range of 6 years to 11 years and 11 months attending the Cotegipe Elementary School, who had lived in the community for at least one year, were invited to participate in the present study. The school principal provided us an updated list of all children enrolled and regularly attending the classes, a total of 110 children. We sent out invitations to the mothers or legal guardians of all children in the specified age range (N=80). Five other children who lived in a street separated

from the village core, on the northwest side and downwind of the plant were also included. They attended an elementary school on the boundaries of Simões Filho town. Despite the fact that they attended a different school, they were included in the study group because our pervious study had shown that the children from this area presented the highest hair Mn concentrations. A total of 85 children were enrolled in the study, but two children were excluded from the study group for medical reasons: one boy had a history of seizure and one girl had hearing problems and used a hearing aid. The final study group was comprised of 83 children.

Parents who had previously accepted to participate in the first phase of the project has already signed the informed consent forms. Parents of those children who did not participate in the first evaluation were informed about the objectives of the study and provided written consent.

The Simões Filho town education department gave us permission to use the school premises to set up the study base and the school principal gave us full support and provided two rooms where we carried out the assessments activities. The present investigation has been aproved to the Federal University of Bahia ethical committee.

Questionnaires: A team of three psychology students, with clinical and psycho-diagnostic experience, were trained and monitored by a leading psychologist (C.O.N.) to administer interviews to the mothers on socio-economic characteristics, family structure, child development, behavior and illness. A second questionnaire, translated and adapted from the HOME Inventory (Home Observation for Measurement of the Environment Scale, NLSY79 Child HOME-SF), comprised of 20 items was used to assess the

quality of family environment. It included indicators of cognitive stimulation, parent-child interaction and general interpersonal interactions. A simple score was derived by summing the number of positive answers obtained for the twenty questions, with values ranging from 0 to 14. The score was transformed into a percent scale.

Anthropometry: A single person performed all weight and height measurements. Children took off their shoes but kept their clothes for both weight and height assessments. Weight was measured using an upright scale (CATSYS 2000 System®, Snerkkersten, Denmark) connected to a computer, with a capacity to weigh 150 kg in 100-g increments. Height was taken using a measuring board. Body mass index (BMI) was calculated by dividing the weight in kilograms by the square of the height in meters. Height-for-age (HA) z-score was calculated using the AnthroPlus software (WHO, 2009) based on the WHO reference 2007 for 5-19 years,

Blood measurements. Venous blood samples were collected from the cubital vein of 70 participating children into sodium–EDTA vacuum tubes proper for metal analysis (Vacutainer, Bencton & Dickson, USA). Thirteen children (15.7%) refused or were not available to provide blood samples. We adapted the method described by Montes et al., (2002). Blood sample was diluted 1:5 with matrix modifier (1% ammonium-dihydrogenphosphate in 0.1% Triton X-100 solution). After homogenization in a vortex, it was centrifuged for 10 minutes at 14,000 rpm. Quality control of blood Mn analysis was assured by measuring human blood reference materials QMEQAS07B-03 and QMEQAS07B-06 (Centre de Toxicologie/INSPQ, Canada). Samples were measured in duplicate; every measurement consisted of two injections into graphite furnace, in all

cases standard deviation was lower than 10%; if otherwise, sample was reanalyzed.

As lead is an ubiquitous contaminant and a recognized neurotoxin, associated with effect on cognition and behavior in children at low blood lead levels (BLL), it was also measured by electro thermal atomic absorption spectroscopy with Zeeman background correction (GTA-120, Varian Inc.). An additional blood sample, collected with a no additive vacuum tube (Vacuntainer, BD) was obtained for serum iron determination. After blood clotting, tubes were centrifuged 9000 rpm for twenty minutes. Serum iron was determined by automated method using a commercial kit (Roche Hitachi 747, Roche®).

Hair measurements. Children and mothers and caregivers were invited to provide hair samples. A tuft of hair of approximate 0.5 cm diameter was cut off with a surgical stainless steel scissor as close as possible to the scalp in the occipital region, after tying with a Teflon string at the proximal end. For boys with short hair (less than 2 cm in length), an equivalent amount was trimmed directly into the sterile sampling plastic bag. The mother's hair sample was taken from the same region. We noted hair treatments: dying, perms and ironing. We followed the procedure described by Wright et al*.,* (2006) to clean up hair. Human hair reference material from the International Atomic Energy Agency (IAEA-085) was analyzed along with hair samples for quality control purpose. Detailed information on hair sample treatment and analysis can be found in Menezes-Filho et al., (2009).

Mothers or caregivers of seventy-seven children were willing to provide hair samples. One mother refused for religious reasons, three mothers of four other children refused for personal reasons and a father of one child had hair

too short to be sampled.

Manganese Determination. In every sample, Mn was analyzed by electrothermal atomic absorption spectroscopy with Zeeman background correction (GTA-120, Varian Inc.). The furnace temperatures were optimized using SRM procedure, which indicated the ashing and atomization temperatures of 700ºC e 2400ºC, respectively. The analyses were performed with the wave length 279.5 nm, slit width of 0,2 nm. Calibration was accomplished using the auto-mix procedure with standard Mn solution in 0.2% nitric acid made freshly from a stock solution 1 mg/mL (AccuStandard, New Haven, USA, traceable to NIST). All glassware and plasticware were thoroughly decontaminated by soaking for 24 hours in 3% neutral detergent (Extran®, Merck), followed by soaking overnight in 10% HNO_3 and finally rinsed with Type I pure water (Milli-Q, Millipore). Reagent blanks were analyzed along with samples in every batch. The detection limit was 0.1 µg/L. Routine checks of accuracy and precision were accomplished using specific reference material samples described above.

Psychological Measures

Children's cognition: The Wechsler Intelligence Scale for Children, version III - WISC-III (Wechsler, 1991), previously validated for Brazilian children (Figueiredo 2002) and suitable for children ≥ 6 years of age was administered by the same psychologist (C.O.N.) over a period of four weeks in July 2008. The Verbal IQ was generated with the recommended five subtests [Information (INF), Similarities (SIM), Arithmetic (ARM), Vocabulary (VOC) and Comprehension (COP)] by the WISC-III manual. Additionally, the Digit Span

(DSP) test was applied. The five nonverbal subtests [Figure completion (FCP), Coding (COD), Figure arrangements (FAG), Cubes (CUB) and Object assembling (OAB)] were used to obtain the Performance IQ score. The supplementary subtests: Search for Symbols (SFS) and Mazes (MAZ) were applied for the factorial analysis. We also determined one of the optional factorial scores, the Comprehension IQ score, comprised of the verbal subtests: INF, SIM, VOC and COM. Testing followed the procedure recommended in the WISC manual. The location was as quiet and isolated environment as possible, given the poor quality of the school building. Total testing time ranged between 50 to 80 minutes. The tester was unaware of children's degree of Mn exposure.

Maternal Cognition: Raven's Standard Progressive Matrices (Raven et al., 1983) was used to assess mother's or caregiver's intelligence. This instrument is free of cultural influences and has been validated for the Brazilian population. It measures general intelligence and reasoning ability. It comprises 60 nonverbal items, divided into five series of 12 items of increasing degree of difficulty. The test was applied individually to each mother or caregiver following the procedure described in the manual. It was abbreviated between series if guessing was detected to be too frequent.

Statistical Analyses

Each child was coded with respect to area of residence and house number. Descriptive statistics were used to determine the distribution of socio-demographic information, bioindicators of manganese and lead exposure and cognitive function parameters. Frequency, as total number (N) and percent (%) were used to describe the categorical variables. The continuous variables were

presented as arithmetic mean (AM), if normally distributed otherwise geometric mean (GM), standard deviation (SD), minimal and maximum,

Pearson or Spearman's correlation tests were applied to evaluate bivariate correlation between co-variables and exposure bioindicators.

Since the distributions of hair and blood metal levels were skewed, data were log10 transformed for further analyses. Backward stepwise regression models were used to identify variables that were potentially associated with intellectual quotients (0.100 to enter; >0.05 to exclude). These variables were then included in linear regression models. A significance level (p=0.05) was used and residual analysis performed to verify model's parameters. Blood lead level was dichotomized into ≤2 µg/dL (low BLL) and >2 µg/dL (high BLL), according to the action level proposed by Gilbert and Weiss (2006) and an interaction term [logMnH*BLL(>2 µg/dL)] was created in order to test for interaction of Mn and Pb effect on cognition. Residuals from the models were assessed in standardized residual vs predicted plot for heterocedasticity and non-linearity and in a half-normal plot for non-normality. All statistical analyses were performed using SPSS version 13 software.

Results

Sample Characteristics

Table 1 presents a summary description of the demographic and anthropometric characteristics of the study subjects. Parents or legal guardians of the 83 children who provided information, 94% were mothers, one was a father (1.2%), one was grandmother (1.2%) and three were stepmothers

(3.6%). Approximately half of the children lived in a structured family with mother and father together. The ethnical composition is representative of the population around the All Saints Bay area, which is comprised of 80% of Brazilian-Africans who have black curly hair. The socio-economic status is very low; the main income source is from cultivating cassava and rudimental processing and commercialization of manioc flour. The average monthly income is U$168, ranging from 25 to 444 US dollars. Families who maintain children at school receive social benefits as a monthly bonus. This low SES is reflected in the low nutritional status, the mean age-for-height z-score, which reflects chronic malnutrition, was -0.16 (range -2.39 to 2.54). Four boys and two girls (7.3%) could be classified as suffering from stunted growth, their HA z-scores were below -2.0 (WHO, 2006). FeS level (N=58) was normally distributed with mean 65.6 µg/dL (range 11-165 µg/dL), 41.4% of the children had FeS below the normal range (55-120 µg/dL) and could be classified as iron deficient (Takemoto et al., 2004). Those iron deficient children had mean HA z-score of 0.13 versus -0.37 in children of normal FeS, even though this difference was not statistically significant (p=0.060).

Exposure Characteristics

Table 2 presents the descriptive statistics of metal exposure indices. MnH levels varied from 0.1 µg/g to 86.68 µg/g, with GM of 5.83 µg/g. A large proportion (77.1%) was above 3.0 µg/g, the upper cutoff limit that had been associated with hyperactive behavior (Bouchard et al., 2007). Age was not significantly correlated with MnH levels.

Blood manganese levels were in general in the normal range: 4-14 µg/L (ATSDR, 2000). Of those, 96% were below 14 µg/L. Mn blood concentration

was normally distributed with a mean of 8.2 µg/L (2.7-23.4 µg/L). No significant correlation was observed between MnH and MnB levels. No statistical difference in the means of MnB levels was observed between children with iron deficiency (8.7±4.52 µg/L) versus normal FeS level children (7.9±3.24 µg/L). On the other hand, MnH level means were 15.94±19.68 µg/g and 8.69±8.23 µg/g, respectively. This difference presented a borderline statistical significance (p=0.059).

BLL had a geometric mean of 1.43 µg/dL (N= 70, ranging 0.5 to 10.35 µg/dL); only one child had PbB slightly above 10 µg/dL (CDC, 1991).

As observed with children's MnH levels, mother's levels were similarly high and were not normally distributed, geometric mean was 3.50 µg/g (range 0.1-77.45 µg/g).

Table 3 presents the correlations between biomarkers. No relation was observed between MnB and MnH, BLL and FeS. Mother's and/or care-givers' Mn hair levels were correlated with children's MnH levels (Spearman's rho=0.294, p=0.010).

Children's intellectual function

Data in table 4 shows the summary of the Wechsler Scale score. The children's mean Full-Scale IQ was 85.5 (SD = 13.5, range = 50–121), the mean Verbal IQ was 89.7 (SD=14.2, range 55–127), and the mean Performance IQ was 81.5 (SD=13.3, range = 45–117). Maternal education years was significantly correlated with children's Full-Scale IQ, Pearson correlation coefficients r=0.300, p=0.006 and Performance IQ, r=0.364, p=0.001, but not with Verbal IQ. Maternal intelligence directly measured by Raven score

presented significant correlation with Full-Scale, Performance and Verbal IQ's; r=0.311 p=0.004, r=0.341 p=0.002 and r=0.221 p=0.044, respectively. Height-for-age z-score was positively correlated with Verbal IQ r=0.239, p=0.030 and a tendency with Full-Scale IQ r=0.209, p=0.068. Family income was also positively correlated with Full-Scale IQ (r=0.232, p=0.045) and Performance IQ (r=0.229, p=0.044). The mean Comprehension IQ score was 91.3 (SD=14.4, range 59-128). The Comprehension IQ was a slightly correlated with maternal education years (r=0.193) but not significantly (p=0.080). None of the IQ scores were significantly related to the HOME inventory score.

Manganese exposure indices and children's IQ

In bivariate analyses MnB or MnH levels were not statistically significant correlated with any of the children's IQ scores. In multiple regression analyses, MnB did not enter significantly into any of the models that were tested. On the other hand, negative relations were observed with MnH levels. A model for each IQ subscale (Table 5) was run adjusting for maternal education and height-for-age z-score showing a significant inverse association between MnH and Full-Scale (p=0.033), Verbal (p=0,019) and Factorial Comprehension (p=0,013) IQ. Residual Full-Scale, Verbal and Comprehension IQ scores versus Mn hair levels, adjusted for the aforementioned co-variables are displayed in Figures 2a, 2b and 2c, respectively. The regression models show that Mn levels in hair explained 5.6%, 6.8% and 7.5%, accordingly, of the variability in children's intelligence scores independently of maternal education and their nutritional status. No interaction or confounding effects were found to be significant with BLL and low serum iron levels.

Mn effect on maternal Intelligence

The mean mother's IQ raw score was 15.9 (SD=9.1, range 5-46). It was significantly positively correlated with years of formal education (Pearson Coefficient r=0.540, p<0.001), family income (r=0.378, p=0.001) and the quality of family environment, measured by the HOME inventory scale (r=0.245, p=0.026). On the other hand, it was significantly, negatively correlated with age (r=-0.358, p=0.001) and with the log of maternal MnH levels (rho=-0.288, p=0.011).

The summary of the multiple linear regression analysis is presented in Table 6. The model for the association between maternal cognition and MnH levels adjusted for education years, family income and age explained 43.2% of the variance in mother's cognition. The model showed that MnH was negatively associated with maternal cognition deficit (β coefficient -2.689,), marginally significant (p=0.055). The partial correlations for education years, age, logMnH and family income were 20.6%, 3.6%, 5.6% and 9.3%, respectively. Figure 3 shows the scatter plot of the residualized maternal intelligence scores versus Mn hair levels, adjusted for maternal education, family income and age.

Discussion

Findings of this study showed that for these children living in the vicinity of a manganese alloy production plant, Full-Scale IQ, and especially Verbal IQ, were negatively associated with hair Mn concentrations, which vary with distance from the plant. The children may be doubly affected, since manganese exposure was also negatively associated with maternal intelligence, when taking into account age, education and family income.

Children's MnB levels were in the majority in the normal range (4-14 µg/L) specified by the Agency for Toxic Substances Disease Registry (ATSDR, 2000). Only three children (3.1%) had MnB above this range. Despite that, MnB levels were approximately twice as high as the levels observed in children of similar age, ethnical and socio-economical background living 7 km away in upwind direction from the same alloy plant (Unpublished data). The MnB concentrations observed are lower than those reported by Wasserman et al., 2006 Mn (12.8±3.2 µg Mn/L) for children exposed to Mn in well water in Bangladesh.

The MnH concentrations observed here are considerably higher than those reported in other studies; 76.8% were above the 3 µg/g cut-off that was used by Bouchard et al (2007) in their study of children exposed to Mn through well water; MnH in that study ranged from 0.28 µg/g – 20.0 µg/g. MnH in the present study are, on average, six times higher than the levels reported for the general Brazilian population (0,25 -1,15 µg/g) (Miekeley et al., 1998). They are also considerably higher than those observed in children living near a waste site in the USA (mean 0.47 µg/g, range 0.89 – 2.15 µg/g).

In the present study MnH and not MnB predicted the IQ deficits. Wasserman et al (2006) likewise did not observe an association between MnB and cognitive function; in their study, poorer performance was associated with Mn content in well water. Wright et al., 2006 reported decrements in verbal functions associated with MnH concentrations in children living near a toxic waste site and Bouchard et al., (2007) reported a positive relation between hair Mn and behavioral problems in children exposed to Mn through drinking water. In the present study, the two bioindicators of MnB and MnH were not correlated, which raises the question of what each represents in terms of Mn toxicity. No

relation was observed between MnB and well water in the study performed by Wasserman et al., (2006) although Bouchard et al., (2007) reported that children whose water supply contained more Mn presented higher concentrations of MnH; MnB was not assessed in that study. MnB may be subjected to greater homeostatic control, while MnH may represent excess Mn. Toxicokinetic studies on Mn are required to elucidate these findings.

Our results show that the elevated Mn exposure is significantly and inversely associated with intellectual function, more pronounced with the verbal domain as observed by Wasserman et al., (2006), Wright et al., (2006) and Kim et al, (2009). The verbal IQ reflects the crystallized intelligence. Crystallized intelligence is related to general knowledge, it demonstrates the extension and depth of information acquired normally through school. Generally it is used to solve problems similar to those experienced in the past. (Primi et al., 2001). Cognitive deficits have been reported in adults exposed to airborne Mn (for review see Zoni et al, 2007), but it is only recently that the mechanisms of action have been elucidated. In non-human primates exposed to Mn by intravenous injection and inhalation. Schneider et al., (2006) observed that chronic manganese exposure was associated with cognitive deficits such as impaired spatial working memory and behavioral alterations consisting of compulsive-like behaviors. Burton and Guilarte (2009), in a review article of their non-human primate studies suggested that changes in gene expression (i.e. tumor suppressor p53, amyloid beta precursor-like protein (1APLP1)) and markers of neurodegeneration in the frontal cortex (i.e. copper homeostasis dysregulation and extracellular accumulation of toxic peptide beta amyloid (Aβ)) may explain subtle cognitive deficits and other early manifestations of Mn

neurotoxicity in humans related to working memory and neuropsychiatric behaviors.

Children's development is strongly influenced both by their families and by the social forces and cultural values in the society they live. Early maternal as well as paternal influences are crucial in children's development (Barros et al., 2009). In our study, we estimated maternal intelligence directly (Raven progressive matrix score) and by a proxy variable (education years). Both variables were significantly, positively, correlated with child's cognition outcomes. The quality of family environment was estimated by an adapted version HOME inventory, which was correlated with maternal education, but was not significantly associated with the IQ scores. Maternal education was an important covariable in the association of children's exposure to Mn and neuro-psychological outcomes in studies in France (Takser et al., 2003), in the USA (Wright et al., 2006) and in Bangladesh (Wassermann et al., 2006). In a study with children from the outskirt of Salvador, Brazil, Andrade et al., (2005) observed that the better the quality of stimulation in the family, the better the child's cognitive performance. They pointed out that maternal years of schooling had a positive association with the quality of stimulation of the child in the family.

The association between Mn levels and cognitive function was markedly affected by the nutritional status of the child. We observed that the chronic nutritional index could explain a significant amount of child's Verbal IQ variance (6.6%). This index encompasses growth and stature, which is inversely related to protein, calcium and iron deficiency during early childhood (Abrams et al., 2005). Malnutrition is a well recognized risk factor for intellectual deficit. For

example, Niehaus et al., (2002) observed that height-for-weight z-score was positively correlated with the TONI-III (Test of Non-verbal Intelligence) score; Wasserman et al., (2006) reported that anthropometric parameters, such as stature and head circumference that reflect nutritional status, explained 17.7% of the Verbal IQ in Mn exposed children. Fonseca et al., (2008) also observed significant correlations between height-for-age z-scores and several WISC-III subtests in Amazonian children exposed to methylmercury. The association between height and cognitive outcomes was also observed in a study with Pb exposed Mexican children. The authors commented that the association of height-for-age z-score with cognitive performance might be explained to some extent by parent and teacher perceptions of ability in taller children (Kordas et al., 2004).

Women environmental exposure to Mn in reproductive age has been investigated by few groups. It has been shown that Mn blood levels physiologically increases during gestational period independent of iron status, peaking in the third trimester (Tholin et al., 1995), High MnB levels at delivery were also observed by Takser et al., (2003) and Ljung et al., (2009). Mergler et al., (1999) in a community based study in Quebec, Canada, observed that higher MnB levels (>7.5 µg/L) were significantly associated with poorer learning and memory in women and men. In Mexico, a pilot study in a community exposed to high air levels of Mn due to a mining activity, it was observed a significant risk increasing of deficient cognitive performance of 12 times (Mini-mental test score <17), where the majority (86%) of the subjects were women (Santos-Burgoa et al, 2001). Another recent study in the same region observed

significant association between elevated Mn air levels and attention deficit; it was not observed a gender difference (Sollis et al., 2009).

This investigation is the first to demonstrate not only children's intellectual impairment, but also that mothers' cognition is being affected. In the reviewed literature of children cognition and Mn exposure (Menezes-Filho et al., 2009) at least three studies attempted to have a measurement of maternal cognition, but none had also measured maternal exposure. Our data show that MnH levels accounted for 5.6% in the variance of Raven score and the beta coefficient of -2.689, meaning that independently of education years, age and family income, each 2 µg/g increase in MnH levels would translate into a 0.81 loss in the intelligence score. About 40% of the mothers lived in the community since they were children and the alloy plant has been emitting high levels of Mn in the air for almost four decades. Our results may suggest that children from Cotegipe village are being doubly affected. Directly, due to Mn effects on their own brain and indirectly, as consequence of Mn effect on their mother's cognition, resulting in a diminished ability to provide better stimulating environment. The implications of small shifts in intelligence quotient score and a slightly increased tendency to aggression are not so easily conveyed or grasped as a picture of deformed limbs, even though the importance of such changes is becoming more evident (Weiss and Landrigan, 2000).

Walker et al., (2007) reviewed the proximal risk factors for child development in the developing countries. Among the most consistent in the literature, inadequate cognitive stimulation was the most important psychosocial determinant along with maternal depression and exposure to violence. The authors also emphasized the other risk factors for impaired child development

were biological (stunting, iodine and iron deficiencies, malaria, intrauterine growth retardation) and exposure to metals The community in Cotegipe village is of vey low socio-economic status and needy of attention for sanitary and education interventions. There is a high unemployment index, problems of alcoholism, which are related with violence, common in communities in the outskirts of metropolitan area in developing countries. In addition to these social factors, children are obliged to live in such polluted environment.

Despite the fact that this study bears the limitation of a cross-sectional study and causal inferences cannot be made on the relationship of Mn exposure and cognitive deficits, this investigation has an important strength: we were able to test all of the children in the desired age range attending the only local elementary school, thus avoiding selection bias. Another limitation is that the HOME inventory adaptation had not been validated previously. The quality of the home environment was not associated with the IQ scores. However, it was significantly correlated with maternal intelligence.

Some measures are urgent for the well being of that community: sewage and waste water collection in order to avoid dumping into the estuary of Aratu bay, a mangrove ecosystem; systematic garbage collection for the entire community and environmental education campaign to prevent incineration of domestic waste. Previously, we have demonstrated that children from families that used to incinerate garbage had higher BLL (Data to be published). Better implementation of the Brazilian health program in Cotegipe village (PSF – Family Health Program) in order to prevent malnutrition, intestinal parasitosis and anemia, which prevail in 18.8% of the children (Menezes-Filho et al., 2009).

In the face of the evidences presented here that maternal and children's cognitive functions are being affected by Mn exposure, we strongly recommend cognitive strategy interventions. These interventions are designed to improve performance through compensatory procedures or through more efficient functioning of weak or deficient processes (Morris and Mather, 2008). An intervention program with mothers in the Northeastern, Brazil, observed positive association in cognitive and motor development in children whose mothers were included in the intervention group (Eickmann et al., 2003). Barros et al., (2009) demonstrated a clear interaction between stimulation and maternal schooling, indicating not only that stimulation has a stronger effect in children of less-educated mothers, but also that by effectively stimulating these children they can achieve a very similar result to those who are more stimulated and have high schooling mothers.

We also suggest that a more comprehensive investigation should be carried out including the whole population of Simões Filho town, which urban area lies inside the 3 km radius from the alloy plant, We propose that this study have a prospective design in which mothers and their children be followed up since the first pre-natal examination. In this way, it would be possible to study Mn maternal-fetus transfer and generate irrefutable data on the relationship between environmental Mn exposure and neuro-behavioral effects on children.

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Annexes

Table 1. Characteristics of study participants.

	N	Mean	SD	Min	Max
Age (months)	83	106.1	19.8	74	147
Height (cm)	83	130.2	10.8	108	155
Weight (Kg)	83	26.8	6.9	16.2	49.9
BMI (kg/m^2)	83	15.6	2.0	11.8	22.9
HA z-score	83	-0.16	1.21	-2.39	2.54
Boys	44 (53.0%)				
Ethnicity					
African-Brazilian	62 (78.1%)				
Non African-Brazilian	21 (21.9%)				
Parents living together	47 (56.6%)				
Years of maternal education	82	6.9	4.0	0	14
Maternal age at birth	82	24.5	6.4	14.3	46.1
Mother's Raven score	82	15.9	9.1	5	46
Family income (U$/month)	75	168	103	21	444
HOME Inventory (%)	82	51.8	15.2	6.7	80
Number of children in the home	82	3.6	2.3	1	15
Fe Serum	58	65.7	31.8	11	164

U$ exchange rate=1.868 BRR (Brazilian Reais) on Aug.02.2009

Table 2. Bioindicators of exposure in children and mothers

Exposure Indices	N	Mean	SD	Min	Max
Mn hair (µg/g) GM	83	5.83	11.5	0.10	86.68
Mn blood (µg/L) AM	70	8.2	3.6	2.7	23.4
BLL (µg/dL) GM	70	1.43	1.90	0.5	10.35
Mother's MnH (µg/g) GM	77	3.50	12.76	0.10	77.45

Table 3. Spearman's rho correlation coefficient matrix among metal biomarkers. Data are coefficients, p-values and N.

	MnH Child	MnB	BLL	MnH Mother	FeS
MnH Child	1.000	0.058	0.247*	0.294**	-0.150
	0.	0.636	0.039	0.010	0.260
	83	70	70	77	58
MnB		1.000	0.061	0.218	0.108
		0.	0.624	0.079	0.437
		70	68	66	54
PbB			1.000	-0.145	0.066
			0.	0.244	0.644
			70	66	52
MnH Mother				1.000	-0.192
				0.	0.152
				77	57
FeS					1.000
					0.
					58

** p = 0.01 (2-tailed), * p = 0.05 (2-tailed).

Table 4. Summary of the Wechsler test scores

IQ scores	N	Mean	SD	Min	Max
Full-Scale	83	85.0	13.5	50	121
Verbal	83	89.7	14.2	55	127
Performance	83	81.5	13.3	45	117
Factorial Comprehension	83	91.3	14.4	59	128

Table 5. Summary of the linear multiple regression models for children's IQ.

Intelligence Quotient	Unstandardized Coefficients	T Stat.	P value
Full-scale IQ			
Intercept	82.80	24.551	<0.001
Maternal Education (Years)	0.992	2.817	0.006
Height-for-age z-score	1.651	1.414	0.161
LogMnH	-5.782	-2.172	0.033
	N=83 r^2=0.147 F=4.554 p=0.005		
Verbal IQ			
Intercept	90.10	25.324	<0.001
Maternal Education (Years)	0.751	2.023	0.046
Height-for-age z-score	2.453	1.993	0.050
LogMnH	-6.723	-2.395	0.019
	N=83 r^2 =0.138 F=4.232 p=0.008		
Performance IQ			
Intercept	74.90	22.547	<0.001
Maternal Education (Years)	1.230	3.549	0.001
Height-for-age z-score	0.646	0.562	0.576
LogMnH	-2.408	-0.918	0.361
	N =83 r^2=0.146 F=4.506 p=0.006		
Factorial Comprehension			
Intercept	91.98	25.141	<0.001
Maternal Education (Years)	0.748	1.958	0.054
Height-for-age z-score	1.736	1.370	0.174
LogMnH	-7.326	-2.537	0.013
	N =83 r^2=0.123 F=3.688 p=0.015		

Table 6. Linear regression model for mother's cognition.

Variable	Unstandardized Coefficients	t Stat.	P value
Intercept	12.664	2.843	0.006
Education Years	0.970	4.111	<0.001
Age	-0.176	-1.563	0.123
LogMnH	-2.689	-1.965	0.055
Family Income	0.022	2.586	0.012

Notes: N =70, r^2 = 0.432, F=12.37, p< 0.001

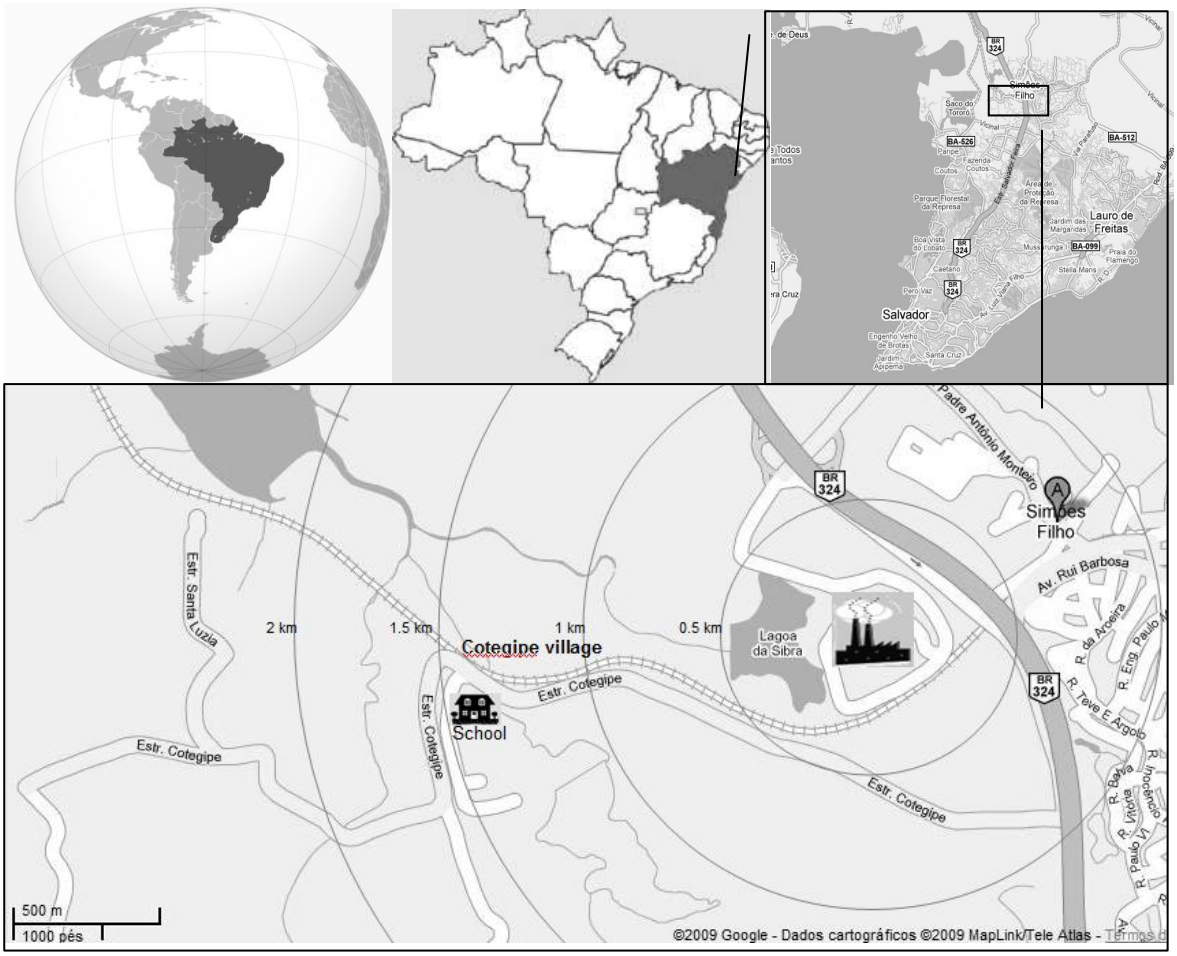
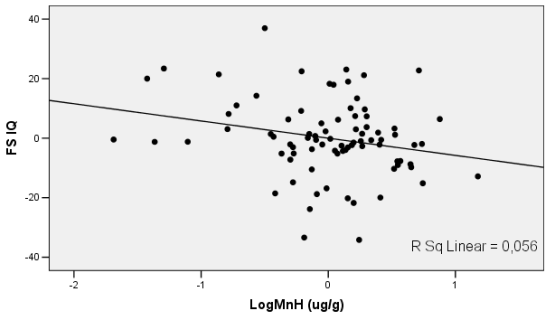
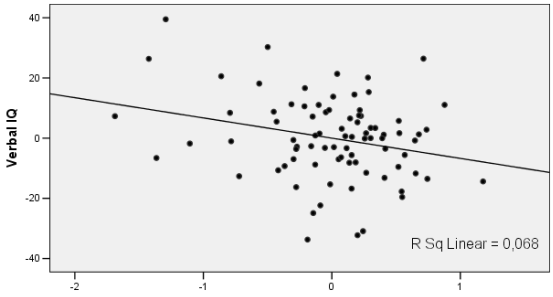


Figure 1. Schematic map of the Cotegipe village in Simões Filho town in the metropolitan area of Salvador, Bahia, Brazil.

a)



b)



c)

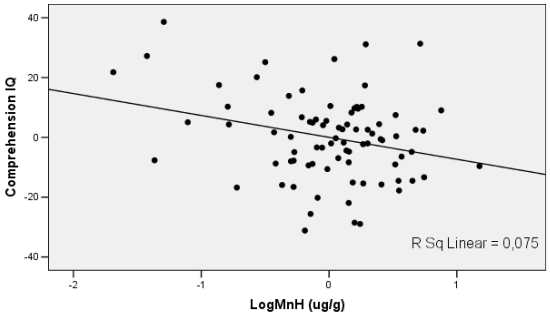


Figure 2. Residualized children's Full-Scale (a), Verbal and Comprehension IQ (c) scores versus Mn hair levels, adjusted for maternal education and nutrition status

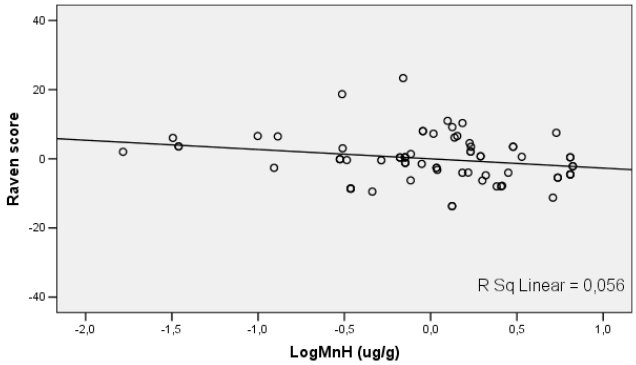


Figure 3. Residualized maternal intelligence versus Mn hair levels, adjusted for education years, monthly family income and age.

Considerações Finais

A revisão da literatura mostrou que o tema efeitos neuropsicológicos em crianças decorrentes da exposição excessiva ao Mn é relativamente novo e tem atraído recentemente a atenção de diversos grupos de pesquisadores no mundo. Vale ressaltar que os primeiros estudos datam do final da década de 1970. Pelo nosso conhecimento além do nosso estudo, estão sendo desenvolvidos projetos no México, Coréia, Itália e Estados Unidos da América.

Ao revisarmos a literatura, encontramos, apesar de esparsas, um crescente número de evidências que documenta uma correlação inversa entre os níveis de Mn no organismo, sobretudo o indicador Mn no cabelo, e diversos efeitos neuro-psicológicos (cognição, atenção e memória) e efeito neuro-motor. Os achados epidemiológicos recentes aliados a experimentação com modelos animais, especificamente com primatas, têm elucidado os mecanismos pelos quais Mn afeta o comportamento, função dos neurotransmissores (por exemplo: dopamina e ácido gama-butírico) e neuropatologia. Para maiores detalhes consultar o artigo de revisão de Burton and Guilarte (2009).

Os resultados da nossa pesquisa evidenciam que os níveis de contaminação ambiental e de exposição da comunidade de Cotegipe são muito elevados, quando comparados com os níveis em uma área controle. Tanto os níveis de Mn na fração respirável do material particulado, quanto na poeira domiciliar mostram que esta comunidade vem sendo contaminada pelo Mn carreado pelo ar. Os dados demonstram que crianças que habitam em casas mais próximas e na direção dos ventos têm níveis de Mn no organismo significativamente mais elevados do que as demais.

Tomando como base esse gradiente de exposição, planejamos o desenho do estudo do efeito na cognição esperando comparar os indivíduos mais expostos com aqueles com menor grau de exposição dentro da mesma comunidade. Os nossos resultados mostram que os níveis elevados de Mn, independentes da escolaridade materna, do estado nutricional, têm uma associação inversa significativa com o seu desempenho intelectual, sobretudo na escala verbal. Apresentamos evidências, pela primeira vez, de que a exposição ambiental excessiva ao Mn tem efeito não somente na cognição das crianças, mas também na função intelectual materna. Assim sendo,

poderíamos levantar a hipótese de que o efeito do Mn no desenvolvimento intelectual das crianças desta comunidade pode estar ocorrendo duplamente. Um efeito em consequência dos níveis elevados no próprio organismo e outro indireto decorrente do déficit cognitivo provocado nas suas mães, que tenderiam a uma inadequada estimulação neuro-psicológica das suas crianças. Aliado ainda a uma hipotética transferência materno-fetal.

Os achados desses estudos suportam a necessidade do desenvolvimento de um estudo com desenho prospectivo. Este permitiria responder a hipótese levantada no segundo artigo, sobre uma possível contaminação intra-uterina, resultados dos altos níveis de Mn no período da gestação (transferência materno-fetal) e ajudar a elucidar os determinantes dos efeitos neuropsicológicos do Mn desde a fase pré-natal.

Sugerimos aos poderes públicos tomar medidas para implementar melhorias nas condições de sanidade na comunidade, conectando todas as residências ao sistema de esgotamento sanitário, evitando o lançamento de esgoto doméstico na Baía de Aratu. Coleta de lixo eficiente e abrangente, assim como campanha de educação ambiental para evitar que persistam no hábito de queimar lixo doméstico. Nossa avaliação mostrou que metade da comunidade tem esse costume, o qual está relacionado com os níveis mais elevados de chumbo sanguíneo.

Ainda, sugerimos que o órgão ambiental da Bahia deveria condicionar a renovação da licença de operação da indústria, após introduzir melhores tecnologias de produção e de tratamento dos efluentes, sobretudo das emissões atmosféricas, com o objetivo de reduzir ao máximo a concentração de material particulado fino e óxidos de manganês lançado no ar. Como medidas corretivas, a companhia deveria financiar projetos de intervenção cognitiva compensatória, visando melhoria da estimulação do desenvolvimento intelectual das crianças.

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ANEXO 1

Termo de Consentimento Livre e Esclarecido

População Exposta

De acordo com as Normas da Resolução nº 196, do Conselho Nacional de Saúde de 10 de outubro de 1996.

Título da Pesquisa: "Exposição Ambiental e Concentrações Biológicas de Manganês em Crianças que Habitam Área Circunvizinha de uma Metalúrgica de Ferro-Manganês na Região Metropolitana de Salvador, Bahia"

Coordenador da Pesquisa: Prof. José Antonio Menezes Filho

Instituições participantes da pesquisa

- Faculdade de Farmácia, Universidade Federal da Bahia & Escola Nacional de Saúde Pública - Fundação Oswaldo Cruz

O departamento de Análises Clínicas e Toxicológicas da Faculdade de Farmácia da UFBa atua na formação acadêmica, serviços de extensão atendendo a comunidade e em pesquisa básica e aplicada nas áreas de diagnóstico laboratorial de patologias infecciosas ou não, e avaliação da exposição ocupacional e ambiental a substâncias tóxicas. O Centro de Estudos da Saúde do Trabalhador e Ecologia Humana - CESTEH, é um centro da Escola Nacional de Saúde Pública - ENSP, da Fundação Oswaldo Cruz - FIOCRUZ, do Ministério da Saúde, que tem por objetivos realizar pesquisas, atividades de ensino e criar tecnologias, na área da Saúde Pública.

Como voluntário, o(a) Sr(a.) está sendo solicitado(a) a participar de uma pesquisa, patrocinada com recursos da Fundação de Apoio a Pesquisa do Estado da Bahia e de fonte internacional. O estudo prevê a participação de crianças de 1 a 10 anos de idade residentes na Vila de Cotegipe, através da assinatura do termo de consentimento dos pais ou responsáveis, de acordo com o ítem IV.3 da Resolução 196/96.

O (A) Sr.(a.) não é obrigado a participar da pesquisa, e poderá se afastar dela a qualquer tempo, sem qualquer prejuízo de sua relação com o pesquisador ou com a instituição. Todas as informações pessoais serão sigilosas, os resultados de suas análises serão fornecidos unicamente ao Sr(a)., e sua identidade não será revelada em qualquer publicação resultante deste estudo. Os exames e procedimentos aplicados serão gratuitos. **Antes de assinar este termo, o(a) Sr(a.) deve entender as informações sobre a pesquisa e fazer todas as perguntas que achar necessário.**

O problema investigado é a exposição de uma parcela da comunidade, crianças de até 10 anos, a poluição industrial (emanações atmosféricas e rejeitos líquido e sólido de uma siderúrgica).

Objetiva-se avaliar o grau de contaminação do ambiente através da medição da concentração de manganês (Mn) no material particulado em suspensão (Mn no ar),

concentração de Mn na poeira doméstica e também na água. Assim como estudar se existe correlação com os níveis de Mn no organismo das crianças. A exposição prolongada de crianças a concentrações deste metal acima do permissível tem sido associada com efeitos negativos no desenvolvimento intelectual. Será coletada uma mecha de cabelo na região da nuca e amostra de sangue para a dosagem de manganês. Nesta oportunidade, serão realizados exames detalhados para detectar a presença de anemia nas crianças: hemograma completo, dosagem de ferro e ferritina séricos; assim como exame parasitológico (exame de fezes). Os casos detectados de anemia serão imediatamente encaminhados ao Programa de Saúde da Família do Município de Simões Filho com todos os resultados dos exames para serem devidamente tratados. A coleta de sangue será feita com material descartável, a vácuo na veia cubital no braço. No momento da picada a criança sente dor passageira, pode ocorrer em alguns casos hematoma na região. No local será colocado curativo adesivo. As amostras de sangue e cabelo receberão um código que estará relacionado a identificação de cada criança, sendo de conhecimento somente da coordenação do projeto. As amostras serão armazenadas devidamente no Laboratório de Toxicologia (FF/UFBa) e poderão ser utilizadas na pesquisa para avaliar a co-exposição a outros metais.

Caso seja detectado concentração de manganês no sangue acima dos valores de referência, será avaliado o grau de intervenção a ser adotado no ambiente, ou mesmo da possibilidade e indicação de intervenção clínica.

Você receberá uma cópia deste termo, onde consta o telefone e o endereço do pesquisador principal, podendo tirar suas dúvidas sobre o projeto e sua participação, agora ou a qualquer momento.

_____

Prof. José Antonio Menezes Filho – Pesquisador Responsável

Endereço: Laboratório de Toxicologia – Faculdade de Farmácia/UFBA Av. Ademar de Barro s/n Campus de Ondina Salvador Bahia

Tel.: 3235-1580 (r.44)

Comitê de Ética da FIOCRUZ: Tel. (021) 2598-2863

Endreço: Rua Leopoldo Bulhões, 1480 Sala 314

Manguinhos, Rio de Janeiro RJ 21041-210

Declaro que entendi os objetivos, riscos e benefícios da minha participação na pesquisa e concordo em participar.

_____

Responsável

ANEXO 2

Termo de Consentimento Livre e Esclarecido

Grupo de Referência

De acordo com as Normas da Resolução nº. 196, do Conselho Nacional de Saúde de 10 de outubro de 1996.

Título da Pesquisa: "Perfil da Contaminação Ambiental e da População Infantil de uma Comunidade Vizinha a uma Metalúrgica Ferro-Manganês na Região Metropolitana de Salvador, Bahia."
Coordenador da Pesquisa: Prof. José Antonio Menezes Filho
Instituições participantes da pesquisa

- Faculdade de Farmácia, Universidade Federal da Bahia.
- Escola Nacional de Saúde Pública - Fundação Oswaldo Cruz.

O Departamento de Análises Clínicas e Toxicológicas da Faculdade de Farmácia da UFBa atua na formação acadêmica, serviços de extensão atendendo a comunidade e em pesquisa básica e aplicada nas áreas de diagnóstico laboratorial de patologias infecciosas ou não, e avaliação da exposição ocupacional e ambiental a substâncias tóxicas. O Centro de Estudos da Saúde do Trabalhador e Ecologia Humana - CESTEH, é um centro da Escola Nacional de Saúde Pública - ENSP, da Fundação Oswaldo Cruz - FIOCRUZ, do Ministério da Saúde, que tem por objetivos realizar pesquisas, atividades de ensino e criar tecnologias, na área da Saúde Pública.

Como voluntário, o (a) Sr. (a) está sendo solicitado(a) a participar de uma pesquisa, com apoio parcial da *National Instute of Health* (NIH) através da *Fogarty Foundation grant* no. 1 D43 TW000640 e do Programas de Pesquisa (PAPS V) do Ministério da Saúde. O estudo prevê a participação de crianças de 1 a 10 anos de idade residentes na Comunidade de Capiarara, Distrito de Areia Branca no Município de Lauro de Freitas, selecionada como referência (população controle) para fins de comparação com a comunidade de Cotegipe em Simões Filho, a qual está exposta às emanações de indústria metalúrgica de ligas ferro-manganês. Solicita-se a sua participação como voluntário e assinatura do termo de consentimento informado, de acordo com o item IV.3 da Resolução 196/96.

O (A) Sr. (a) não é obrigado (a) a participar da pesquisa, e poderá se afastar dela a qualquer tempo, sem qualquer prejuízo de sua relação com o pesquisador ou com a instituição. Todas as informações pessoais serão sigilosas, os resultados de suas análises serão fornecidos unicamente ao Sr. (a), e sua identidade não será revelada em qualquer publicação resultante deste estudo. Os exames e procedimentos aplicados serão gratuitos. **Antes de assinar este termo, o (a) Sr. (a) deve entender as informações sobre a pesquisa e fazer todas as perguntas que achar necessário.**

O problema investigado é a exposição de uma parcela da comunidade, crianças de até 10 anos, a poluição industrial (emanações atmosféricas e rejeitos líquido e sólido de uma siderúrgica); para tal fim faz-se necessário comparar as concentrações dos metais de interesse (manganês e outros) nas amostras de sangue e cabelo de uma população com semelhantes características sócio-demográficas.

Objetiva-se avaliar o grau de contaminação do ambiente através da medição da concentração de manganês (Mn) no material particulado em suspensão (Mn no ar), concentração de Mn na poeira doméstica e também na água. Assim como estudar se existe correlação com os níveis de Mn no organismo das crianças. A exposição prolongada de crianças a concentrações deste metal acima do permissível tem sido associada com efeitos negativos no desenvolvimento intelectual. Será coletada uma mecha de cabelo na região da nuca e amostra de sangue para a dosagem de manganês. Nesta oportunidade, serão realizados exames detalhados para detectar a presença de anemia nas crianças: hemograma completo, dosagem de ferro e ferritina séricos; assim como exame parasitológico (exame de fezes). Os casos detectados de anemia serão imediatamente encaminhados ao Programa de Saúde da Família do Município de Lauro de Freitas no Posto de Saúde Areia Branca Dr. Antonio Carlos

Rodrigues com todos os resultados dos exames para serem devidamente tratados. A coleta de sangue será feita com material descartável, a vácuo na veia cubital no braço. No momento da picada a criança sente dor passageira, pode ocorrer em alguns casos hematoma na região. No local será colocado curativo adesivo. As amostras de sangue e cabelo receberão um código que estará relacionado a identificação de cada criança, sendo de conhecimento somente da coordenação do projeto. As amostras serão armazenadas devidamente no Laboratório de Toxicologia (FF/UFBa) e poderão ser utilizadas na pesquisa para avaliar a co-exposição a outros metais. Para avaliar possíveis efeitos neuro-psicológicos serão aplicados testes psicológicos (questionários com papel e lápis). Estes testes medem alguns aspectos relacionados com o movimento, atenção, linguagem, memória, aprendizagem, percepção, pensamento e conduta; as crianças terão atividades como ler, escrever e manipular objetos como cubos, quebra-cabeças e cartões. E a você pediremos que responda a alguns questionários sobre a história de desenvolvimento de seu filho (a), sua conduta e dados gerais. A aplicação de destes testes durarão entre 2 e 3 horas, repetidas e duas sessões. A aplicação de cada teste durará cerca de 30 minutos. Estes testes serão realizados por psicólogos na escola da comunidade.

Caso seja detectada concentração de manganês no sangue acima dos valores de referência, será avaliado o grau de intervenção a ser adotado no ambiente, ou mesmo da possibilidade e indicação de intervenção clínica.

Você receberá uma cópia deste termo, onde consta o telefone e o endereço do pesquisador principal, podendo tirar suas dúvidas sobre o projeto e sua participação, agora ou a qualquer momento.

_____

Prof. José Antonio Menezes Filho – Pesquisador Responsável

Endereço: Laboratório de Toxicologia – Faculdade de Farmácia/UFBA
Avenida Barão de Jeremoabo, s/n Campus Universitário de Ondina, Salvador, Bahia.
Tel.: 3283-6960/9144-7719
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Endereço: Rua Leopoldo Bulhões, 1480 Sala 314. Manguinhos, Rio de Janeiro RJ 21041-210

Declaro que entendi os objetivos, riscos e benefícios da minha participação na pesquisa e concordo em participar.

_____

Responsável

ANEXO 3

Questionário 1 – Dados Sócio-econômicos e hábitos gerais

PROJETO COTEJIPE / CAPIARARA
Questionário Adulto/Responsável

Número do Questionário:	**Data da entrevista:** / /	**Horário de Início:** :
Entrevistador:	**Código do Entrevistador:**	

Nome Completo do Responsável:		
Endereço Completo: _____		
Telefone para contato:		
Coordenadas – GPS (localização da casa)	**E: 38º**	**S:** 12º

IDENTIFICAÇÃO:					
Q 1	Sexo:	1. ( ) Masculino		2. ( ) Feminino	
Q 2	Data de Nascimento:	Idade:			
Q 3	Raça:	1. ( ) Branca	2. ( ) Negra	3. ( ) Amarela	4. ( ) Outras

SÓCIO-ECONÔMICO						
Q 4	o de moradia:	1. ( ) Lona	2. ( ) Alvenaria	3. ( ) Madeira	4. ( ) Casa de taipa	
5	o de cobertura:	1. ( ) Telha	2. Forro Sim ( ) Não ( )	3. ( ) Laje	4. ( ) Palha	
Q 6	Tipo de piso:	1. ( ) Chão batido	2. ( ) Cimento "vermelhão"	3. ( ) Madeira	4. ( ) Cimento comum 5. ( ) Cerâmica/lajota	
Q 7	De onde o Sr (a) obtém água de beber?	1. ( ) Encanada da estação de tratamento	2. ( ) De cisterna própria	3. ( ) Córregos Qual?	4. ( ) Nascente	
Q 8	Qual o tratamento que dá a água de beber?	1. ( ) Ferve	2. ( ) Filtra	3. ( ) Côa	4. ( ) Clora 9. ( ) NS/NR	
Q 9	Onde faz as necessidades fisiológicas?	1. ( ) Sanitário com fossa	2. ( ) Sanitário s/ fossa	3. ( ) Mato	4. ( )Rio/Lagoa	
Q 10	Qual o destino do lixo?	1. ( ) Queima	2. ( ) Enterra	3. ( ) Joga no rio	4. ( ) Coletado	
Q 11	De onde tira o sustento?	1. ( ) Agricultura /Roça	2. ( ) Feira / Comerciante	3. ( ) Pesca	4. ( ) Aposentado / Pensionista 5. Outros	
Q 12	Você sabe ler e escrever?	1. ( ) Sim	2. ( ) Não	99. ( ) NS / NR		
Q 13	Até que ano você estudou na escola?	1. ( ) não foi à escola	2. ( ) fundamental incompleto	3. ( ) fundamental completo	4. ( ) Médio incompleto	

		5. ( ) Médio completo		6. ( ) Mais que ensino médio.	
Q 14	Quantos anos mora nesta localidade?	1. Anos:		9. ( ) NS / NR	
Q15	Cria algum animal?	1. ( ) Gato	2. ( ) Cachorro	3. ( ) Galinha	4. ( ) Porco 5. ( ) Outros 9. ( ) Não
Q 16	Costuma lavar as frutas e verduras antes de comer?	1. ( ) Sim	2. ( ) Não	9. ( ) NS / NR	
Q 17	Como costuma comer as verduras?	1. ( ) crua	2. ( ) cozida	3. ( ) Não come.	

Q 18	Quais as refeições feitas diariamente?	1. ( ) café da manhã	2. ( ) almoço	3. ( ) Jantar
Q 19	O que costuma comer / freqüência semanal 1x, 2x, 3x	a. Carne ( ) Peixe ( ) Frango ( ) Fígado ( ) b. Tomate ( ) Abóbora ( ) Cenoura ( ) Batata ( ) Beterraba ( ) Outros ( ) c. Laranja ( ) Banana ( ) Manga ( ) Goiaba ( ) Caju ( ) Outros ( ) d. Leite ( ) Ovos ( )		
Q 20	Junto com almoço ingere com freqüência:	1. ( ) Chá preto e/ou café	2. ( ) Banana	3. ( ) Frutas cítricas 9. ( ) NS/NR
Q 21	Usa alguma erva para fazer chá como medicamento?	1. ( ) Sim	2. ( ) Não	9. ( ) NS/NR
Q 22	O Sr(a) consome hortaliças plantadas no local?	1. ( ) sim	2. ( ) não	9. ( ) NS/NR
Q 23	Alguém da família tem contato com agrotóxicos?	1. ( ) sim	2. ( ) não	9. ( ) NS/NR Se sim, quem:_____, produto:
Q 24	Recebe visita do agente de saúde?	1. ( ) Sim	2. ( ) Não	9. ( ) NS/NR Se sim, freqüência:
Q 25	Vai ao médico?	1. ( ) Sim	2. ( ) Não	9. ( ) NS/NR. Se sim, freqüência:
Q 26	Alguém na família já teve anemia?	1. ( ) Sim	2. ( ) Não	9. ( ) NS/NR. Se sim, quem:
Q 27	Houve casos recentes de diarréia?	1. ( ) Sim	2. ( ) Não	9. ( ) NS/NR Se sim, sangramento nas fezes?
Q 28	Existem casos na família de:	1. ( ) Hipertensão	2. ( ) Diabetes	3. ( ) Doença de Chagas.
Q 29	Alguém na família apresenta algumas destas queixas?	1. ( ) Nervosismo 2. ( ) Dificuldade de engolir 3. ( ) Tontura 4. ( ) Fraqueza 5. ( ) Palpitação		6. ( ) Enjôo 7. ( ) Dores nas pernas 8. ( ) Dor de cabeça 9. ( ) Sonolência 10. ( ) Falta de ar
Q 30	Algum caso de asma alérgica?	1. ( ) sim	2. ( ) Não	9. ( ) NS/NR Se sim, quem:
Q 31	Alguém em sua família toma medicamento regularmente?	1. ( ) sim	2. ( ) Não	9. ( ) NS/NR Se sim, quem:_____, medicamento:
Q 32	Alguém na família consome bebida alcoólica?	1. ( ) sim	2. ( ) não	9. ( ) NS/NR Se sim, freqüência semanal:
Q 33	Conhece algum caso de:	1. ( ) Esquistossomose (barriga d´água, xistose)	2. ( ) Amarelão	3. ( ) Calazar 4. ( ) Doença de Chagas

Q 34	Conhece o inserto barbeiro?	1. ( ) sim	2. ( ) não	9. ( ) NS/NR Se sim, Já o viu em sua casa?:
Q 35	Usa algum método para evitar filhos?	1. ( ) Sim	2. ( ) Não	9. ( ) NS/NR Se sim, qual?
Q 36	Orienta os filhos sobre sexualidade?	1. ( ) Sim	2. ( ) Não	9. ( ) NS/NR

TABAGISMO NO DOMICÍLIO				
Q 37	Quem é que fuma em casa?	1. ( ) Ninguém **(Se NINGUÉM, vá para a pergunta Q 42)** 2. ( ) Mãe	3. ( ) Pai 4. ( ) Mãe e Pai	5. ( ) Outros
Se a entrevistada for a Mãe e na pergunta Q 37 a resposta foi 2. Mãe , responda as perguntas Q 38 e Q 39. Se na pergunta Q 37 a resposta foi 3. Pai, responda as perguntas Q 40 e Q 41. Se na pergunta Q 37 a resposta foi 4. Mãe e Pai, responda as perguntas Q 38 a Q 41.				
Q 38	A Sra. fuma quantos cigarros por dia?	9. ( ) NS / NR		
Q 39	Quantos horas por dia a Sra. costuma ficar em casa com o seu filho(a)?	Horas:	9. ( ) NS / NR	
Q 40	O Pai da criança fuma quantos cigarros por dia?	9. ( ) NS / NR		
Q 41	Quantos horas por dia o Pai da criança costuma ficar em casa com ela?	Horas:	9. ( ) NS / NR	
Se a entrevistada for a Mãe, vá para a pergunta Q 42. Caso contrário, ENCERRE a entrevista.				
Q 42	A Sra. fumou durante a gravidez de seu filho(a)?	1. ( ) Sim	2. ( ) Não **(Se NÃO, ENCERRRE a entrevista)**	99. ( ) NS / NR **(Se NS / NR, ENCERRE a entrevista)**
Q 43	Quantos cigarros por dia a Sra. fumava durante a gravidez de seu filho(a)?	99. ( ) NS / NR		

Data final da entrevista: / /	**Horário de Término:** :

Recusa do Questionário	
Outros	

A2	C1
A3	C2
A4	C3
A5	C4
A6	C5
A7	C6
A8	C7
A9	C8

ANEXO 4

Questionário 2 – Dados antropométricos e hábitos da criança

PROJETO COTEJIPE / CAPIARARA

Questionário Individual Infantil – até 10 anos

Número do Questionário: _____	**Data da entrevista:** ___/___/____	**Horário de Início:** ___:___ hs
Entrevistador: _____	**Código do Entrevistador:**_____	

Nome Completo da Criança:
Nome Completo do Responsável:

IDENTIFICAÇÃO:					
Q 1	Sexo:	1. ( ) Masculino		2. ( ) Feminino	
Q 2	Data de Nascimento:	_____ Idade: _____			
Q 3	Raça:	1. ( ) Branca	2. ( ) Negra	3. ( ) Amarela	4. ( ) Outras
Q 5	Altura:	____ , ____ ____ metros		9. ( ) NS / NR	
Q 6	Peso:	_____ Kg		9. ( ) NS / NR	
Q 7	Série Escolar:	_____			

HÁBITOS				
Q 8	A criança foi amamentada?	1. Sim ( )	2. Não	3. NS/NR Se sim, até que idade?_____ano_____ meses
Q 9	A criança chupa dedo ou chupeta?	1. ( ) sim Especifique_____		2. Não
Q 10	A criança escova os dentes diariamente?	1. Sim ( ) Freqüência (1x, 2x, 3x) _____		2. Não ( )
Q 11	A criança tem o hábito de comer	1. ( ) reboco 2. ( ) terra 3. ( ) areia 4. ( ) lápis cera 5. ( ) massinha 6. ( ) borracha 7. ( ) plástico 8. ( ) lápis 9. ( ) papel 10. ( ) Outros. Especifique: _____		
Q 12	A criança tem hábito de roer as unhas?	1. Sim ( )	2. Não ( )	9. NS/NR ( )
Q 13	A criança tem hábito de brincar próximo a indústria?	1. Sim ( )	2. Não ( )	9. NS/NR ( )
Q 14	A criança toma banho de rio ou na represa?	2. Sim ( )	2. Não ( )	9. NS/NR ( )

CRITÉRIO DE ASMA				
Q 15	Alguma vez na vida seu filho(a) teve chiado no peito?	1. ( ) Sim	2. ( ) Não **(Se NÃO, vá para a pergunta Q 21)**	99. ( ) NS / NR **(Se NS/NR, vá para a pergunta Q 21)**
Q 16	Nos últimos 12 meses seu filho(a) teve chiado no peito?	1. ( ) Sim	2. ( ) Não **(Se NÃO, vá para a pergunta Q 21)**	99. ( ) NS / NR **(Se NS/NR, vá para a pergunta Q 21)**

Q 17	Nos últimos 12 meses quantas crises de chiado no peito seu filho(a) teve?	1. ( ) Nenhuma	2. ( ) 1 a 3	3. ( ) 4 a 12	4. ( ) Mais de 12
Q 18	Nos últimos 12 meses, com que freqüência seu filho(a) teve o sono perturbado por chiado no peito?	1. ( ) Nunca	2. ( ) Menos de 1 noite por semana	3. ( ) 1 ou mais noites por semana	
Q 19	Nos últimos 12 meses, o chiado do seu filho(a) foi tão forte a ponto de impedir que ele(a) conseguisse dizer mais de 2 palavras entre cada respiração?	1. ( ) Sim	2. ( ) Não	99. ( ) NS / NR	
Q 20	Nos últimos 12 meses, seu filho teve chiado no peito após exercício físico?	1. ( ) Sim	2. ( ) Não	99. ( ) NS / NR	
Q 21	Alguma vez seu filho(a) teve asma?	1. ( ) Sim	2. ( ) Não	99. ( ) NS / NR	
Q 22	Nos últimos 12 meses, seu filho(a) teve tosse seca à noite sem estar gripado ou com infecção respiratória?	1. ( ) Sim	2. ( ) Não	99. ( ) NS / NR	
Q 23	Algum médico disse que seu filho(a) tinha problema de pulmão?	1. ( ) Sim	2. ( ) Não **(Se NÃO, vá para pergunta a Q 18)**	99. ( ) NS / NR **(Se NS / NR, vá para a pergunta Q18)**	
Q 24	Qual médico e quando?	a. Qual?_____	b. Quando?_____		
Q 25	Alguma vez algum médico disse que seu filho(a) tinha tuberculose pulmonar?	1. ( ) Sim	2. ( ) Não **(Se NÃO, vá para a pergunta Q 20)**	99. ( ) NS / NR **(Se NS / NR, vá para a pergunta Q20)**	
Q 26	Quando?	a. _____Dias	b. _____Meses	c. _____Anos	
Q 27	Alguma vez seu filho(a) já apresentou mancha no pulmão ou escarrou sangue?	1. ( ) Sim	2. ( ) Não **(Se NÃO, vá para o próximo módulo)**	99. ( ) NS / NR **(Se NS / NR, vá para próximo módulo)**	
Q 28	Quando?	a. _____Dias	b. _____Meses	c. _____Anos	

Data final da entrevista: ___/___/____	**Horário de Término:** ___:___ hs

Recusa do Questionário	
Outros	

ANEXO 5

QUESTIONARIO 3: ANAMNESE – PSICODIAGNÓSTICO INFANTIL

Data da anamnese: _____/____/_____ Entrevistador: _____

Nome do entrevistado: _____ Parentesco:_____

I – IDENTIFICAÇÃO DA CRIANÇA:

1- Nome da criança:_____ Data de nasc.:____/_____/_____

2 -Sexo: (1) masculino (2) feminino

3- Filiação:

Mãe:_____ Escolaridade: _____Profissão: _____

Pai:_____ Escolaridade:_____Profissão: _____

4- Os pais vivem juntos? (1) sim (2) não.

Obs.:_____

5 -Escolaridade: _____ Nº de irmãos:_____ Ordem de nasc.:_____

II – DADOS GERAIS:

6- Com quem reside atualmente?

Parentesco	Idade	Parentesco	Idade

7 - Endereço: _____ _____ Telefone _____

8 - Mudou de casa? (1) sim Ano?_____ (2) não (3) não sabe.

9 - Mudou de cidade? (1) sim Ano?_____Origem:_____(2) não(3) não sabe.

10 - Tipo de residência:

(1) própria (2) alugada (3) cedida/familiares (4)Instituição

11- Fonte de renda familiar: (1) salário (2) trabalhos eventuais (3) pensão (4) aposentadoria

(5) auxílio de terceiros (6) Outros_____

Total da renda R$:_____

III – DESENVOLVIMENTO:

16 - Gestação: (1) Planejada (2) Inesperada (3) Indesejada

17 - Reação: (1) Normal (2) Boa (3) Ruim (4) Indiferente (5) Medo

(6) Outras: _____

18 - Problemas com a gravidez: (99) Nenhuma/Não se aplica

(1) stress (2) Desemprego (3) Doença (4) Acidentes (5) Problemas emocionais (6) problemas conjugais (7) Problemas de saúde. Qual? _____

(8) Violência (9) Outros_____

19 - Fez pré natal? (1) sim (2) não

20 - Uso de substâncias? (1) sim (2) não

21 – Se sim, Qual?

Tabaco	Trimestre pré-gravidez	1º trimestre	2º trimestre	3º trimestre	Lactação
(1) Diariamente – Média cig:					
(2) Semanalmente – Média cig:					
(3) Mensalmente – Média cig:					
(4) Menos de 1 vez /mês – Média cig:					
Álcool	Trimestre pré-gravidez	1º trimestre	2º trimestre	3º trimestre	Lactação
(1) Diariamente – Média doses:					
(2) Semanalmente – Média doses:					
(3) Mensalmente – Média doses:					
(4) Menos de 1 vez /mês – Média doses:					
Outras drogas. Qual_____	Trimestre pré-gravidez	1º trimestre	2º trimestre	3º trimestre	Lactação
(1) Diariamente – Média:					
(2) Semanalmente – Média:					
(3) Mensalmente – Média:					
(4) Menos de 1 vez /mês – Média:					
Medicações					
(1) Diariamente – Média:					
(2) Semanalmente – Média:					
(3) Mensalmente – Média:					
(4) Menos de 1 vez /mês – Média:					
Nome das medicações:					

23 -Nascimento: (1) Normal (2) Cesária (3) Prematuro, meses: _____

24 - Ficou na incubadora? (1) sim (2) não Tempo:_____

25 - Problemas no parto /gestação? _____

26 - A criança: (Marque todas as opções pertinentes e respectiva idade de interrupção)

Atividade	Início	Interrupção	Observações:
Amamentação			
Uso de chupeta			
Uso de mamadeira			
Papinha			
Alimentos sólidos			
Engatinhar			
Andar			
Falar			
Controle do xixi			
Controle do cocô			

27 - A criança tem atualmente alguma dificuldade para:(99) Nenhuma/Não se aplica

(1) Andar (2)Correr (3) Alimentar-se (4)Tomar banho (5)Vestir-se

(6) Enxergar (7) Ouvir (8) Brincar (9) subir escadas/degraus (10) Outra:

_____

28 – histórico de aborto materno? (1) Sim Tipo:_____ (2)Não

IV – HÁBITOS DIÁRIOS

29 - Quantas refeições diárias? _____

30 - Alimenta-se só? (1) sim (2) não. Porque?_____

Sono:

31 - Onde dorme?_____ _____

32 - Com quem? _____

33 - Hora que dorme: _____ ____ Hora que acorda: _____

34 - Dorme bem? (1) sim (2) não. (3) não sabe

35 - Sono agitado? (1) sim (2) não. (3) não sabe

36 - Acorda com freqüência durante a noite? (1) sim (2) não. (3) não sabe

37 -Faz xixi na cama? (1) sim (2) não. (3) não sabe

V - HISTÓRIA CLÍNICA:

38 - Tem algum diagnóstico médico? (1) sim (2) não.

Qual?_____ Idade do diagn.:_____

39 - Passou por cirurgia? (1) sim (2) não. Quando? _____

40 - Qual? _____

41 - Como foi a recuperação? _____

42 - Diagnósticos: _____

_____

43 – A criança faz uso de medicação? (1) sim (2) não (3) não sabe

44 -Medicação	Motivo da Indicação	Desde quando

45- Teve algum acidente? (1) sim (2) não Qual? _____

46 - Tomou todas as vacinas? (1) sim (2) não (3) não sabe

VI – DADOS SOCIAIS

47 - A criança foi para creche? (1) sim (2)não.Quando? _____

48 - Quem cuida dela com mais freqüência?_____ _____

49 - Precisou de cuidados especiais? (1) sim (2) não (3) não sabe

50 - Como se relaciona com os pais? _____

_____

51 - Como se relaciona com os irmãos? _____

_____

52 - Quem tem mais autoridade com a criança? _____

53 – Se seu filho comete uma travessura, faz algo que não devia, como é corrigido?

(1) conversa (2) apanha (3) fica de castigo (4) fica sem poder fazer algo que gosta

(5) não é corrigido/ ignora Outros._____

54 - Quais os familiares mais próximos? _____

55 - Tem manias (roer unhas, ranger dentes)? (1) sim (2) não (3) não sabe

56 - Quais? _____

57 - Com quem brinca (só ou acompanhada)? _____

58 - Quem são seus amigos, de onde?

(1) escola (2) vizinhança (3) outras atividades (4) irmãos (5) parentes

59 - São muitos? Nº_____ (1) sim (2) não (3) não sabe

60 - Do que brinca? _____

_____

61 - Como se comporta com estranhos?

(1) adapta-se rápido (2) estranha (3) ignora (4) retrai-se (3) não sabe

62 - Tem curiosidade sexual? (1) sim (2) não

63 - Quais os lugares que freqüenta?

(1) escola (2) praça (3) casa de terceiros (4) clube (5) quadra (6) igreja

64 -Quais dessas atividades pratica?

(1) esportes (2) leitura (3) ver Tv (4) aulas extras (inglês, música)

(5) uso de computador (6) jogos eletrônicos (7) andar de bicicleta (8) jogar bola

(9) outras_____

65 - Perdeu algum ente querido? (1) sim. Quando? _____ Quem? _____

Como reagiu? _____ (2) não.

VII– DADOS ESCOLARES

66 - Quando foi para escola? _____

67 - Quem levou? _____

68 - Como foi a adaptação? _____

69 - Já mudou de escola? (1) sim (2) não. Quantas vezes? _____

70 - Cumpre as atividades escolares? (1) sim (2) não (3) às vezes

71 - Costuma ser repreendido, na escola? (1) sim. Porque? _____

(2) não (3) às vezes

72 - Gosta da professora? (1) sim (2) não (3) não sabe

73 - Gosta dos colegas? (1) sim (2) não (3) não sabe

74 – Gosta da escola? (1) sim (2) não (3) não sabe

75 - Quando tem problemas na escola(comportamento, nota) qual a reação da família? _____

_____

_____

76 - Pegou recuperação alguma vez? (1) sim. Quantas vezes? _____ (2) não (3) não sabe

77 - Já foi reprovado? (1) sim. Quantas vezes? _____ (2) não (3) não sabe

78 - Quais as principais dificuldades escolares? _____

_____

OBS:

Atitude do entrevistado: (1) cooperativo (2) disperso (3) confuso (4) resistente (5)ansioso

VIII – OUTROS:_____

ANEXO 6

Questionário 4. Inventário HOME Adaptado

SEÇÃO 1: O LAR
PARTE C: PARA CRIANÇAS QUES TÊM AO MENOS 6 ANOS, MAS MENOS DE 10 ANOS
Para _____ quem fez o sexto aniversário mas não o décimo.

1. Mais ou menos quantos livros seu filho(a) tem?
(MARQUE UMA)

Nenhum.....1
1 ou 2..... 2 = 0
3 a 9 3

10 ou mais..... 4 = 1

---

2. Mais ou menos com que freqüência você lê pra seu filho? **NÃO PERGUNTAR CRIAN≥10 ANOS**
(MARQUE UMA)

Nunca.....1
Várias vezes ao ano..... 2
Várias vezes por mês..... 3 = 0
Cerca de uma vez por semana..... 4

Ao menos uma vez por semana..... 5
Todos os dias.....6 = 1

3. Com que frequência você espera que seu filho(a) faça as seguintes tarefas?
(MARQUE UM NÚMERO PARA CADA QUESTÃO.) Se ao menos 3 dos 4 itens (de a a d) são >1, então Q3 (rec) = 1 , outras Q3(rec) = 0.

	Quase Nunca	**Menos da metade Das vezes**	**Metade das vezes**	**Mais da metade das vezs**	**Quase Sempre**
PARA TODAS AS IDADES					
a. Fazer sua cama?	1	2	3	4	5
b. Limpar seu quarto?	1	2	3	4	5
c. Limpar sua bagunça?	1	2	3	4	5
PARA 6 a <10 ANOS					
d. Tomar banho sozinho?	1	2	3	4	5
e. Arruma seus brinquedos?	1	2	3	4	5
PARA ≥ 10 ANOS					
f. Ajuda a manter áreas comuns limpas e arrumadas.	1	2	3	4	5
g. Realiza tarefas domésticas (lava prato, banheiro, compras)	1	2	3	4	5
h. Ajuda a administrar seu próprio tempo (acordar, se arrumar, tc)	1	2	3	4	5

If Q3e ≥ 2 then Q3e(Rec) = 1
If Q3e = 1 then Q3e(Rec) = 0

4. Tem instrumento musical (por exemplo violão, tambor, pandeiro, etc) que sua criança possa usar em casa?

Sim .....1 = 1
Não .....0 = 0

5. Mais ou menos com qual frequência que seu fillho lê por diversão?

Todos os dias 1
Várias vezes na semana..... 2 = 1

Várias vezes no mês..... 3
Várias vezes ao ano ..... 4 = 0

Nunca.....5

6. A sua família estimula seu filho(o) iniciar a e manter uma atividade de lazer (artesanato, etc)
Sim .....1 = 1
Não .....0 = 0

7. Com que frequência sua família se reúne com outros parentes ou amigos?
Uma ves ao ano.....1
Algumas vezes ao ano ..... 2 = 0
Quase uma vez por mês..... 3

Duas ou três vezes ao mês ..... 4 = 1
Quase uma vêz por semana ou mais..... 5

8. Tente se lembrar, durante um dia de semana normal em sua casa. Quanto tempo seu filho gasta vendo TV (em sua casa ou em outro lugar)?

(ENTRAR HORAS POR/DIA DE SEMANA.) ☐ ☐

Menos de 1 hora por dia de semana..... 0

9. Agora tente se lembrar sobre um dia de final de semana em sua casa. Quanto tempo você diria seu filho gasta vendo TV (em sua casa ou em outro lugar)?

(ENTRAR HORAS POR/DIA DE SEMANA.) ☐ ☐

Menos de 1 hora por dia de semana..... 0

10. Seu filho(a) mantém contato com seu pai, padrasto ou a figura paterna?
Sim 1
Não 0

11. Esta pessoa é pai biológico, padrasto, ou figura paterna (tio por exemplo)?
Pai biológico 1
Padrasto 2
Figura paterna 3
Sem pai, padrasto ou figura paterna 4

12. Qual a relação com **você**?
(MARQUE SOMENTE **UMA**)
Seu marido.....1
Seu ex-marido 2
Seu Companheiro.....3
Seu ex-companheiro.....4
Seu namorado.....5
Seu ex-namorado.....6
Seu noivo.....7
Seu amigo.....8
Seu pai.....9
Seu avô.....10
Seu irmão.....11
Seu tio 12
Outro (Quem).....13

Sem pai, padrasto, ou figura paterna.....14

13. Com que frequência seu filho(a) mantém contato com ele?
(MARQUE SOMENTE **UMA**)

Uma vez ao dia ou mais..... 1
Ao menos 4 vezes por semana ..... 2 = 1

Cerca de uma vez por semana..... 3
Cerca de uma vez por mês..... 4
Poucas vezes por ano ou menos ..... 5 = 0
Nunca.....6
Sem pai, padrasto, ou figura paterna..... 7

14. Com que frequência seu filho(a) realiza com ele em atividades fora de casa?
(MARQUE SOMENTE **UMA**)

Uma vez ao dia ou mais..... 1
Ao menos 4 vezes por semana ..... 2 = 1

Cerca de uma vez por semana..... 3
Cerca de uma vez por mês..... 4
Poucas vezes por ano ou menos ..... 5 = 0
Nunca.....6
Sem pai, padrasto, ou figura paterna..... 7
Não sei.....98

15. Com que frequência seu filho(a) faz as refeições com ambos os pais (padrasto ou figura paterna)?
(MARQUE SOMENTE **UMA**)

Mais de uma vez ao dia..... 1
Uma vez ao dia ..... 2 = 1

Várias vezes na semana..... .3
Cerca de uma vez por semana..... 4
Cerca de uma vez por mês..... 5 = 0
Nunca.....6
Sem pai, padrasto, ou figura paterna..... 7

16 Quando sua família assiste a TV juntos, você ou o pai (padrasto ou figura paterna) da criança discutem os programas de TV com eles?

Sim.....1 = 1
Não.....0 = 0
Não tem TV..... ……..2 = -4 recode

17. Quão próximo seu filho(a) se sente de . . .
RESPONDA PARA CADA ITEM

	Extremamente próximo	**Bem próximo**	**Razoavelmente próximo**	**Nem um pouco**	**Não tem esse parente**
A. . . . você?	1	2	3	4	
B. . . . seu/sua pai biol.?	1	2	3	4	5
C. . . . seu/sua madrasta?	1	2	3	4	5
D. . . . seu/sua padrasto?	1	2	3	4	5

18.As vezes as crianças ficam zangadas com seus pais que os fazem dizer coisas como “Te odeio” ou xingar num momento de raiva. Como você geralmente reage nessas vezes?
(MARQUE TODAS AS PERTINENTES)

Se Q22for codificada 2 ou “outra” = 0, então Q22(Rec) = 0 else Q22(Rec) = 1

Põe de Castigo 1
Dá uma surra ou palmada.....2
Conversa com a criança.....3
Dá a ela tarefa doméstica.....4
Manda pra o quarto por 1 hora.....6
Corta a mesada..... 7

Note: “Outra”

Respostas recodificada como 0 if harsh or 1 if not harsh

Proíbe de ver TV ou outros privilégios.....8
Coloca a criança de "escanteio"..... 10
Outra (Diga qual)_____ 9

19. Se seu filho(a) trouxer um boletim com notas baixas, que atitude você provavelmente tomaria... (MARQUE UM NÚMERO PARA CADA QUESTÃO).

	Muito Provável	*Talvez*	*Não tô certa do quanto*	*Improvável*	*De forma alguma*
a. entraria em contato com o professor ou diretorl?	5	4	3	2	1
b. chamaria a atenção?	5	4	3	2	1
c. ficaria mais atenta às suas atividades?	5	4	3	2	1
d. puniria a criança?	5	4	3	2	1
e.conversaria com a criança?	5	4	3	2	1
f. espera pra vê se a criança melhora por si só?	5	4	3	2	1
g. fala para a criança passar mais tempo com os deveres de classe?	5	4	3	2	1
h. passa mais tempo ajudando com os deveres de casa?	5	4	3	2	1
i. limita ou reduz as atividades extra escola (brincar, esporte, lazer, etc).?	5	4	3	2	1

j. Outra (Favor indique quais).

20. Quantas vezes na última semana você…

NÚMERO DE VEZES/ÚLTIMA SEMANA

a. Teve que dar uma surra ou palmada no seu filho? ≥ 2 = 0/0 ou 1 = 1 ☐☐

b. Botou de castigo? ☐☐

c. Proibiu de ver TV ou tirar outras regalias? ☐☐

d. Elogiou seu filho(a) por ter feito algo válido? ☐☐

e. Cortou a mesada? ☐☐

f. Demonstrou afeto físico(beijo, abraço, carinho no cabelo, etc)? ☐☐

g. Mandou a criança pro quarto? ☐☐

h. Fez comentário positivo sobre a criança com outro adulto (esposo, amiga, colega de trabalho, visita ou parente)? ☐☐