REJANE CORRÊA MARQUES

"AMAMENTAÇÃO EXCLUSIVA E ESTENDIDA COMO UM FATOR DE PROTEÇÃO À EXPOSIÇÃO AO MERCÚRIO DERIVADO DE VACINAS CONTENDO TIMEROSAL"

TESE SUBMETIDA À UNIVERSIDADE FEDERAL DO RIO DE JANEIRO VISANDO A OBTENÇÃO DO GRAU DE DOUTOR EM CIÊNCIAS

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Tese apresentada ao Programa de Pós-Graduação em Ciências Biológicas (Biofísica) do Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, como parte dos requisitos necessários à obtenção do título de Doutor em Ciências.

Orientador: Prof. Dr. Olaf Malm
Co-orientador: Prof. Dr. José Garrofe Dórea

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Rio de Janeiro, 19 de dezembro de 2007.

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Volte para o seu lar. Volte para lá!

ARNALDO ANTUNES

RESUMO

MARQUES, Rejane Corrêa: **Amamentação exclusiva e estendida como fator de proteção à exposição ao mercúrio derivado de vacinas contendo timerosal.** Rio de Janeiro, 2007. Tese (Doutorado em Ciências Biológicas – Biofísica) – Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, 2007.

Crianças são expostas ao mercúrio (Hg) proveniente das mães (através da placenta e aleitamento materno), do ambiente (alimentos), e em muitas partes do mundo, das vacinas contendo timerosal (VCT) recebidas durante a imunização. O timerosal contém 49.6% etilmercúrio (EtHg) por peso. Devido às incertezas associadas a um possível aumento de déficits neurodesenvolvimentais entre crianças imunizadas, as vacinas conservadas com timerosal não são usadas desde 2004 nos Estados Unidos da América (EUA) e na União Européia (UE), mas são amplamente produzidas e utilizadas em países em desenvolvimento, caso do Brasil. Nós investigamos o impacto do timerosal no cabelo de crianças que receberam VCT de acordo com o calendário de imunização preconizado pelo Ministério da Saúde do Brasil. Usando o Hg total no cabelo como marcador de exposição pós-natal ao Hg orgânico (Hg inorgânico e MeHg do leite materno; EtHg do timerosal) nós estudamos sua associação com a escala de Gesell (QD) mensurada aos 6, 36 e 60 meses e a influência da amamentação exclusiva no crescimento e desenvolvimento de crianças de Porto Velho, Rondônia. As vacinas forneceram uma exposição EtHg de 12,5 µg ao nascimento (mínima) a 37,5 µg aos seis meses (máxima), podendo perfazer um total de 325 µg de Hg em cinco anos. Enquanto as mães apresentaram as concentrações mais altas de Hg no parto, as crianças apresentaram valores mais altos aos seis meses após completar o esquema de imunização com VCT para o período. Posteriormente, a tendência descendente do Hg no cabelo demonstrado pelas crianças coincidiu com o desmame e menos freqüentemente com o período de vacinação. O tempo de amamentação e o Hg no cabelo foram, cada um, significativamente correlacionados com QD, mas de maneiras opostas: o tempo de amamentação foi positiva e significantemente associado com o QD aos cinco anos; as concentrações de Hg no cabelo foram negativa e significativamente correlacionadas com QD aos seis meses ($r=-0.3329$; $p=0.0022$) e cinco anos ($r=-0.8029$; $p=0.0106$), mas não aos 36 meses ($r=-0.1722$; $p=0.1218$). O resultado dos QD aos cinco anos dependeu do QD aos três anos, que por sua vez, foi influenciado pelas variáveis do desenvolvimento e de exposição ao Hg. O QD aos seis meses foi significantemente influenciado pela exposição pré-natal (Hg no cabelo materno e infantil) e pós-natal aos seis meses. Nós acreditamos que o aleitamento materno é uma importante estratégia para a proteção do SNC de crianças frente aos desafios da exposição precoce ao Hg. O presente estudo foi financiado pelo Ministério da Saúde do Brasil (MS) e Organização das Nações Unidas para Educação, Ciência e Cultura (UNESCO), processo SC27824/2005/914-BRA2000-Decit-PRODOC.

Palavras-chave: Timerosal; etilmercúrio; vacinas; crianças; aleitamento materno; neurodesenvolvimento; Escala de Gesell.

ABSTRACT

MARQUES, Rejane Corrêa: **Amamentação exclusiva e estendida como fator de proteção à exposição ao mercúrio derivado de vacinas contendo timerosal.** Rio de Janeiro, 2007. Tese (Doutorado em Ciências Biológicas – Biofísica) – Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, 2007.

Children are exposed to Hg from mothers (via placenta and lactation), environment (food), and in many parts of the world by thimerosal-containing vaccines (TCV) during immunization. Thimerosal contains 49.6% Hg by weight. Because of uncertainties associated with a possible rise in neurodevelopmental deficits among vaccinated children, TCV have not been used since 2004 in the USA and the EU but are widely produced and used in other developing countries. This longitudinal study (birth to 5 years) investigated the impact of thimerosal on the total Hg in hair of breast-fed infants that received TCV according to the immunization schedule recommended by the Ministry of Health of Brazil. Using total HHg as a marker of post-natal organic Hg exposure (inorganic and methylmercury from breast milk, and ethylmercury from thimerosal) we studied its association with Gesell schedules measured at 6m, 36m and 60m, and the influence of exclusive breastfeeding on growth of this sample of children from Porto Velho, Western Amazon. TCV provided an ethylmercury (EtHg) exposure of 12.5 µgHg (minimum) up to 37.5 µgHg (maximum). The cumulative exposure by age 5 years could reach 325 µgHg. While mothers had the highest HHg concentrations at childbirth, infants showed the highest HHg values at 6 months after the recommended six shots of immunization with TCV; after that, the downward trend in HHg shown by children coincided with both weaning and less frequent vaccination period (5 years). Length of lactation and HHg were each significantly correlated with Gesell scores, but in opposite ways: length of lactation was positive and significantly correlated with all Gesell scores at 60m; HHg concentrations were negative and significantly correlated with Gesell scores at 6m ($r=-0.3329$; $p=0.0022$) and 60m ($r=-0.8029$; $p=0.0106$), but not at 36m ($r=-0.1722$; $p=0.1218$). The DQ outcome at 60m depended on DQ at 36m that in turn was influenced by infants developmental and Hg exposure variables. DQ at 6m was significantly influenced by prenatal (maternal and infant HHg at birth) and post-natal Hg exposure at 6 months. Until a more refined approach to recognize sensitive children to TCV-EtHg exposure, the ND benefit of breastfeeding should suffice to recommend it in situations of uncertainty. United Nations Educational, Scientific, and Cultural Organization (UNESCO) and Ministry of Health of Brazil (MS) supported this work, grant SC27824/2005/914BRA2000 Decit PRODOC.

Key words: Thimerosal, ethylmercury neurodevelopment, vaccines, Gesell scores, breastfeeding.

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LISTA DE SIGLAS E ABREVIATURAS

AAP – AMERICAN ASSOCIATION OF PEDIATRICS

AIG – ADEQUADO PARA A IDADE GESTACIONAL

ANVISA – AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA

ATSDR – AGENCY FOR TOXIC SUBSTANCES AND DISEASE REGISTRY

CDC – NATIONAL CENTER FOR DISEASE CONTROL AND HEALTH PROMOTION

CEP – COMITÊ DE ÉTICA EM PESQUISA COM SERES HUMANOS

DDA – DISTÚRBIO DO DÉFICIT DE ATENÇÃO

DHHS – DEPARTMENT OF HEALTH AND HUMAN SERVICES

dT – VACINA DUPLA ADULTO DIFTERIA-TÉTANO

DT – VACINA DUPLA INFANTIL DIFTERIA-TÉTANO

DTP – VACINA TRÍPLICE BACTERIANA (DIFTERIA, TÉTANO E COQUELUCHE)

DTP + HIB – VACINA QUÁDRUPLA BACTERIANA, ASSOCIANDO DTP À Hib

EDGA – ESCALA DESENVOLVIMENTAL DE GESELL E AMATRUDA

EtHg – ETILMERCÚRIO

EUA – ESTADOS UNIDOS DA AMERICA

FDA – FOOD AND DRUGS ADMINISTRATION

GIG – GRANDE PARA A IDADE GESTACIONAL

Hep B – VACINA CONTRA HEPATITE B

Hg – MERCÚRIO

Hib – *Haemophilus Influenzae* tipo b

IOM – INSTITUTE OF MEDICINE

ISRC – IMMUNIZATION SAFETY REVIEW COMMITTEE

LOAEL - NÍVEL MAIS BAIXO EM QUE EFEITO ADVERSO FOI OBSERVADO

MeHg – METILMERCÚRIO

SRC ou MMR – VACINA TRÍPLICE VIRAL (SARAMPO, RUBÉOLA E CAXUMBA)

MS – MINISTÉRIO DA SAÚDE

NCHS – NATIONAL CENTER FOR HEALTH STATISTICS

ND – NEURODESENVOLVIMENTAIS

OMS – ORGANIZAÇÃO MUNDIAL DA SAÚDE

OPAS – ORGANIZAÇÃO PAN-AMERICANA DE SAÚDE

PC – PERÍMETRO CEFÁLICO

PIG – PEQUENO PARA A IDADE GESTACIONAL

PNI – PROGRAMA NACIONAL DE IMUNIZAÇÃO BRASILEIRO

PUFA – POLYUNSATURED FATTY ACIDS.

QD – QUOCIENTE DE DESENVOLVIMENTO

QI – QUOCIENTE DE INTELIGÊNCIA

RN – RECÉM-NASCIDO

SIDA – SÍNDROME DA IMUNO DEFICIÊNCIA HUMANA ADQUIRIDA

SNC – SISTEMA NERVOSO CENTRAL

TDAH – TRANSTORNO DE DÉFICIT DE ATENÇÃO E HIPERATIVIDADE

UE – UNIÃO EUROPÉIA/COMUNIDADE EUROPÉIA

UFRJ – UNIVERSIDADE FEDERAL DO RIO DE JANEIRO

UNESCO – ORGANIZAÇÃO DAS NAÇÕES UNIDAS PARA EDUCAÇÃO, CIÊNCIA E CULTURA

UNIR – FUNDAÇÃO UNIVERSIDADE FEDERAL DE RONDÔNIA

VCT – VACINAS CONSERVADAS COM TIMEROSAL

VICP – VACCINE INJURY COMPENSATION PROGRAM

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1 INTRODUÇÃO

O mercúrio (Hg) é encontrado no ambiente em diversas formas químicas: Hg elementar, inorgânico, e orgânico (etil-, metil-, alquil-, ou fenil-Hg). A química do Hg modula sua toxicidade e metabolismo. Enquanto o Hg inorgânico atua principalmente nos rins, o Hg metálico volátil e, sobretudo, o metilmercúrio (MeHg) afetam o sistema nervoso central (SNC). As atuais questões sobre a toxicidade do Hg estão focadas nos prováveis danos neurológicos em baixos níveis de exposição. Conquanto a exposição a algum nível de Hg seja universal, a avaliação quantitativa mostrou que os três maiores contribuidores da exposição ao Hg para a população geral são: dieta (basicamente peixe), amálgama dental e alguns produtos farmacológicos (Clarkson e Magos, 2006; Dórea, 2007; Marques *et al.*, 2007a).

As formas químicas do Hg são cruciais no entendimento e na avaliação da exposição infantil e do risco de toxicidade. A vulnerabilidade do cérebro humano em desenvolvimento é a mais importante janela para os insultos dos compostos de Hg (Castoldi, 2003; Dórea, 2007). A gênese e o desenvolvimento do SNC durante a gravidez e lactação podem ser afetados por múltiplas causas, variando de nutrição materna a exposição a substâncias tóxicas. Nesta janela de tempo específica, o Hg pode afetar funções neurocomportamentais. A exposição moderada pode resultar em sintomas de atraso (não observados ao nascimento) no andar e falar, além da persistência de reflexos perinatais (Davidson *et al.*, 1998; Grandjean *et al.*, 1997; WHO, 1976; 1990). Pondera-se que a exposição materna ao MeHg (do consumo de peixes) pode afetar o neurodesenvolvimento, diminuir a inteligência e provocar

alteração do comportamento da prole (Clarkson, 1998; Grandjean *et al.*, 2006). O recém-nascido (RN) é particularmente vulnerável a estes efeitos porque seu SNC ainda está se desenvolvendo.

Outra fonte de preocupação relacionada ao Hg é o seu uso, na forma de timerosal, como agente anti-séptico de escolha para prevenir a contaminação bacteriológica em vacinas, apresentando concentração de timerosal até 0,01% (Amanna e Slifka, 2005). Como o etilmercúrio (EtHg) recebido na vacinação não é parte da alimentação, ele pode atravessar a barreira e detoxificação do sistema entero-hepático, não sendo fisiologicamente processado, podendo migrar para o cérebro (Cory-Slechta, 2005; Dórea, 2007; Marques *et al.*, 2007a). Devido a sua lipossolubilidade, o EtHg pode se acumular em seres humanos e cruzar a barreira hematoencefálica e placentária, apresentando uma meia-vida curta no sangue. Pode depositar-se no SNC, onde é transformado posteriormente em Hg inorgânico acumulando-se no cérebro animal e humano (Clarkson, 2002).

Em razão da limitada base de informação disponível, concluiu-se que os efeitos nocivos do EtHg podem ser análogos àqueles do MeHg, outro organomercurial de conhecida neurotoxicidade (Malm, 2001; Clarkson *et al.*, 2007). É fato que o Hg é tóxico para o cérebro humano em desenvolvimento, e, por isso, evitar a exposição durante os períodos críticos do desenvolvimento do SNC tem sido alvo de estratégias de saúde pública. Para proteger crianças dos efeitos nocivos do Hg há recomendações para se evitar a ingestão de peixes durante a gravidez (Cohen *et al*., 2005, Dórea, 2007) e, apesar do uso de vacinas conservadas com timerosal (VCT) ter sido banido nos Estados Unidos (EUA) e União Européia (UE), a Organização Mundial da Saúde (OMS) consideram-nas seguras para uso pediátrico (CDC, 2001; WHO, 2000, 2007).

Sem uma sistemática síntese do conhecimento, as restrições para o consumo

de peixe se tornaram regra para prevenir os efeitos neurotóxicos do metilmercúrio (MeHg) em recém-nascidos (Cohen *et al.*, 2005; Dorea, 2007). Contudo, o consenso precedeu as evidências no caso de exposição à EtHg de VCT, quando o Comitê de Avaliação sobre Segurança da Imunização do Instituto de Medicina dos EUA (Immunization Safety Review Committee – IOM/ISRC) concluiu que a hipótese de que as VCT poderiam estar associadas com desordens neurodesenvolvimentais (DN) era biologicamente plausível (US CDC, 1999; 2001). Apesar dos estudos epidemiológicos não mostrarem associações entre VCT e autismo, há base para as preocupações referentes a outras DN.

A capacidade de um recém-nascido de lidar com o Hg é modulada pelo seu grau de imaturidade, ou seja, limitada pela produção da bile e função renal, vias metabólicas subdesenvolvidas. Essas injúrias são acentuadas mais tarde por diversos fatores associados com a exposição ao Hg: fonte (formas químicas de Hg inorgânica e orgânica), rota (enteral/aleitamento materno versus parenteral/VCT) e via (intrínseco na matriz protéica do leite humano vs. extrínseco na forma química pura-EtHg) (Dorea, 2007).

RN e crianças (especialmente aquelas de países menos desenvolvidos) representam a população de maior risco para DN (devido à imaturidade anatômica, fisiológica e bioquímica) e aquelas mais expostas ao Hg – quando imunizadas com VCT e após consumo de peixe materno (Marques *et al.*, 2007b). Devido à grande variabilidade na suscetibilidade infantil e circunstâncias modificáveis (desenvolvimento anatômico e funcional), há um risco presumível de DN transitória sutil como seqüela de VCT. Entretanto, como os efeitos sutis da exposição ao Hg sobre o SNC podem se manifestar muito tempo depois da exposição e, porquanto este problema está vinculado à ausência de marcadores apropriados (de exposição e efeito), há sérias

dificuldades em estabelecer causa e efeito (ou mesmo relações). Por isso, a conexão entre DN e exposição precoce a esquemas de vacinação com VCT continua intricada (Marques *et al.*, 2007c; 2007d; 2007e). Portanto, isto é importante para distinguir casos de baixo risco e alto risco (relacionados com a variação na imaturidade ou suscetibilidade de crianças a termo).

A breve duração da amamentação é considerada um fator de risco para sinais de distúrbio do déficit de atenção (DDA) e de hiperatividade. Presume-se que haja um ganho de 3.2 pontos no quociente de inteligência – QI (após ajustamento para o QI materno) entre seis meses e 15 anos (Anderson, *et al.*, 1999; Dorea, 2007). O risco irrefutável da dose de timerosal (0.01%) usado nas vacinas infantis é mascarado pela insuficiência de conhecimento científico sobre a toxicocinética e toxicodinâmica do EtHg em crianças com diferentes taxas de maturidade (Dorea, 2007, Marques *et al.*, 2007e). Interações complexas são entraves para definir se a segurança da dose de EtHg é superestimada devido a interação antagonista com o aleitamento materno ou subestimada devido a alimentação alternada e outras co-exposições em RN e crianças (Dórea, 2007; Marques et *al.*, 2007d; 2007e).

Os estudos recentes associando exposição ao Hg e DN em crianças dependeram das concentrações desse metal no cabelo. O cabelo é composto de uma proteína complexa formada por aminoácidos que se ligam ao MeHg. Admite-se que a ocorrência de DN é resultado do consumo de peixe contaminado com MeHg, que rapidamente de liga ao cabelo (Cernichiari *et al.*, 2007). Além disso, estudos epidemiológicos direcionados para a exposição ao consumo de peixes têm usado concentrações de Hg no cabelo como um indicador confiável.

Entretanto, durante os primeiros cinco anos de vida, crianças podem ser expostas ao Hg nas mudanças das suas fontes de alimentação: do leite materno (ou

fórmulas) para dietas de desmame e, mais tarde, de outras fontes alimentares (que podem incluir o peixe) quando passar a se alimentar com “comida de adulto”. Essas crianças podem ser expostas, também, a outra forma de Hg: ao EtHg quando imunizadas com vacinas conservadas com timerosal (Marques *et al.*, 2007a, 2007e).

Crianças amazônicas são expostas a MeHg via transferência placentária e leite materno (Barbosa *et al*., 1998; Barbosa e Dorea, 1998; Marques *et al.*, 2007f). Neste contexto, o aumento no risco de DN associado à MeHg naturalmente presente nos peixes dos rios amazônicos foi fortemente sugerido (Grandjean *et al.*, 1999). A probabilidade do aumento de DN nestes estudos foi baseada nas concentrações de Hg no cabelo (biomarcador de consumo de peixe). Nós levantamos a hipótese que, pelo menos nos primeiros seis meses de vida, as concentrações de Hg no cabelo podem ser influenciadas pelo pesado calendário vacinal (com vacinas conservadas com timerosal) (Marques *et al.*, 2007a). Além disso, durante os primeiros cinco anos, a susceptibilidade a fatores modificadores permanece desconhecida, mas as mudanças fisiológicas e alimentares (amamentação, desmame e dieta familiar tradicional) provavelmente influenciam essas mudanças (Marques *et al.*, 2007e).

As incertezas sobre a segurança das VCT e a controvérsia sobre a associação entre o EtHg das vacinas e DN, incluindo autismo, são questões polêmicas que envolvem tanto os cientistas quanto a sociedade (Bernard *et al.*, 2001; Bigham e Copes, 2005; Clements e Mcintyre; 2006; Coleman, 2003; COMED, 2007; Geier e Geier, 2007a; Malm, 2001; Mutter *et al.*, 2005; Marques *et al.*, 2007b; 2007c; 2007d). Por isso, vacinas VCT não são administradas em crianças norte-americanas desde 2002 e européias desde 2004. Contudo, por recomendação da OMS, o timerosal ainda é amplamente utilizado como conservantes de vacinas produzidas para os países em desenvolvimento, como o Brasil (Migowski, 2007; Marques *et al.*, 2007b).

1.1 TIMEROSAL

O timerosal (ou tiomersal) é um composto organomercurial proveniente do sal sódico do ácido o (etilmercuritio) benzóico que contém 49.6% etilmercúrio (EtHg) por peso. É um pó cristalino de cor branca a amarelo-clara, solúvel em água e em metanol, e insolúvel em éter e benzeno (Prado *et al.*, 2004). É representado pela fórmula estrutural $C_9H_9HgNaO_2S$ e sua estrutura química é a seguinte:

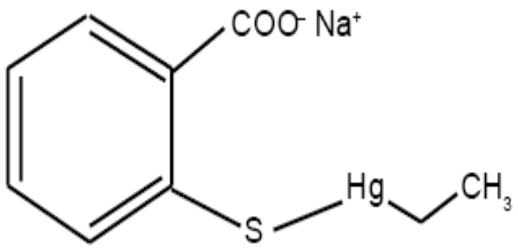


Figura 1.Estrutura Química do Timerosal

Também conhecido como: “thiomersal” (Europa), “thimerosol”, “thimerosal” (EUA), “merthiolate”, mertiolate, tiomersal, timerosol, etilmercúrico de sódio e mertiolato de sódio, foi historicamente adicionado a vacinas como agente antimicrobiano (0.005 a 0.01%). Transforma-se no organismo em EtHg (CH3-CH2-Hg+) e tiosalicilato, sendo um composto químico altamente instável (Figura 2).

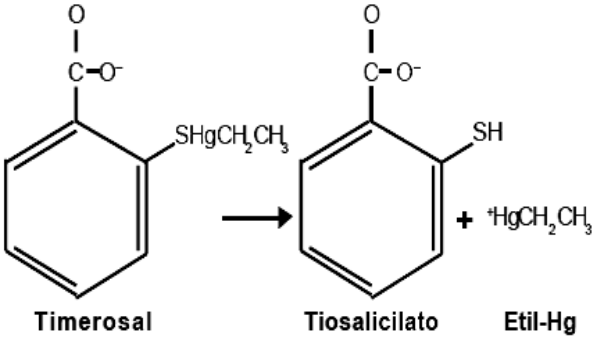


Figura 2. Estrutura química do EtHg

A fórmula patenteada pela Eli Lilly Company é utilizada como adjuvante farmacotécnico com função de conservante em produtos farmacêuticos, sendo

amplamente empregada em vacinas e em líquidos protetores de lentes oftálmicas (Geier *et al.*, 2007; Prado *et al.*, 2004; Migowski, 2007). Durante vários anos, este fármaco foi usado como agente bacteriostático e fungistático tópico, sendo apresentado na forma de tintura (solução hidroalcoólica a 0,1%), geralmente, indicado para anti-sepsia de pequenas escoriações e ferimentos. A literatura é significativa sobre as propriedades anti-sépticas do timerosal, comparando-o com os outros compostos químicos empregados como anti-sépticos e concluindo que sua ação é moderada (Litter, 1986; Prado *et al.*; 2004).

O timerosal, usado para uso tópico, chegou a ser um dos medicamentos de maior venda livre, pelo fato de ser único no mercado e estar aliado a uma forte propaganda que prometia muito mais eficácia do que realmente tinha (Ball *et al.*, 2001; Geier *et al.*, 2007; Prado *et al.*, 2004; Rodrigues e Saito; 1991; Valle *et al.*, 1991). A avaliação risco/benefício ficava evidente quando se comparava o coeficiente fenólico do timerosal com os demais anti-sépticos. Litter (1986) cita que o coeficiente fenólico da polivinilpirrolidonaiodo é 200, enquanto o timerosal apresenta 40. Prado *et al.* (2004) descreve que o fármaco tem pouca atividade contra bactérias gram-negativas, sendo as *Pseudomonas* e *Staphylococcus aureus* resistentes aos derivados de amônio quaternário.

No Brasil, em 8 de junho de 2001, o Diário Oficial da União publicou a resolução nº 528, do Ministério da Saúde (de 17 de abril do mesmo ano), que suspendeu a venda de produtos à base de timerosal (como o mertiolate e o mercurocromo) em todo o país. A Agência Nacional de Vigilância Sanitária (ANVISA) proibiu o uso desse composto alegando tratar-se de uma substância mercurial que, segundo diretrizes internacionais, ofereceria risco de toxicidade aos usuários. Em seu comunicado à imprensa, a ANVISA diz que a decisão foi tomada “tendo em vista

a tendência mundial da diminuição da exposição de seres humanos a produtos à base de derivados de mercúrio", e determina à imediata "proibição da utilização de derivados de mercúrio em medicamentos fabricados no Brasil, **exceto vacinas**". O comunicado esclarece ainda que "o tiomersal (derivado de mercúrio) não será usado como remédio, mas **apenas** como conservante de vacinas, por recomendação da Organização Mundial de Saúde". (grifo meu)

A retirada abrupta do timerosal por exigência da ANVISA veio trazer a público uma série de questionamentos, entre eles, por qual razão este fármaco ficou tanto tempo no mercado, se desde 30 de junho de 1980 já existia a Portaria n. 07/Dimed (DO n. 125 de 7/7/1980), revogada pela Portaria 10/80, proibindo a fabricação de medicamentos contendo substâncias compostas de Hg isoladas ou associadas e dava prazo de 30 dias para as empresas modificarem a formulação sob pena de revogação do respectivo registro. O fabricante do produto referência (Merthiolate), ao tomar conhecimento da Resolução 528/2001 da ANVISA, imediatamente substituiu o timerosal por um composto derivado de amônio quaternário, o cloreto de benzalcônio, que segundo Prado *et al.* (2004) também apresenta fracas propriedades anti-sépticas.

1.2 VACINAS

Entre as intervenções de saúde pública que tiveram maior impacto sobre a saúde das pessoas estão a água potável e as vacinas. Graças a pioneiros como Edward Jenner e Louis Pasteur, as vacinas evitam doenças e morte de milhões de pessoas no mundo inteiro, todos os anos. Porém, há ainda um longo caminho a percorrer. Quando se discute a medicina moderna, poucos temas são tão polêmicos quanto à vacinação. É uma história repleta de jogos políticos, grandes empresas, pais desesperados, crianças doentes e morrendo, conflitos de interesse e a segurança

nacional – que envolve a controvérsia do aborto, uma vez que muitas vacinas são criadas utilizando células de fetos abortados (Allen, 2007; Kennedy, 2005; Moreira, 2002, Temporão, 2003; Weisberg, 2007).

O assunto é polêmico.

A oposição às vacinas não é algo realmente "novo". E apesar de contestada desde suas origens, a vacina acumula defensores entre a maioria dos profissionais de saúde, cientistas e autoridades que atuam na esfera da saúde coletiva, bem como uma quantidade significativa de ferrenhos adversários. Hoje, as vacinas e os protocolos tradicionais de imunização estão sob fogo cruzado e as pessoas tem se perguntado se os riscos são dignos dos benefícios que elas proporcionam. Contudo, é inegável que as vacinas são a parte de benéfica de uma estratégia global de saúde preventiva que reduziu grandemente as mortes por doenças infecciosas e levou à erradicação da varíola, a eliminação da poliomielite na maioria dos países e ao controle de muitas outras doenças, incluindo sarampo, rubéola, tétano e difteria (Allen, 2007; Moulin, 2003; Ponte, 2003; Stewart e Devlin, 2006; Weisberg, 2007).

Então, não é surpreendente que haja um debate atual sobre a segurança e a importância das vacinas, pois isso já acontecia antes mesmo da primeira vacina ser introduzida. A vacina foi desenvolvida a partir de uma antiga técnica praticada na Ásia – a “variolização” – que consistia em infectar um paciente são com um fluido de uma pústula de varíola humana. Isto geralmente reduzia a severidade da infecção, pois causava uma manifestação moderada e controlada da varíola, tornando os inoculados imunes à doença (Stewart e Devlin, 2006; Wilson e Marcuse, 2001).

Esta prática foi introduzida no Reino Unido, em 1721, por Lady Mary Wortley Montagu, esposa de um diplomata britânico na Turquia. Lá, ela notou que algumas senhoras não tinham cicatrizes da doença. Prontamente, ela inoculou seus dois

filhos e se tornou uma ativista da variolização (Stewart e Devlin, 2006; Weisberg, 2007; Wilson e Marcuse, 2001). A popularidade da variolização aumentou pouco a pouco, apesar da forte oposição de muitos médicos que igualavam a técnica ao assassinato de uma criança, ao expô-la a doença (Stewart e Devlin, 2006). Benjamin Franklin inicialmente se opôs a variolização, mas mudou de idéia após seu filho de 4 anos, não vacinado, morrer em decorrência da varíola. Ele escreveu em sua autobiografia que "lamentou amargamente" não ter vacinado seu filho (Stewart e Devlin, 2006; Weisberg, 2007).

A vacinação foi, inicialmente, realizada em 1796 por Edward Jenner. Ele observou que ordenhadores de vaca mostravam-se imune à varíola. Todas essas pessoas tinham se contaminado com *cowpox*, uma doença do gado semelhante à varíola, pela formação de pústulas, mas que não causava a morte dos animais. Após uma série de experiências, constatou que estes indivíduos mantinham-se refratários à varíola, mesmo quando inoculados com o vírus. Com o pus retirado de uma pústula de uma ordenhadora que apresentava *cowpox*, ele inoculou o filho de seu jardineiro. O garoto contraiu uma infecção benigna e, dias depois, estava recuperado. Meses depois, expôs Phipps a um pus varioloso e o menino, felizmente, não desenvolveu a doença (Jenner, 1798).

Era a descoberta da vacina.

Jenner publicou sua pesquisa em 1798, *Variolae Vacciniae*, mas persistia uma grande oposição na classe médica e na população em geral (Stewart e Devlin, 2006; Wilson e Marcuse, 2001). Grupos religiosos alertavam para o risco da degeneração da raça humana pelo contágio com material bovino, apelidado de “vacalização” ou “minotaurização” (Fig. 3). Essa generalizada oposição a vacina obrigatória de Jenner foi deflagrada por rumores e boatos que atribuíam as mais variadas complicações a

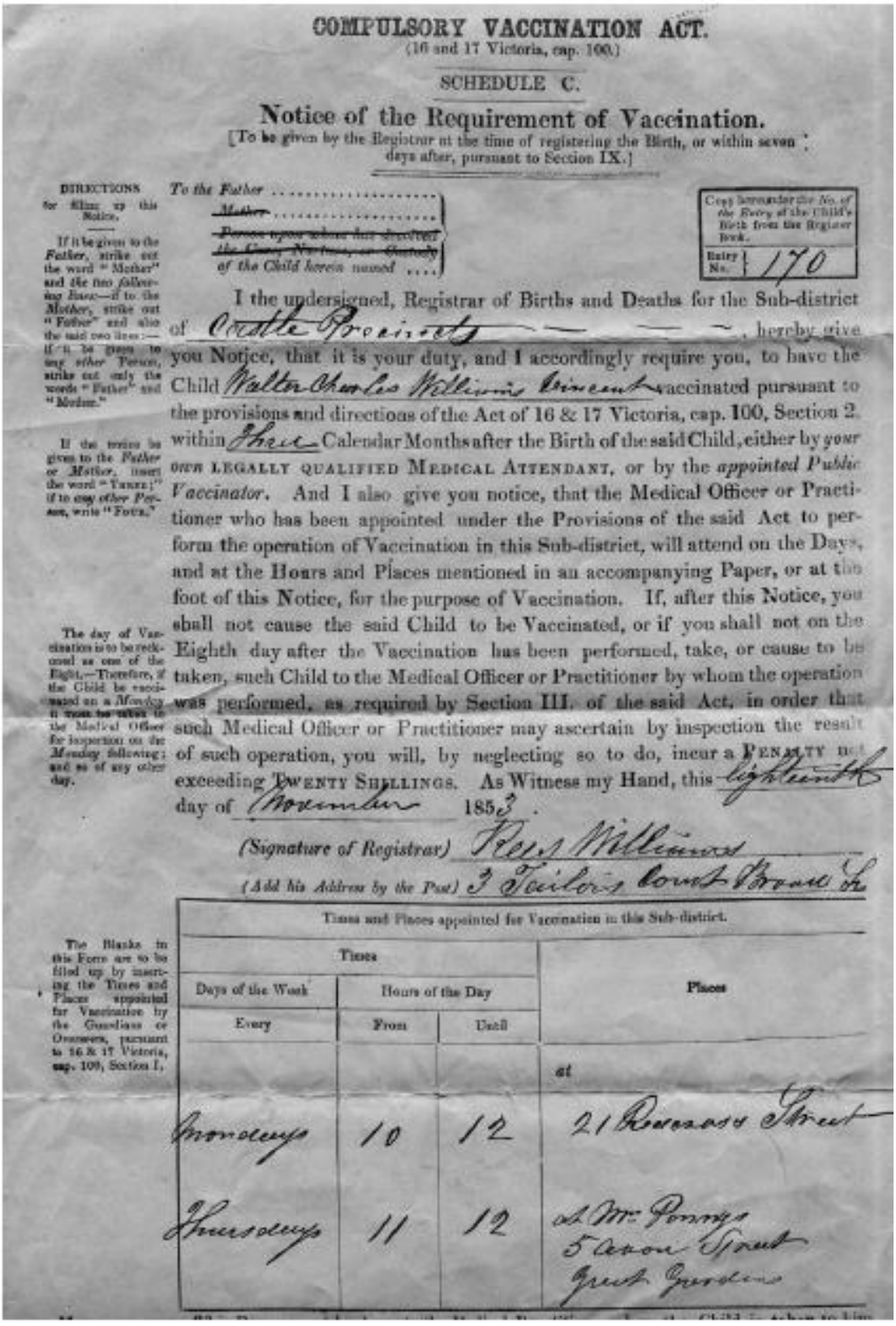
vacinação: de febres, amputação do braço e até mesmo a morte por “envenenamento do sangue meses mais tarde”.

As semelhanças entre a oposição à vacina contra a varíola e as controvérsias atuais em torno da vacinação são óbvias.

Figura 3.The Cow-Pock or The Wonderful Effects of the New[1]

No mundo inteiro se criou leis para a vacinação obrigatória, como o ato do parlamento inglês para promover a expansão e tornar compulsória a prática da vacinação (*Act to further extend and make compulsory the practice of vaccination*), de 1853. Este ato obrigava a toda criança ser vacinada dentro de três meses após o nascimento. No registro de nascimento da criança, os pais eram notificados sobre a obrigatoriedade da vacinação para se obter os registros de nascimento, casamento e morte (Fig. 4). Se a vacinação fosse bem-sucedida, um certificado de vacinação era entregue (Fig. 5).

[1] De James Gillray (1757-1815), foi publicado na Inglaterra em 12 de junho de 1802 pela Anti-Vaccine Society. O quadro mostra Edward Jenner entre pacientes no Small Pox and Inoculation Hospital, em St Pancras (Londres), e sugere a transformação de indivíduos vacinados em vacas, após a vacinação.



COMPULSORY VACCINATION ACT.
(16 and 17 Victoria, cap. 100.)

SCHEDULE C.

Notice of the Requirement of Vaccination.
[To be given by the Registrar at the time of registering the Birth, or within seven days after, pursuant to Section IX.]

DIRECTIONS for filling up this Notice.

If it be given to the *Father*, strike out the word "Mother" and the two following lines—if to the *Mother*, strike out "Father" and also the said two lines:—if it be given to any *other Person*, strike out only the words "Father" and "Mother."

If the notice be given to the *Father* or *Mother*, insert the word "THREE;" if to any *other Person*, write "FOUR."

The day of Vaccination is to be reckoned as one of the Eight,—Therefore, if the Child be vaccinated on a *Monday* it must be taken to the Medical Officer for inspection on the *Monday* following; and so of any other day.

The Blanks in this Form are to be filled up by inserting the Times and Places appointed for Vaccination by the Guardians or Overseers, pursuant to 16 & 17 Victoria, cap. 100, Section I.

To the Father .....
~~Mother~~ .....
~~Person upon whom has devolved the Care, Nurture, or Custody of the Child herein named~~ ....

Copy hereunder the No. of the Entry of the Child's Birth from the Register Book.
Entry No. 170

I the undersigned, Registrar of Births and Deaths for the Sub-district of Castle Precincts, hereby give you Notice, that it is your duty, and I accordingly require you, to have the Child Walter Charles Williams Vincent vaccinated pursuant to the provisions and directions of the Act of 16 & 17 Victoria, cap. 100, Section 2. within Three Calendar Months after the Birth of the said Child, either by your own LEGALLY QUALIFIED MEDICAL ATTENDANT, or by the *appointed Public Vaccinator*. And I also give you notice, that the Medical Officer or Practitioner who has been appointed under the Provisions of the said Act to perform the operation of Vaccination in this Sub-district, will attend on the Days, and at the Hours and Places mentioned in an accompanying Paper, or at the foot of this Notice, for the purpose of Vaccination. If, after this Notice, you shall not cause the said Child to be Vaccinated, or if you shall not on the Eighth day after the Vaccination has been performed, take, or cause to be taken, such Child to the Medical Officer or Practitioner by whom the operation was performed, as required by Section III. of the said Act, in order that such Medical Officer or Practitioner may ascertain by inspection the result of such operation, you will, by neglecting so to do, incur a PENALTY not exceeding TWENTY SHILLINGS. As Witness my Hand, this eighteenth day of November 185 3.

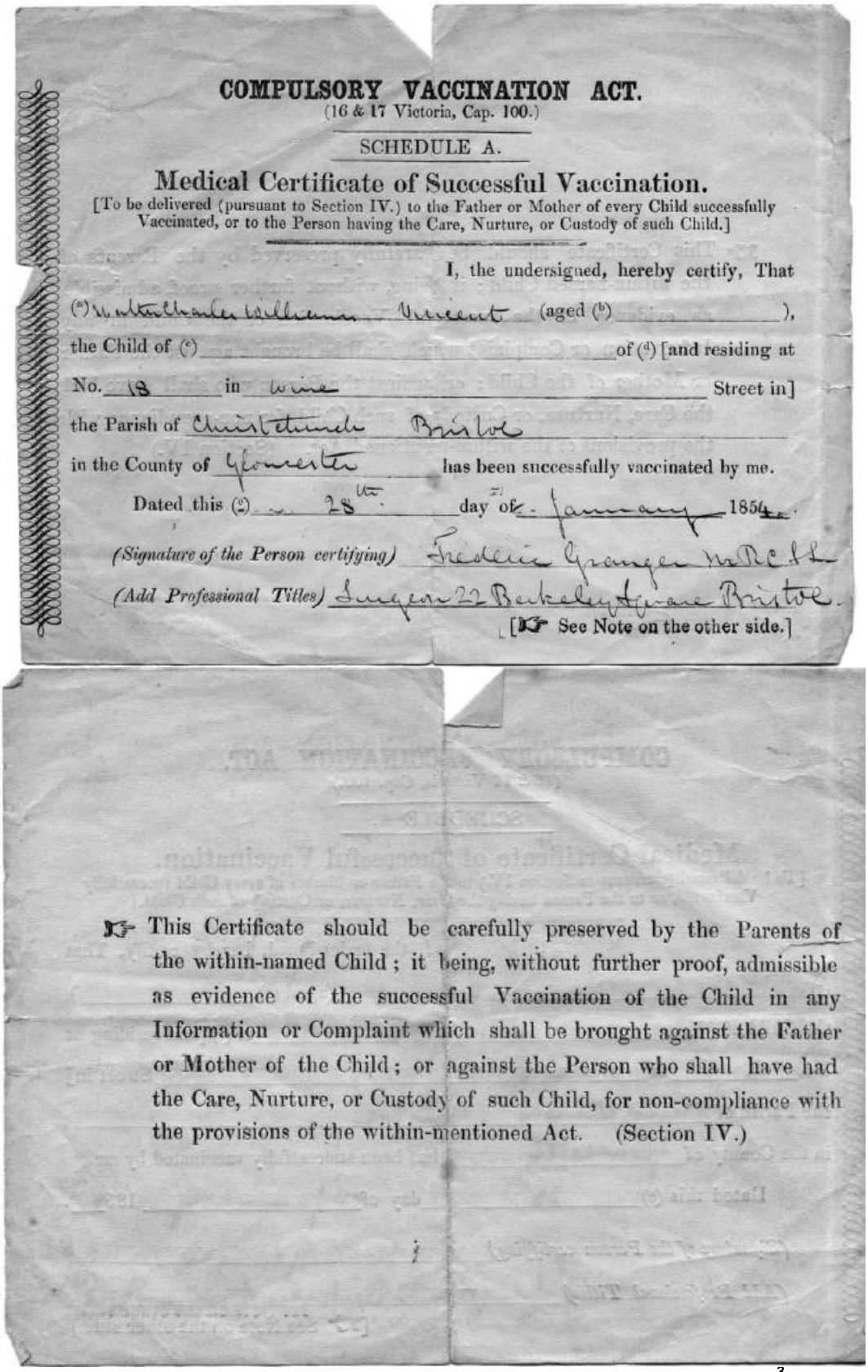
(Signature of Registrar) Rees Williams

(Add his Address by the Post) 3 Tailors Court Broad St

Times and Places appointed for Vaccination in this Sub-district.

Times			Places
Days of the Week	Hours of the Day		
Every	From	Until	
			at
Mondays	10	12	21 Berners Street
Thursdays	11	12	at Mr Penny's 5 Avon Street Great Portland

[2]Retirado de Stewart e Devlin, 2006.



COMPULSORY VACCINATION ACT.
(16 & 17 Victoria, Cap. 100.)

SCHEDULE A.

Medical Certificate of Successful Vaccination.

[To be delivered (pursuant to Section IV.) to the Father or Mother of every Child successfully Vaccinated, or to the Person having the Care, Nurture, or Custody of such Child.]

I, the undersigned, hereby certify, That

(a) Walter Charles William Vincent (aged (b)), the Child of (c) of (d) [and residing at No. 18 in Wine Street in] the Parish of Christchurch Bristol in the County of Gloucester has been successfully vaccinated by me.

Dated this (e) 28th day of January 1854.

(Signature of the Person certifying) Frederic Granger MRCSL

(Add Professional Titles) Surgeon 22 Berkeley Square Bristol.

[☞ See Note on the other side.]

☞ This Certificate should be carefully preserved by the Parents of the within-named Child; it being, without further proof, admissible as evidence of the successful Vaccination of the Child in any Information or Complaint which shall be brought against the Father or Mother of the Child; or against the Person who shall have had the Care, Nurture, or Custody of such Child, for non-compliance with the provisions of the within-mentioned Act. (Section IV.)

Figura 5. Certificado de vacinação bem-sucedida de Walter Charles Willian Vicent.[3]

[3] Retirado de Stewart e Devlin, 2006.

O experimento clínico de Jenner de imunização com o vírus da varíola e a publicação de sua pesquisa, deu origem a imunologia, mas nem um entendimento das bases de sua eficácia, nem a aceitação universal da sua prática foi seguido. Ao contrário, ceticismo e alarme público e científico é, sempre, a primeira reação (Allen, 2007; Bazin, 2001; Wilson e Marcuse, 2001).

A vacina jenneriana é um exemplo excepcional de empirismo científico, mas também um símbolo sujeito a diversos significados e apropriações. A fluidez da significação, traduzida na diferença entre discurso, regulamentação e prática, longe dos rigores do laboratório bacteriológico que se consolidaram depois de 1880, faz da vacinação um palco significativo de métodos e dinâmicas sociais inscritos nas práticas da saúde (Porto e Ponte, 2003; Saavedra, 2004; Wilson e Marcuse, 2001).

Acusada de desviar a atenção e investimentos de problemas econômicos e sociais básicos cujos impactos prevaleceriam sobre o quadro sanitário e qualidade de vida da população, também é criticada por aqueles que defendem que o uso continuado de uma substância biologicamente ativa é incerto e generalizante quando se considera a multiplicidade e variabilidade do organismo humano (Ponte, 2003). O debate envolve grupos religiosos, políticos e científicos, dos mais variados matizes (Clements e Mcintyre, 2006; COMED, 2007; Geier e Geier, 2006a; Moulin, 2003; Ponte; 2003; Temporão, 2003) e por vezes, ao longo da história, tem aproximado oponentes de posições mais convergentes, quando grupos antivacinistas afirmam não serem contra a vacinação e sim contra a vacinação sistemática, corroborando com aqueles que, acreditando no uso das vacinas pelas autoridades de saúde pública como algo imprescindível, admitem a flexibilização dos índices de cobertura vacinal, reduzindo-os em alguns casos à vacinação de bloqueio (Moulin, 2003; Ponte, 2003).

As vacinas são, provavelmente, o principal instrumento de saúde pública já

posto à disposição dos tomadores de decisão, apesar da oposição que sofre desde suas origens. O século passado foi ameaçado pelo fantasma das grandes epidemias que atingiriam milhões de indivíduos devido à intensa movimentação das pessoas; à devastação florestal, à eclosão de guerras e à possibilidade do uso de patógenos em atentados terroristas e em conflitos entre nações (Ponte, 2003; Temporão, 2002; Wilson e Marcuse, 2001).

Nos últimos anos houve um grande número de publicações sobre o desenvolvimento e o uso de vacinas para evitar o surgimento epidemias cada vez mais abrangentes (Clements e Mcintyre, 2006; COMED, 2007; Moulin, 2003, Ponte, 2003; Temporão, 2003; WHO, 2006c). Contribuem para esse processo previsões bastante pessimistas de segmentos da epidemiologia que acreditam, por exemplo, que o mundo será, em futuro próximo, assolado por uma pandemia de influenza que ceifará milhares de vidas e arruinará a economia de muitos países. Verdadeiras ou falsas tais previsões passaram a povoar o imaginário de setores da opinião pública, fortalecendo a posição daqueles que defendem a ampliação do uso de vacinas.

Se Shakespeare tinha razão ao escrever que o mundo é um palco, então, certamente há muitos atores neste palco. E eles estão freqüentemente interpretando diferentes scripts. Na verdade, eles têm interesses muito diferentes.

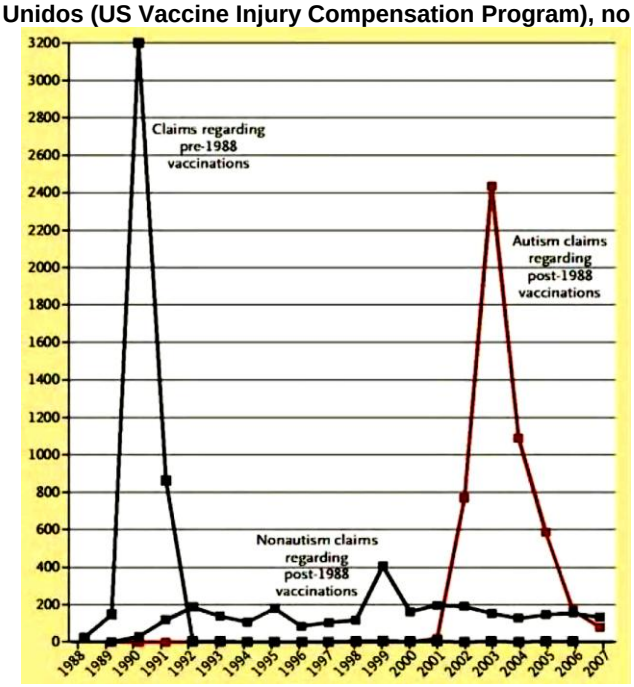
1.2.1 Eventos Adversos e Vacinas Conservadas com Timerosal

A utilização de conservantes em vacinas foi instituída logo após a ocorrência de acidentes trágicos de contaminação de frascos multidoses sem conservantes. Na Austrália, em janeiro de 1928, durante a campanha de vacinação contra a difteria, de 21 crianças vacinadas por via subcutânea, 12 morreram nas 24-48 horas devido à infecção por estafilococos (Zambrano, 2004). Em 1968 os EUA instituíram o uso de conservantes (incluindo o timerosal) em frascos de vacinas multidoses, exceto as de

vírus vivos atenuado, como a SRC (tríplice viral, contra sarampo, rubéola e caxumba), pólio oral e febre amarela (Bernier *et al.*, 1981; Simon *et al.*, 1993; Migowsk, 2007).

A vacina mais arrolada a eventos adversos pós-vacinais é o componente *pertussis* da vacina tríplice bacteriana DTP ou quádrupla bacteriana que associa a DTP à vacina Hib. O *pertussis* é responsável por eventos adversos, que, mesmo raros, podem ser graves (Edlich *et al.*, 2007; Fombonne, 2001; Geier e Geier, 2006a; 2007b; Maya e Luna, 2006). Evidências preliminares sugerem que o timerosal pode estar associado a dificuldades na aquisição da linguagem, ao Transtorno de Déficit de Atenção e Hiperatividade (TDAH) ou Distúrbio de Déficit de Atenção (DDA) (Blaxill, 2004; Doja e Roberts, 2006; Mutter *et al.,* 2005). Milhares de famílias registraram queixa junto ao Programa de Compensação as Injúrias Vacinais (VICP) do governo norte-americano (figura 6) e, atualmente, todos os fabricantes e distribuidores de VCT estão sendo processados nos EUA (Kennedy JR, 2005; Sugarman, 2007).

Figura 6. Gráfico das reivindicações apresentadas junto ao Programa de Compensações as Injúrias Vacinais dos Estados Unidos (US Vaccine Injury Compensation Program), no período de 1989-2007[4].



[4] Retirado de Sugarman, 2007. Vacinações após 1988 (Post-1988) são aquelas que ocorreram em ou após 1 de outubro de 1988; vacinações pré-1988 são aquelas que ocorreram antes de 1 de outubro de 1988 (pre-1988). Os dados são da Administração dos Serviços e Recursos de Saúde dos EUA (US Health Resources and Services Administration).

Desde o dia 11 de junho de 2007, em Washington, um Tribunal Federal de Reclamações (*Court of Federal Claims*) está sendo confrontado com uma questão controversa e altamente emocional: saber se vacinas podem ter causado autismo em milhares de crianças norte-americanas. O processo envolve queixas de milhares de famílias que atribuem às vacinas a responsabilidade pelo autismo diagnosticado nos seus filhos. A maioria dessas famílias afirma que o timerosal das vacinas é o responsável pelas disfunções na interação social e de comunicação de suas crianças, fruto da alteração do espectro autista (COMED, 2007; Edlich, 2007; US Vaccine Court, 2007).

Esse é um dos mais importantes processos judiciais na história médica norte-americana. Ali, um júri especial composto de três juízes começou a ouvir as evidências para apoiar – ou refutar – a hipótese de que o Hg em vacinas causou autismo ou sintomas semelhantes ao autismo em crianças. É a primeira vez em que a evidência sobre o dano autista das vacinas é analisada em uma corte legal.

Tecnicamente, este não é, de todo, um julgamento. É um "*Autism Proceeding Omnibus*", sem culpados. Os juízes não são chamados de juízes, mas "*Special Masters*"; as famílias demandantes e seus advogados são denominados de “peticionários”. E o réu, denominado "inquirido", não é uma droga potente, e sim, o Departamento de Saúde e Serviços Humanos dos EUA (*United States Department of Health and Human Services* – DHHS), representado pelos bem–pagos advogados do Ministério de Justiça Norte-Americano.

Os três juízes do Tribunal de Vacinação (*Vaccine Court*) mergulharam nas águas intensas e contraditórias da querela vacina-autismo, sabendo que devem emergir do outro lado, cada um com sua própria decisão sobre o nexo causal. Por fim, devem emitir juízo sobre algumas das 4900 queixas.

Em sua defesa o CDC apresentou um estudo onde foram avaliados quatro anos da sua vasta base de dados sobre eventos adversos pós-vacinais e não se encontrou qualquer ligação entre timerosal e autismo (US Vacinne Court, 2007). O outro lado foi impedido de ver os dados brutos originais, de modo a reproduzir o que os pesquisadores do CDC descobriram. A reprodução exata dos dados originais é impossível porque, de algum modo, "desapareceram" e não estão mais disponíveis para a re-análise, num provável crime de violação da lei federal norte-americana de qualidade dos dados (COMED, 2007, Marques, 2008).

Conceitos importantes em vacinas (Bigham e Copes, 2005; Clements, 2004)

→ "*Thimerosal-free*" (livre de timerosal): Indica que a vacina não contém timerosal, como conservante ou remanescente do processo de fabricação da mesma.

→ "*Preservative-free"* (livre de conservante): Indica que não se adicionou timerosal como conservante na vacina, mas ele foi usado no processo de fabricação. Podem estar presentes traços de Hg (< que 0,5 µg/0,5 mL) no produto final.

→ *Redução do timerosal*: Indica que estão tentando diminuir expressivamente a quantidade de timerosal. A vacina não pode ser considerada "isenta de timerosal".

1.2.2 Quantificando o timerosal das vacinas pediátricas (Marques *et al.*, 2007b)

O timerosal tem aproximadamente 50% de Hg por peso. Uma solução de 0,01% (1 parte por 10.000) de timerosal contém 50 µg de Hg por dose 1 mL ou 25 µg de Hg por dose de 0,5 mL (Quadro 1). Por exemplo, cada dose da vacina combinada DTP+Hib disponível no calendário oficial de imunizações brasileiro (Brasil, 2007) pode conter entre 25 a 50 µg de Hg/dose (0,5 mL), dependendo do fabricante. A vacina pediátrica contra a hepatite B (Hep B) tem 12,5 a 25 µg de Hg/0,5 mL, também a depender do laboratório fabricante.

QUADRO 1. Dose de mercúrio em vacinas para uso infantil

VACINA	Concentração de Timerosal[5]	Hg µg/0.5 ml
DTaP	.01%	**25**
DTP	.01%	**25**
DT	.01%	**25**
dT	.01%	**25**
TT	.01%	**25**
DTwP-Hib	.01%	**25**
Hib	.01%	**25**
Hep B	.005%	**12.5**
Influenza	.01%	**25**
Antimeningocócica	.01%	**25**
Antipneumocócica	.01%	**25**

1.3 Limites de Exposição ao Mercúrio: Limitações e Certezas

Atualmente não há limites de toxicidade estabelecidos para o EtHg. Os estudos sobre toxicidade consideram que o MeHg e EtHg podem ser equivalentes (Bigham e Copes, 2005; Clarkson, 2003, 2007; Magos, 1985; US ATSDR, 1999; US EPA, 1997). Entretanto, os limites de toxicidade fixados para o MeHg se referem a uma exposição crônica, por via alimentar e em população adulta, não para uma administração subcutânea ou intramuscular em RN e crianças, população mais sensível que a adulta para a exposição a metais pesados. Ou seja, estamos utilizando limites de exposição crônica por via oral, provenientes de exposição ambiental ao Hg, frente a exposições intermitentes por via subcutânea e intramuscular de timerosal, que por sua vez, gera EtHg.

As exposições intermitentes e altas podem presumir um risco maior do que baixas doses diárias. Por outro lado, se desconhece qual a quantidade de EtHg se pode considerar segura em uma única dose (caso das VCT), ainda que Magos (1985, 2001, 2003) tenha interpolado dados de relatos de caso publicados. Embora

[5]Uma concentração de 1:10,000 é equivalente a concentração 0.01%. Uma concentração de 1:10,000 contém 25 microgramas de Hg por cada 0.5ml.

nenhum nível de ingestão diária tolerável tenha sido proposto para o EtHg, várias agências publicaram limites máximos de exposição ao MeHg que fornecem uma orientação para “tomadores de decisão” no governo de populações cronicamente expostas. No geral, esses limites são propostos com o objetivo de proteger o feto.

Os limites máximos de exposição são calculados das concentrações no cabelo ou sangue que estão em um estado constante e são propostos para a aplicação em longo prazo. Para fins de comparação, apresentamos no Quadro 2 os limites de referência das seguintes Agências Internacionais de Saúde: OMS (WHO, 1996), *Environmental Protection Agency* (US EPA, 1997), *Food and Drugs Administration* (US FDA, 1982), *Agency for Toxic Susbtances Disease Registry* (US ATSDR, 1999).

QUADRO 2. Limites de exposição ao metilmercúrio na dieta de um indivíduo adulto.

Environmental Protection Agency (EPA)	0,1 µg/kg/dia
Agency for Toxic Susbtances Disease Registry (ATSDR)	0,3 µg/kg/dia
Food and Drug Administration (FDA)	0,4 µg/kg/dia
Organização Mundial da Saúde (OMS)	3,3µg/kg/semana=0,47µg/kg/dia

Os limites máximos de exposição não representam os níveis absolutos acima do qual a toxicidade ocorre. Como estas recomendações são propostas para exposições crônicas, de longo prazo, não para uma exposição máxima de um único dia, deve-se tomar cuidado ao avaliar exposições calculadas sobre uma base apropriada de tempo. Uma vez que as diretrizes para os limites de exposição são derivadas de dados científicos similares, as diferenças entre agências refletem as variadas suposições e fatores de incerteza que são aplicados na transformação dos dados científicos em recomendações de políticas públicas. Após essas ressalvas, atualmente a única possibilidade de estimar os limites de exposição ao EtHg das VCT é utilizando os valores descritos para MeHg.

Não obstante diferirem em suas margens de segurança, os limites de exposição ao MeHg reconhecidos pelas agências internacionais são todos da mesma

ordem de magnitude: microgramas/kilo/dia (µg/kg/dia). Para elucidar os cálculos de exposição, adotamos como exemplo uma criança do sexo masculino, no percentil 50 de média de peso corporal, de acordo com a idade. Este cenário supõe que aos seis meses a criança recebeu todas as vacinas do calendário preconizado pela OMS e Ministério da Saúde do Brasil (MS), que corresponde a uma dose cumulativa de EtHg de 187.5 μg^{-1}. A exposição ao Hg do nascimento até os 18 meses pode chegar a 212,5 μg^{-1} se todas as vacinas que contém timerosal forem administradas (Quadro 3).

QUADRO 3. Exposição ao mercúrio de vacinas pediátricas conservadas com timerosal.

Idade da criança/ Peso médio[a]	Vacina	Timerosal/Hg (µg/dose)	Exposição ao Hg[b]	OMS[c]	ATSDR[c]	EPA[c]	FDA[c]
0 meses/3,4 kg	Hep B	25/12,5	3,68	7,83	12, 27	36,80	9,20
1 mês/4, 5 kg	Hep B	25/12,5	2,78	5,91	9,27	27,80	6,95
2 meses/5,6 kg	DTP	50/25	4,46	9,49	14,87	44,60	11,15
2 meses/5,6 kg	Hib	50/25	4,46	9,49	14,87	44,60	11,15
Aos 2 meses a administração da DTP e Hib é simultânea[d]		37,5	6,70	14,26	22,33	67,00	16,75
4 meses/7 kg	DTP	50/25	3,57	7,60	11,90	35,70	8,93
4 meses 7 kg	Hib	50/25	3,57	7,60	11,90	35,70	8,93
Aos 4 meses a administração da DTP e Hib é simultânea [d]		37,5	5,36	11,40	17,87	53,60	13,40
6 meses/8,3 kg	Hep B	25/12,5	1,51	3,21	5,03	15,10	3,78
6 meses/8,3 kg	DTP	50/25	3,01	6,40	10,03	30,10	7,53
6 meses/8,3 kg	Hib	50/25	3,01	6,40	10,03	30,10	7,53
Aos 6 meses a administração da Hep B, DTP, Hib é simultânea[d]		62,5	7,49	15,94	24,97	74,90	18,73
18 meses/11,2 kg	DTP	50/25	2,23	4,74	7,43	22,30	5,58

[a]Percentil 50; [b]µg de Hg/kg/dia; [c]Número de vezes que supera o limite de segurança estabelecido pelas agências em µg/kg/dia; [d]no Brasil, desde 2002 foi implantada a vacina tetravalente DTP + Hib, com 50 µg de timerosal.

1.4 A Plausibilidade Biológica

Desde 1999, duas abordagens diferentes foram seguidas com relação às VCT nos programas de imunização infantil. Os EUA, UE e outros países desenvolvidos adotaram medidas para eliminar a exposição infantil ao Hg das vacinas. Desde

2004, nenhumas das vacinas monovalentes ou multivalentes de rotina recomendadas e usadas para proteger crianças em idade pré-escolar nos EUA ou na UE contêm timerosal. A eliminação do timerosal dos programas rotineiros de imunização infantil nestas jurisdições foi alcançada, essencialmente, pelo uso exclusivo da monodose (dose única), formato das vacinas sem conservantes (AAP, 1999; EMEA, 1999; 2000; IOM, 2004; US CDC, 1999; 2000).

Contudo, a maioria dos países ainda usa VCT em seus programas de imunização infantil, uma vez que a OMS continua a defender o uso do timerosal nas vacinas pediátricas, incluindo para a administração em crianças desnutridas, prematuras ou com baixo peso. A base da posição da OMS é que os estudos farmacocinéticos e epidemiológicos não apresentaram evidências convincentes da toxicidade do EtHg pela exposição através das VCT. E argumenta que o uso destas vacinas, particularmente nas regiões com elevadas cargas de doenças, provou-se altamente eficaz na proteção de crianças (WHO, 2006a; 2007).

As duas diferentes abordagens quanto à questão do timerosal nas vacinas continuam a gerar confusão entre os pais e trabalhadores de saúde que administram vacinas. Mesmo nas jurisdições aonde timerosal foi eliminado das vacinas infantis administradas rotineiramente, ainda há VCT que podem ser recomendadas para algumas crianças de alto risco. Por exemplo, vacinas contra influenza, doença de Lyme, doença pneumocócica invasiva ou contra a raiva humana (Fombonne *et al.*, 2006; Geier e Geier, 2007a; COMED, 2007).

Diversas publicações científicas relacionam o aumento no número de casos de DN – tais como atraso na linguagem, alterações de conduta e síndrome autista – a maior exposição que sofre a população infantil ao Hg orgânico das vacinas, devido a um calendário de imunização cada vez mais repleto de VCT (Bernard, 2001; Blaxill,

2004; Blaxill *et al.*, 2004; Doja e Roberts, 2006; Geier e Geier, 2006a; 2007b; Herman *et al.,* 2006; Maya e Luna, 2006; Mutter *et al.*, 2005; Shevell e Fombonne, 2006).

O estudo realizado pelo IOM, publicado em 1 de outubro de 2001, concluiu que a evidência científica era imprópria para aceitar ou descartar uma relação de causa e efeito entre a exposição de crianças ao timerosal presente nas vacinas infantis e transtornos do desenvolvimento neurológico como, síndrome autista, transtornos de hiperatividade e atrasos da linguagem, pois seria necessário realizar mais estudos para se estabelecer ou rechaçar uma relação causal (US CDC, 2001). Contudo, concluíram que DN relacionadas à exposição ao Hg de VCT é uma hipótese biologicamente plausível. Além disso, recomendou esforços para abolir o timerosal das vacinas como medida preventiva de saúde pública para reduzir a exposição ao Hg em RN e crianças. Dois anos depois um estudo epidemiológico mostrou um incremento no risco relativo de sofrer de DN e enfermidades cardíacas com doses maiores de Hg. Isto indicaria que quanto maior a dose de Hg maior a probabilidade de sofrer danos durante o desenvolvimento neurológico (Geier e Geier, 2003a).

A plausibilidade da associação entre a exposição ao EtHg derivado das VCT e efeitos para a saúde foi baseado no seguinte:

→ Presumidas similaridades na farmacocinética e efeitos toxicológicos do EtHg e do MeHg.

→ Reações de hipersensibilidade após baixas doses de exposição a produtos contendo timerosal.

→ Aumentos mensuráveis de Hg no sangue de crianças após imunização com VCT.

→ Evidência de um efeito dose-resposta a altas doses de exposições ao EtHg tanto alimentares quanto ocupacionais, agudas ou crônicas.

1.4.1 A farmacocinética e toxicologia do MeHg e do EtHg

Preocupações a respeito da segurança do timerosal (que contém EtHg) foram baseadas em estudos que sugerem efeitos adversos em crianças com exposição *in utero* ao MeHg em níveis antes considerados seguros (Grandjean, 1997; US CDC, 1999; 2000; WHO, 1991). A similaridade da farmacocinética e toxicológica foi postulada em função das estruturas químicas e efeitos para a saúde similar em doses elevadas. O metabolismo e mecanismos toxicológicos de ação do EtHg e MeHg são complexos e diferenças significantes na farmacocinética entre esses dois compostos estão sendo reconhecidas.

Duas diferenças importantes são a meia-vida significantemente mais curta do EtHg no sangue e menor movimento de EtHg através da barreira hematoencefálica. Magos (2003) estimou uma meia-vida corrigida alometricamente de 18 dias para o Hg administrado como timerosal, que estava dentro de 10% dos níveis mensurados de Hg no sangue relatados por Pichichero *et al.* (2002). Os dados apresentados por Magos indicam que a acumulação transiente do Hg no sangue é resultado da vacinação com VCT nos intervalos de dose de 4-6 semanas comumente indicados para imunização infantil em países em desenvolvimento.

Os efeitos toxicológicos da exposição ao MeHg in utero são relevantes para o EtHg?

Estudos de longitudinais são baseados na exposição ao MeHg. Esses estudos acompanharam crianças cronicamente expostas no período pré-natal e pós-natal (via alimentar) a baixas doses de Hg e foram realizados nas ilhas Seychelles (Davidson *et al.*, 1998; Myers, *et al.*, 2003), nas ilhas Faroes (Grandjean *et al.*, 1997, 1999, 2007), e na Nova Zelândia (Crump *et al.*, 1998). Nas Seychelles, as exposições crônicas *in utero* e em baixas doses resultaram de mães que tinham uma dieta

essencialmente baseada em peixes, enquanto o consumo de peixes das mães das Ilhas Faroes mudava intermitentemente para o consumo de carne e gordura de baleias piloto.

O estudo das Seychelles usou cabelo materno e da criança para avaliar a exposição pré-natal e infantil ao Hg e escalas neurodesenvolvimentais para avaliar os efeitos, não encontrando nenhum dano neurológico em crianças até os nove anos de idade (Myers, *et al.*, 2003). O estudo das Faroes usou sangue umbilical e cabelo infantil para avaliar a exposição pré e pós-natal ao Hg, respectivamente, e testes neurodesenvolvimentais de domínio específico para avaliar os efeitos, relatando déficits neurológicos sutis em escores de memória, atenção e linguagem entre as crianças testadas quando completaram sete anos de idade. Segundo os autores, a exposição pós-natal ao Hg foi menos preditora desses efeitos do que a exposição pré-natal. Os resultados dos testes neurocomportamentais não foram consistentes para predizer disfunções tardias (Grandjean *et al.*, 1997, 2007). O estudo da Nova Zelândia correlacionou a exposição pré-natal ao MeHg – estimado de análises de amostras maternas coletadas durante a gravidez – com resultados de testes psicológicos e educacionais aplicados em crianças de seis e sete anos de idade. Um possível efeito sutil do Hg foi detectado após a exclusão de um par mãe-filho *"outlier"* das análises (Crump *et al.*, 1998).

A exposição infantil ao Hg de VCT difere da exposição dos estudos das Ilhas Faroes, Seychelles e Nova Zelândia em diversos aspectos-chaves. Primeiro, as circunstâncias da exposição são diferentes da exposição pós-natal associada apenas com a vacinação infantil. Em segundo lugar, a rota de exposição (parenteral vs oral) é diferente, e terceiro, a exposição da vacinação é intermitente, enquanto nesses três estudos é contínua.

1.4.2 Reações de hipersensibilidade após exposição a baixas doses de timerosal

O timerosal é associado a reações alérgicas dérmicas de contato (hipersensibilidade tardia). Entre 1% a 16% dos indivíduos testados exibiram alergia dérmica. Hipersensibilidade imediata (anafilaxia) e desordens imunes (glomerulonefrite) também foram relatadas em associação com exposição aos produtos contendo timerosal (Bigham e Copes, 2005, Cox e Forsyth, 1988; Ellis, 1947; Forstrom *et al.,* 1980; Geier *et al.*, 2007).

1.4.3 Evidências da relação dose-resposta na exposição a altas doses de EtHg

Na China no início dos anos 1980, o consumo do arroz tratado com EtHg causou uma multiplicidade de sintomas neurológicos incluindo fraqueza, vertigens, torpor, parestesia e ataxia, identificados nas pessoas moderadamente afetadas em níveis de exposição de 0.5 mg/kg de peso. Nascimento *et al*. (1990) não apenas relatou uma morte em conseqüência da ingestão de timerosal, como também advertiu sobre o onipresente perigo do envenenamento por timerosal. Magos (2001) relatou que nenhum efeito adverso foi observado em níveis de Hg no sangue entre 140 e 650 ng/L em cinco adultos, avaliados 11-22 dias após exposição a doses variadas de EtHg de alimentos contaminados, infusão ou aplicação tópica de produtos farmacêuticos contendo EtHg. O LOAEL (níveis mais baixos em que efeitos adversos foram observados) no sangue foi de 1000 ng/L. Embora as relações dose-resposta tenham sido construídas de exposições pré-natal ao MeHg, nenhuma relação dose-resposta foi estabelecida para exposições pós-natal a EtHg nas doses utilizadas em vacinas.

1.5 Timerosal e Distúrbios do Desenvolvimento Neurológico

O Centro para Prevenção e Controle de Doenças dos EUA (CDC) entre o final dos anos 1980 e durante os anos 1990 expandiu o número de doses de VCT a serem administradas em crianças americanas. A vacinação de rotina foi gradualmente ampliada de uma administração de cinco doses da vacina contra DTP para, no final, incluir três doses da vacina hep B (a primeira dose administrada nas primeiras 24h de vida), e de quatro doses da Hib. Adicionalmente, o CDC também passou a indicar que três doses da vacina contra influenza fossem administradas a populações infantis específicas (a primeira dose administrada aos seis meses de idade). Logo, segundo o calendário de imunização americano, a exposição total ao Hg das VCT poderia ter sido de 200 µg de Hg nos primeiros seis meses da vida (Geier e Geier, 2003; Marques, 2008).

O IOM, por determinação do congresso americano, realizou no início dos anos 1990 um estudo que envolveu queixas contra a vacina contra coqueluche. O grupo investigou dezoito tipos de efeitos adversos associados à vacina, entre os quais morte súbita de bebês, agitação e espasmos, encefalite, meningite, autismo, anafilaxia e diabetes. Após vinte meses avaliando estudos de casos, estatísticas epidemiológicas, experiências com animais e estudos laboratoriais, a comissão descartou toda e qualquer relação causal entre a vacina e autismo. Reconheceu evidências de que ela pode provocar agitação, encefalite e choque anafilático e deixou sem resposta o resto das perguntas alegando insuficiência de dados (COMED; Sugarman, 2007).

Vários autores afirmam que o papel do Hg nos danos ao sistema neurológico e imunológico associados a atrasos de desenvolvimento já está comprovado (Doja e Roberts, 2006; Holmes *et al.*, 2003; Geier e Geier, 2006b; 2007a; 2007b; Mutter *et al.*, 2004, Walker *et al.*, 2006). Mesmo assim, os laboratórios produtores continuam

enviando milhões de doses de VCT (sob os auspícios da OMS) para países em desenvolvimento, onde a regulamentação e fiscalização de medicamentos não proíbem o uso de timerosal como conservante (WHO, 2002; 2007).

1.5.1 Estudos Laboratoriais

Estudos em humanos: mudanças do Hg no sangue após o uso de VCT em crianças

Os estudos em Rochester (EUA) sugerem que a meia-vida do EtHg é de apenas 7 dias – comparados aos 40-50 dias para o MeHg – e é excretado no intestino, não se acumulando ativamente no corpo. Pichichero *et al.* (2002) comparou amostras de fezes, sangue e urina de crianças entre 2 e 6 meses de idade, expostas a VCT (DTP e Hep B, algumas receberam Hib) e controles que receberam vacinas livres de timerosal. A administração de VCT não elevou os níveis sanguíneos de Hg acima do limite considerado seguro. O EtHg parece ser eliminado rapidamente do sangue através das fezes após injeção parenteral de VCT. Os autores, porém, não conseguiram medir quanto do EtHg cruzou a barreira hematoencefálica (impossibilidade virtual em humanos, sobretudo crianças) nem se houve algum dano neurológico decorrente da imunização. Eles concluíram que o timerosal nas doses usadas em vacinas oferece pouco risco para crianças a termo, mas não deve ser administrado ao nascimento em crianças prematuras e de baixo peso ao nascer (não incluídas no estudo).

Stajich *et al.* (2000) descreveram que a imunização com uma única dose da vacina Hep B (12.5 $\mu g/g^{-1}$ de EtHg), resultaram em aumento no nível médio de Hg no sangue de prematuros quando medido 2-3 dias após a vacinação. Ainda que os prematuros tenham recebido doses mais altas (µg/kg) que as crianças a termo, as concentrações sanguíneas de Hg pré-vacinação entre prematuros e a termo foram mais altas que a razão de correspondência da concentração de Hg sanguíneo pós-

vacinação. Isto indicaria que os prematuros excretaram uma proporção maior da dose de Hg/kg de peso do que crianças a termo. Para os autores, permanece incerto se os níveis mais altos de Hg no sangue, subseqüentes a imunização com VCT, detectados nas crianças prematuras ou de baixo-peso oferecem qualquer risco toxicológico mensurável.

Pichichero e Stajich supõem que a menor massa corporal aumentaria em proporção às concentrações de timerosal. Assim, é provável que todo o EtHg já tenha sido excretado pelo corpo quando a dose vacinal seguinte for administrada; e os níveis de pico são determinados pela quantidade de EtHg administrada. Logo, a pergunta seguinte é qual a proporção (se houver) de Hg no sangue cruza a barreira hematoencefálica, que níveis de EtHg no cérebro são alcançados, e se o nível é lesivo.

Estudos em modelos animais

Burbacher *et al.* (2005) mediram a distribuição sistêmica e no cérebro do Hg total e inorgânico em macacos não expostos ao timerosal e compararam-nos com macacos expostos ao EtHg. Os níveis de Hg no sangue em macacos evidenciaram que a meia-vida do EtHg é muito mais curta quando comparado ao MeHg.

1.5.2 Estudos Epidemiológicos

A literatura anterior a 1999 consiste, principalmente, de estudos não toxicológicos do MeHg e de síndromes clínicas associadas a aplicação tópica de produtos farmacêuticos contendo timerosal. Desde 1999, houve um aumento esperado nos estudos sobre avaliação de danos neurodesenvolmentais causados pelo timerosal. Porém, apenas 12 publicações apresentaram dados novos expressivos ou novas abordagens para dados existentes. Seis concluíram que não há evidências de insulto neurológico ou psicológico relacionado ao Hg de VCT. Em

contraposição, Geier e Geier publicaram 6 artigos apontando que essa relação existe. Como há poucos artigos originais e o debate científico em torno do assunto se articula neles, cada um foi revisado e sumarizado no Quadro 4.

QUADRO 4. Fontes de dados epidemiológicos primários investigando possíveis associações entre vacinas conservadas com timerosal e aparecimento de sintomas neurológicos.

Autor (s)	Tipo de Estudo	Ligação com alterações neurodesenvolvimentais	Possíveis associações
Andrews (2004)	Coorte retrospectiva	Não confirmado	Convulsões, espasmos
Heron *et al.* (2004)	Coorte Prospectiva	Não confirmado	Nenhuma
Haviid *et al.;* (2003)	Coorte retrospectiva	Não confirmado	Nenhuma
Madsen (2003)	Coorte retrospectiva	Não confirmado	Nenhuma
Verstraeten *et al.* (2003)	Coorte retrospectiva	Inconclusivo: Não confirmado	Desordens na linguagem, na fala, déficit de atenção, convulsões, espasmos.
Stehr- Green (2003)	Ecológico	Não confirmado	Nenhuma
Geier (2003a; 2003b; 2004a; 2005; 2006a; 2006b)	Coorte ecológica e retrospectiva	Ligação confirmada em seis artigos	Diversas associações confirmadas

2 A HIPÓTESE

2.1 As vacinas são seguras?

O método introduzido por Jenner, em 1796, veio a ser chamada de “vacinação”, de “*Vacca*”, a palavra latina para vacas e a substância usada para vacinar foi chamada de “vacina”. Vacinas são injeções que contém uma parte ou o todo de um patógeno que aumenta a resposta imune, mas não deve causar a doença. São administradas para induzir o desenvolvimento da resposta imune e proteger o indivíduo contra um patógeno ou toxina (Prescott, 1996); para trabalhar estimulando o corpo a produzir anticorpos, proteínas que defendem o corpo de uma invasão de genes perigosos. Agora, mais de 200 anos depois, avançamos de uma época em que a vacinação era um evento raro, e as teorias de Jenner sobre a imunização não eram amplamente aceitas, para os anos 2000, quando as vacinas se tornaram tão banais que a maioria das crianças recebe várias delas antes de chegarem ao primeiro aniversário. Seres humanos em todo o mundo, de todas as idades são vacinados contra muitas doenças O resultado dessa vacinação em massa tem sido uma acentuada diminuição das doenças que assolaram a população mundial.

Entretanto, nas últimas décadas um número significante pessoas tem se preocupado com a segurança das vacinas. Este movimento vem da apreensão de que algumas vacinas podem ter complicações, muitas vezes sérias, com seqüelas permanentes. As cepas virais atenuadas usadas nas vacinas contra poliomielite, sarampo, rubéola e varicela tem o potencial para transformar-se em cepas mais virulentas e causar sérias enfermidades em crianças ou cuidadores susceptíveis.

Como qualquer vacina tem o potencial de aumentar uma resposta anafilática em indivíduos alérgicos, essas preocupações têm levado muitos pais a recusarem vacinar suas crianças. Somam-se a isso, as baixas taxas de vacinação em populações que tem dificuldade de acesso aos serviços de saúde e uma fração crescente de crianças que estão novamente suscetíveis a essas doenças.

A maioria das pessoas acredita que as vacinas são seguras e eficazes. Como dito antes, elas certamente ajudaram a erradicar ou reduzir a ocorrência de doenças letais, como a varíola, pólio e coqueluche. Contudo, devem-se creditar às vacinas o declínio dessas doenças ou elas são ineficazes e a ausência ou diminuição destas foi devido à melhoria nas condições de vida?

Esta é a uma das controvérsias que o mundo enfrenta hoje.

Médicos, microbiologistas e outros profissionais da saúde irão atribuir o declínio nos casos relatados de doenças ao aumento da vacinação em massa. Para eles, é muito melhor vacinar e correr o risco de complicações de uma vacina que não vacinar. Os ativistas contra a imunização dizem que isso não vale à pena, pois os índices de mortalidade já tinham começado a declinar significativamente antes das vacinas se tornarem disponíveis. E defendem que injetar um patógeno letalmente conhecido em um hospedeiro saudável é como colocar um revólver e puxar o gatilho esperando que ele não dispare.

O que nos é divulgado é que o controle das epidemias nos dias atuais é resultado da vacinação em massa. Em 1967, a OMS recebeu registro de 131.000,00 casos de varíola, em 42 países. Em maio de 1980 a varíola foi oficialmente considerada erradicada no mundo como resultado das campanhas de imunização em massa (Scheibner, 1993). Com esta descoberta nós fomos aconselhados a encerrar os programas de vacinação contra a varíola. A utilidade das vacinas foi

comprovada e o sucesso alcançado.

Mas foi isso mesmo?

As estatísticas de muitos países indicam que a varíola já estava declinando antes dos programas de vacinação tornarem-se obrigatórios (Miller, 1993). Isto pode ser atribuído à melhoria na educação, renda, condições sanitárias e em outras reformas governamentais que levaram a melhoria na qualidade de vida das pessoas. Não obstante, uma vez que os programas de imunização se tornaram compulsórios, mortes por doenças evitáveis por vacina diminuíram.

Todavia, vejamos o exemplo da varíola: a probabilidade de a varíola ser fatal é cinco vezes maior tanto em indivíduos vacinados quanto em não vacinados (Scheibner, 1993). A vacina não deveria supostamente proteger contra a doença?

Similarmente à varíola e a poliomielite, a ocorrência de coqueluche também teve uma diminuição nos casos e mortes associadas à doença (Moulin, 2003). A doença é causada por uma bactéria que afeta o sistema respiratório, acarretando severos ataques de tosse, febre e circulação inadequada de oxigênio (Miller, 1993). Muitos acreditam que a incidência da coqueluche diminuiu devido à melhoria nas condições de vida, mais que ao uso de uma vacina eficaz. A vacina contra a coqueluche, chamada DTP, protege também contra a difteria e o tétano. Diversos estudos afirmam que a DTP pode causar danos cerebrais. Entretanto, O CDC e a associação médica norte-americana continuam afirmando que não há evidências de que a DTP produza danos cerebrais (Freed *et al.,* 1996, US CDC, 1999; 2000; 2001).

Se vacinas são eficazes por que as estatísticas mostram um aumento nas taxas de ocorrência de muitas doenças após o início da vacinação? E se as vacinas são ineficazes, por que a varíola foi totalmente erradicada e a incidência de outras doenças foi grandemente reduzida desde o início do desenvolvimento das vacinas?

Quem está correto?

A controvérsia sobre a vacinação tem origem em um conflito conceitual na área da saúde que marcou o século XIX e remonta aos famosos debates entre dois notáveis cientistas: Louis Pasteur e Claude Bernard. Bernard formulou a teoria segundo a qual a causa de uma doença estava em elementos ambientais, externos e internos, não passando de uma perda de equilíbrio do corpo gerada por diversos fatores. Para ele, o corpo é um "terreno" onde os microorganismos podem ou não atuar de forma lesiva, dependendo das condições que encontram. A doença seria um sinal do esforço do organismo para reequilibrar-se. Pasteur é autor da teoria segundo a qual cada doença possui uma causa única, um vírus ou bactéria que invade o organismo e ali produz um tipo especifico de devastação (Allen, 2007; Bordenave, 2003; Capra, 2001; Moulin, 2003).

Pasteur venceu a disputa. Ele foi um debatedor talentoso e soube tirar proveito do surgimento das epidemias daquele tempo para comprovar a coerência do seu conceito de *causação específica*. Desde então, todo um modelo centrado na microbiologia e, recentemente, na biologia molecular, fundamentou os procedimentos de saúde modernos, incluindo às vacinações em massa. Capra (2001) afirma que, mais tarde, Pasteur reconheceu a importância do "terreno" para as enfermidades, tendo ressaltado a influência dos fatores ambientais e dos estados mentais na resistência às infecções.

Pasteur ou Bernard? No mundo atual, ao que tudo indica, ficou ainda mais difícil resolver velhas incertezas. Os muitos interesses que envolvem o assunto gerarão ainda mais questionamentos e críticas mordazes antes que se chegue a algum acordo. A resposta aos nossos questionamentos não chegará até que haja pesquisas suficientes e imparciais em relação aos efeitos reais das vacinas.

2.1.1 Controvérsias atuais sobre a segurança das vacinas

A comunidade científica tem sido confrontada com as acusações de encobrir provas de que o Hg em vacinas infantis causa autismo (Allen, 2007; Bernard *et al.,* 2001; Blaxill *et al.*, 2003; Colgrove e Bayer, 2003; Kennedy Jr, 2005; Nature Neuroscience, 2001). O timerosal, um conservante de vacinas à base de Hg, está sob vigilância e investigação pública e profissional – e seu uso questionado – devido à crescente conscientização da sua presença em vacinas infantis (Dórea, 2007; Fombrone, 2006; Geier e Geier, 2007a; Marques *et al.*, 2007b). A principal alegação é que o composto exporia crianças aos prováveis efeitos neurológicos atribuídos ao Hg. O uso do mercúrio – um conhecido neurotóxico – em vacinas não é novo. Ele é usado para evitar contaminação por fungos e bactérias desde os anos 30, reduzindo o risco de contaminação na abertura e manipulação dos frascos multidoses (Edlich *et al.*, 2007; Geier e Geier, 2006; US CDC, 1999; 2000; WHO, 2003).

Nos últimos tempos parece ter aumentado – ou pelo menos se tornado mais visível – a ocorrência de eventos adversos de determinadas vacinas, como DPT e Hep B (Zetterstro, 2004; Geier, 2004; Geier e Geier, 2007, Maya e Luna, 2006). Os registros de eventos adversos variam da simples irritabilidade ao desenvolvimento da doença que se pretendia evitar. Há registro de casos extremos em que a vacinação resultou em morte (Brasil, 2007; Edilich *et al.,* 2007; Geier e Geier, 2007b, Sanford e Kimmel, 2002).

O sítio do governo americano para relatos de eventos adversos pós-vacinais recebeu 108.000 queixas entre janeiro e outubro de 2000, encaminhadas para averiguação técnica. A maioria dos relatos dizia respeito a desconfortos leves, como febres e indisposição passageiras, que os cientistas costumam desconsiderar. Mesmo assim, as referências a complicações colaterais graves, inclusive mortes, em

14% das denúncias levou o Serviço de Saúde dos EUA a redobrar a vigilância sobre os fabricantes de vacinas e a interferir nas normas de produção (US CDC, 1999; US DHHS, 2007). Outros países também fecharam o cerco às vacinas nos últimos anos, baixando medidas preventivas (EMEA, 1999; 2000).

Um número crescente de estudos independentes associa a exposição ao Hg a problemas neurológicos em milhões de pessoas. De reduções imperceptíveis do QI a DN mais debilitantes (Geier e Geier, 2007b; Maya e Luna, 2006; Mutter *et al.*, 2004; Trasande *et al.*, 2005). Por outro lado, uma série de outros estudos ligados ao *establishment* da saúde garante que a apreensão é infundada (Clements e Mcintyre, 2006; Fombonne *et al.*, 2006; Migowsk, 2007; Pichichero *et al.*, 2002; Shevell e Fombonne, 2006; Thompson *et al.*, 2007; Trollfors *et al.*, 2006), já que a pequena quantidade contida nas vacinas seria facilmente eliminada pelo organismo.

É difícil acreditar que níveis de mercúrio 60 a 250 vezes mais elevados do que os níveis de resíduos perigosos (ATSDR, 2001; EPA, 1997; FDA, 1982; WHO, 2006) sejam chamados de "minúsculos", como querem os defensores do timerosal. É notório: todas as diretrizes publicadas para exposição ao Hg foram extrapoladas.

Os EUA e países europeus já proibiram o uso do timerosal em vacinas. E apesar da OMS declarar que não há nível seguro para Hg, continua a recomendar sua utilização nas fórmulas vacinais. Agora, se “quantidade” é a palavra-chave, convém lembrar que a quantidade de VCT foi triplicada no começo dos anos 1990, por recomendação da própria OMS (WHO, 2006; 2007). Em 1999, quando a Associação Americana de Pediatria (AAP) e o Departamento de Saúde dos EUA (DHHS) reuniram-se para revisar e avaliar os riscos do timerosal em vacinas infantis a hipótese inicial era: se o EtHg e MeHg são quimicamente similares, por conseguinte devem ter os mesmos efeitos toxicológicos (Magos, *et al.*, 1985; Clarkson, 2007).

Assim, a pergunta que precisava ser respondida era: “O EtHg comporta-se da mesma maneira que o Hg elementar e o MeHg, se acumula e causa os mesmos efeitos ou efeitos similares?” A pergunta é crucial – pois, se a resposta for “sim”, então não há argumentos: vacinas não devem conter Hg. Pode-se contra-argumentar que a pergunta não poderia ser respondida por que dados sobre a toxicidade da exposição humana a baixas doses de EtHg não estavam disponíveis em 1999. Contudo, foram presumidos como similares àqueles causados por outros compostos organomercuriais (Ball *et al.*, 2001; Bigham e Copes, 2005; Blaxill *et al.,* 2004; Halsey, 1999; Halsey e Goldman, 2003).

A controvérsia do timerosal começou de maneira inesperada com uma “epifania[6]” que os limites de exposição para MeHg foram excedidos nas vacinas infantis. Após a apreensão inicial dos prováveis riscos para a saúde das crianças relacionados ao Hg nas vacinas, veio a “constatação” de que o que se sabia sobre a toxicologia do EtHg era insuficiente, a despeito do considerável conhecimento disponível sobre o MeHg. A literatura sobre o assunto estava limitada a casos de timerosal aplicado topicamente em doses que excediam, em muito, quaisquer vacinas (Cox e Forsyth, 1988; Fagan, 1997). Os epidemiologistas e “cientistas de bancada” logo começaram a publicar seus artigos trazendo a luz diferenças entre o perfil toxicológico do MeHg e do EtHg, e a comprovação da segurança do conservante nas vacinas. Ainda assim, a opinião pública permaneceu incrédula. Um conjunto de circunstâncias – desde 1999 – conspirou para criar um ambiente de descrença na população.

O “*mainstream*” científico – sobre imunização e Hg – foi exaustivamente citado pelos grupos de pressão que eram contrários a remoção do produto das vacinas, sempre assegurando que não existia comprovação científica da relação entre DN e

[6] Epifania: manifestação ou percepção da natureza ou do significado essencial de uma coisa (dicionário Houaiss).

o Hg das vacinas. E embora os críticos do timerosal tenham proposto que alguns dos sintomas do autismo e outras DN fossem análogos aos sintomas associados a exposições ao Hg, peritos como Nelson e Bauman (2003) discordaram, indicando que as diferenças clínicas e neuropatológicas entre o autismo e a intoxicação por Hg extrapolam as similaridades e são limitadas a sintomas não específicos, tais como ansiedade, depressão e medos irracionais. As conclusões dos "*experts*" ofereciam fortes evidências contra a plausibilidade biológica e epidemiológica da causação.

A reação da opinião pública foi contrária a da comunidade científica.

Mas, se timerosal não tem relação causal com o aumento dos casos de autismo (Coury e Nash, 2003; Clements e Mcintyre, 2006; Doja e Roberts, 2006; Thompson *et al.*, 2007), ele causa o que? Coury e Nash (2003) afirmam que a epidemiologia das desordens do espectro autista mudou. Um aumento na prevalência foi notado durante as duas décadas passadas. O que não está claro é a causa deste aumento. Múltiplos fatores parecem ser responsáveis. Entre eles às mudanças nos critérios diagnósticos e maior consciência por parte dos cuidadores e pais. Portanto, mais pesquisas são necessárias para ajudar a esclarecer a incidência e prevalência das DN e sua etiologia. Neste ínterim, epidemiologistas e toxicologistas precisam centrar atenção em assegurar que todas as evidências possíveis sejam examinadas e que possam acrescentar novas descobertas sobre a segurança ou não do timerosal em vacinas.

Halsey declarou em 1999 que muitos dos argumentos contra as vacinas estão fundamentados em hipóteses não comprovadas ou em elos causais com provas insuficientes, mas que gradualmente está se dando conta de que existe um risco real para as crianças; e Clarkson, no mesmo documento, admite que talvez tenha que rever os resultados de seus estudos sobre Hg em crianças de Seychelles,

levando em consideração as VCT administradas, a época, nas crianças avaliadas por seu grupo (COMED, 2007).

Geier e Geier (2006a; 2006b; 2006c) afirmam que há uma relação direta entre o Hg nas vacinas das crianças e autismo, contradizendo afirmações do governo norte-americano de que não há relação comprovada entre os dois. Os dados mostram que desde que o Hg foi removido das vacinas infantis, o aumento nas taxas registradas de autismo e outras DN nas crianças não só parou como caiu drasticamente: até 35% nos EUA. Utilizando o próprio banco de dados do governo, os cientistas analisaram casos registrados de DN em crianças, inclusive autismo, antes e depois da remoção timerosal. Esses resultados contradizem diretamente as recomendações de 2004 do IOM, que reavaliou as informações acerca da segurança das vacinas do programa de vacinação norte-americano. Ainda que sem disposição de excluir ou corroborar uma relação causal entre Hg e autismo, o comitê interdisciplinar do IOM concluiu que não há necessidade de mais estudos (IOM, 2004).

Então lembremos. Se já é consenso científico (corroborado pela OMS) que:

a) o mercúrio é tóxico;

b) nos primeiros meses de vida crianças são mais suscetíveis a interferências no desenvolvimento neurológico causado pela exposição ao mercúrio; e

c) prevenir exposição ao mercúrio durante os períodos críticos do desenvolvimento do SNC deve ser objeto de estratégias de saúde pública;

Causa estranheza a posição da OMS, que avaliza as recomendações para restrição ao consumo de peixes durante a gravidez, com o intento de proteger crianças dos efeitos danosos do mercúrio, mas “permite” injetar a substância em crianças. Ora, se parece razoável minimizar a exposição de fetos e crianças a fontes de exposição ao mercúrio, outras duas questões permanecem sem resposta: por

que continuar usando vacinas com mercúrio quando existem alternativas? E a vacina hepatite B, administrada nas primeiras horas de vida, não representa um risco ainda maior para recém-nascidos?

Atualmente a maioria das vacinas Hep B disponíveis para países em desenvolvimento – inclusive em preparações monodoses – contém timerosal (Clements, 2004; Geier e Geier, 2007; Marques *et al.*; 2007b). O mesmo se dá em relação às vacinas DTP e Hib, administradas em três doses a partir do segundo mês de vida. Não obstante a OMS garanta a segurança do uso desse composto à base de Hg em vacinas pediátricas e para grávidas, em janeiro de 2005, começou a promover estudos em modelos animais para avaliar este aspecto (Clements e Mcintyre, 2006; WHO, 2005).

A luz dos fatos há uma quantidade substancial de crianças que recebem doses de mercúrio das vacinas infantis conservadas com timerosal por uma quantidade de anos, já que o composto ainda é adicionado rotineiramente às vacinas administradas a crianças no mundo inteiro. Nos EUA e UE, por exemplo, a vacina influenza, bem como as dT e DT (difteria-tétano), meningite, e tétano monovalente continuam usando o conservante, a despeito das medidas de eliminação do uso do composto em vacinas (Geier e Geier, 2007c).

2.2 Dilema: comungar no dogma ou seguir o instinto científico?

Críticas as vacinas devem ser feitas com cuidado e responsabilidade, a fim de evitar a um problema maior de saúde pública: a queda nos índices de vacinação. Usar os efeitos nocivos do timerosal para clamar contra as campanhas de vacinação fere o bom senso, pois colocaria em risco um programa de saúde bem-sucedido, trazendo muitos prejuízos à população, como o retorno das epidemias do passado.

É fato. Por isso, será necessário mais tempo até que todas as dúvidas sejam esclarecidas e as opiniões hoje antagônicas afluam para um novo entendimento. Não há resposta fácil. Mas a questão é: há dúvidas e desconfiança onde antes parecia só haver certezas e tranqüilidade.

O que deve se deixar claro é que nem todos os que fazem restrições às vacinas querem abolir o seu uso. O ponto consensual é o de que está na hora dos centros tradicionais de pesquisas se disporem a investigar os problemas relacionados às vacinas e reavaliar as práticas atuais nessa área. E é essa uma das maiores complicações.

Sabemos que as vacinas têm benefícios globais. Quem nos diz o que seríamos hoje se não fosse à descoberta das vacinas? Do que reconheço, talvez eu não pudesse estar hoje, aqui, escrevendo sobre esta questão. Mas, isto não compensa o fato de que muitas crianças são mortas ou lesadas após receber vacinas. Há muitas vacinas seguras disponíveis para uso atualmente, porém, elas não são utilizadas. E por quê? Porque a comunidade médica continua a defender as vacinas antigas porque elas são menos dispendiosas e as empresas farmacêuticas – produtoras das vacinas – vão perder dinheiro, visto que as vacinas mais seguras são mais caras e levam mais tempo para serem produzidas.

Para manter a confiança do público nas vacinas, devemos garantir que a sua segurança é levada a sério e que, quando indicado, ações são tomadas no seu devido tempo para reduzir o potencial de risco.

E o debate está apenas começando.

3 OBJETIVOS

3.1 Objetivo Geral

Avaliar as implicações da exposição ao mercúrio derivado de vacinas conservadas com timerosal (VCT) e da dieta (incluindo aleitamento materno) nos atrasos desenvolvimentais de crianças.

3.2 Objetivos Específicos

- Avaliar a exposição pré e pós-natal ao mercúrio sobre o desenvolvimento neuropsicomotor de crianças.
- Avaliar qual o papel da dieta no processo de acumulação do mercúrio no cabelo de crianças.
- Verificar se durante o calendário de vacinação recomendado no Brasil pode-se superar os limites de exposição diária ao mercúrio estabelecido pela Organização Mundial de Saúde e outras agências internacionais.
- Analisar os níveis de exposição ao mercúrio no cabelo de crianças.
- Verificar se a exposição ao Hg das vacinas conservadas com timerosal afetou o crescimento e desenvolvimento de crianças.

4 MATERIAL E MÉTODOS

4.1 Caracterização da Amostra

Os dados iniciais deste estudo não foram coletados com o objetivo de avaliar o impacto da imunização nas DN. O protocolo de pesquisa inicial foi concebido para avaliar o crescimento e desenvolvimento das crianças expostas ao Hg no período pré-natal e até o sexto mês de vida através da alimentação materna (pré-natal) e aleitamento (MARQUES, 2002). Quando o trabalho de investigação foi iniciado não estávamos cônscios da questão das VCT (anexo 1 e 2). A amostra inicial constituiu-se de cem crianças nascidas nas maternidades do Hospital de Base Ary Pinheiro, Hospital Pan-Americano e Regina Pacis, e suas respectivas mães. Os referidos estabelecimentos de saúde estão localizados na cidade de Porto Velho, Rondônia.

Foram selecionadas todas as grávidas que concordaram em participar do estudo. As crianças deveriam alimentar-se exclusivamente com leite materno até os seis meses de idade. Os dados da ficha dos pacientes foram obtidos com a mãe ou responsável legal após prévia autorização por escrito (anexo 7) e nos registros hospitalares. Na mãe, avaliamos hábitos alimentares, antecedentes pessoais (pré-natal, parto, abortos, natimortos, recém-nascidos malformados, doenças) e familiares (passado em garimpo, ingestão de alimentos potencialmente contaminados, e outros), na busca de fatores de risco para exposição ao Hg ou que poderiam afetar o desenvolvimento de seus filhos (anexos 8 e 9).

Os recém-nascidos (RN) foram submetidos a exame de rotina ao nascer, realizados pelo pediatra e/ou enfermeira presentes no momento do parto para

verificação: 1) do índice de Apgar[7] no 1º e 5º minutos (avaliação da vitalidade); 2) da presença de reflexos, 3) da maturidade (prematuro, a termo ou pós-maduro); 4) do peso, estatura, perímetro torácico e cefálico; 5) classificação quanto ao peso: pequeno para a idade gestacional (PIG), adequado para a idade gestacional (AIG) e grande para a idade gestacional (GIG); 6) ausculta do tórax e coração e verificação de anormalidades grosseiras e malformações congênitas.

Os dados antropométricos, peso, estatura e perímetro cefálico (PC), foram comparados aos dados tabulados pela OMS, enquadrados nos z-escores e percentis apropriados (WHO, 2006a), utilizando-se o programa ANTRHO 2005. Os gráficos de crescimento têm sido usados para traçar o crescimento físico de crianças desde 1977 e passaram por duas amplas revisões (WHO, 2000; 2006b). Consistem de uma série de curvas, ilustrando a distribuição de crianças de acordo com as medidas do corpo selecionadas. São considerados os mais apropriados para aplicação clínica, representando um instrumento fundamental na avaliação do crescimento de crianças (Kuczmarski *et al.*, 1998; WHO, 2006a).

Aos seis meses de idade, 86 crianças compareceram ao exame clínico e neuropsicomotor programado, quando amostras de cabelo foram novamente coletadas. Para fins de avaliação usamos apenas 82 pares de mãe-filho. Maiores detalhes aparecem nos anexos 1 e 2. A avaliação foi realizada nos meses de maio e junho de 2001, aos sábados, no horário das 8 às 12 horas, e das 14 às 18 horas. As mães foram convidadas a levar suas crianças ao prédio central da Fundação Universidade Federal de Rondônia (UNIR) para a realização do exame físico e do

[7] A maioria dos hospitais utiliza um sistema de índices (classificação) elaborado pela Dra. Virgínia Apgar em 1952, para fazer uma avaliação clínica das condições físicas do recém-nascido com 1 minuto após o nascimento e novamente com 5 minutos. Essa avaliação inclui freqüência cardíaca, esforço respiratório, tônus muscular, irritabilidade reflexa e cor. Cada sinal pontua de 0 a 2 pontos. A contagem de 7 a 10 indica RN vigoroso, de 4 a 6 RN moderadamente deprimido, de 0 a 3 gravemente deprimido (Ziegel e Cranley, 1986).

desenvolvimento neuropsicomotor. Ao chegar ao local, mãe e filho (a) eram levados a uma sala preparada com balança, mesas e cadeiras, mesa de exame, fitas métricas, tesouras inox, brinquedos, material para acondicionamento do cabelo coletado, fichas dos gráficos de crescimento e desenvolvimento e questionários de cada criança. Nos meses de novembro e dezembro de 2003 e 2005, as crianças submeteram-se à nova avaliação física para verificação dos dados antropométricos e do desenvolvimento neuropsicomotor. Novos questionários, readequados para a idade, foram aplicados. Os dados contidos nos questionários incluem dados sobre hábitos alimentares, situação vacinal, antecedentes pessoais e familiares, e foram obtidos após nova autorização (Anexos 10 e 11).

Nessa fase, a coleta foi realizada de modo a atender a disponibilidade de cada mãe. Inicialmente, as avaliações foram realizadas na Policlínica Hamilton Raulino Gondim, bairro Tancredo Neves, visando atender as mães residentes em bairros próximos. A seguir, as moradoras dos bairros centrais foram convidadas a levar suas crianças ao prédio central da UNIR. Para as mães que faltaram à avaliação foi realizado visita domiciliar. Em cada avaliação, novas amostras de cabelo eram coletadas para estimativa da exposição ao mercúrio.

4.2 A Avaliação do Desenvolvimento Infantil

Na avaliação do desenvolvimento neuropsicomotor, consideramos como padrão de normalidade a Escala Desenvolvimental de Gesell e Amatruda (EDGA) (Gesell, 2003; Gessel e Amatruda, 2002). No estudo da conduta motora avaliamos as reações posturais, prensa palmo-plantar, locomoção e coordenação dos movimentos. Na conduta adaptativa testamos à capacidade construtiva, que é influenciada pelo desenvolvimento motor. Na conduta linguagem, avaliamos todas

as formas de visíveis e audíveis de comunicação. Na conduta pessoal-social avaliamos as reações individuais da criança frente às pessoas e estímulos, dependendo do temperamento da criança e condições ambientais. A EDGA faz uso de tabelas que permitem obter um “quociente de desenvolvimento” (QD) em cada um dos quatro campos avaliados e um geral. O QD é a relação entre a idade maturacional (derivada do desempenho comportamental da criança nas provas) e a idade real (cronológica), expressa em proporção:

$$QD = \frac{\text{IDADE MATURACIONAL X 100}}{\text{IDADE CRONOLÓGICA}}$$

As tabelas são usadas para checar a presença ou a ausência de comportamentos significativos, representados por sinais de positivo (+) e negativo (–), respectivamente. Os dados são comparados à escala elaborada a partir de condutas padrão apresentadas por crianças em determinadas faixas etárias. Deste modo, ao realizarmos comparações quantitativas e qualitativas, comparamos o estado de desenvolvimento com o comportamento adequado para a idade e determinamos se e em que grau ele se desvia. O QD de uma criança perfeitamente média é 100. Valores entre 85 e 68 são considerados limítrofes e tem implicações em termos de acompanhamento. QD abaixo de 68 indica atraso significativo em uma ou mais áreas do desenvolvimento.

Os materiais necessários foram confeccionados ou comprados, considerando as especificações descritas na escala. O tempo necessário para a aplicação das provas foi 20 a 30 minutos, aproximadamente, dependendo da colaboração da criança. As idades – chaves com os comportamentos esperados para os quatros setores do teste de Gesell são apresentadas no anexo 12.

4.3 Exposição estimada ao mercúrio das vacinas (timerosal injetado) e aleitamento materno

A exposição ao Hg derivado de vacinas baseou-se no Calendário de Imunização do PNI. Até os seis meses todos os lactentes receberam o esquema de imunização completo. Após esse período as mães foram orientadas sobre a importância da vacinação e a seguir as recomendações dos serviços de saúde. Entre as vacinas tomadas pelas crianças durante os 5 anos do estudo algumas foram conservadas com 0,01% timerosal. A concentração de Hg nas doses recebidas através de vacinas foi de 12,5 (hep B) ou 25 µg Hg/0.5 mL (outras vacinas).

O esquema de vacinação com VCT e respectivas dosagens de Hg, como indicado pelo fabricante, é apresentado nos anexos 2 a 4. Nós usamos os dados de concentrações de Hg no leite (adaptado de Dórea, 2004), para estimar a exposição ao Hg durante o período em que as crianças estavam sob amamentação exclusiva – média do peso infantil × média da ingestão diária de leite materno (140 mL/kg) × número de dias × média da concentração de Hg no leite materno (1,9 µg/L) – e discutimos os resultados nos anexos 2, 4, 5 e 6.

4.4 Determinação do nível de exposição ao mercúrio

Os níveis de Hg total foram verificados em amostras de cabelo (5 a 10 g) da mãe e filho (nascimento, seis meses, três e cinco anos), sangue materno (4,5 mL), placenta e cordão umbilical (5 g/peso úmido), coletadas na época do parto. As amostras foram analisadas pelo método de espectrofotometria de absorção atômica pela técnica de vapor frio. As técnicas e metodologias analíticas aplicadas no estudo estavam de acordo com as do Laboratório de Biogeoquímica Ambiental da UNIR (Bastos *et al.*, 1998). Mais detalhes aparecem nos anexos 1 a 6.

4.5 Análise Estatística

A análise e interpretação dos dados foram feitas através de estatística multivariada (regressão logística, análise dos componentes principais, por correspondência e prospecção de dados) e os testes de significância baseados em permutação. Para testar as diferenças entre grupos aplicamos métodos apropriados para cada caso. Os dados serão apresentados na forma de tabelas, ilustrações e gráficos, e comparados à bibliografia existente sobre o assunto. Detalhamento deste tópico é apresentado nos anexos 1 a 6.

4.6 Considerações Éticas

Para a primeira fase do estudo, o protocolo deste projeto foi aprovado pelo Comitê de Ética em Pesquisa com Seres Humanos do Núcleo de Medicina Tropical da Universidade Federal do Pará (CEP/NMT/UFPA) para o período de 2000 a 2001. Para as fases seguintes, a pesquisadora submeteu novo protocolo de pesquisa ao Comitê de Ética em Pesquisa com Seres Humanos do Núcleo de Saúde da Fundação Universidade Federal de Rondônia – CEP/NUSAU/UNIR, tendo sido aprovado para o período de 2003 a 2007. Não houve danos à dimensão física, psíquicas, morais, intelectuais, sociais, culturais ou espirituais dos participantes, em qualquer fase da pesquisa e dela decorrente, ou mesmo dano associado ou decorrente deste estudo científico. O estudo cumpriu todas as exigências da resolução CNS 196/96.

5 RESULTADOS

Os dados descritivos das mães e RN são apresentados no anexo 1. Os dados demográficos mostram uma ampla variação do status socioeconômico. A maioria das mulheres foi recrutada de um hospital público (n: 61; Hospital de Base) enquanto 39% vieram de dois hospitais privados (n:13, Hospital Panamericano; n: 26, Hospital Regina Pacis). Uma proporção substancial (39%) dessas mães eram primíparas. Para verificar alterações do desenvolvimento intra-uterino avaliamos as características físicas, habilidade funcional e sinais neuromusculares dos RN.

Na avaliação estatística dos parâmetros antropométricos pelo teste Anova, verificou-se a homogeneidade dos resultados entre os dois sexos. Os dados antropométricos de mães e RN são apresentados nos anexos 1, 2, 3 e 6. Nove RN estavam abaixo das curvas de referência do NCHS para o peso. Após seis meses de aleitamento materno exclusivo, quatro crianças apresentaram sobrepeso e apenas três meninos apresentaram estatura pequena para idade.

Os dados de exposição ao Hg são apresentados nos anexos 1 a 6. O consumo de peixe materno (Hg no cabelo) não dependeu da renda mensal ou educação (anos). Os níveis de Hg no cabelo materno variaram amplamente (0.2 $\mu g.g^{-1}$ to 62.4 $\mu g.g^{-1}$), refletindo extremos de consumo de peixe. Mulheres que relataram baixo consumo de peixe (<2 refeições/semana) apresentaram concentrações medianas de Hg no cabelo mais baixas (3.5 $\mu g.g^{-1}$) que o outro grupo (5.7 $\mu g.g^{-1}$). A maioria das amostras de cabelo (57%) apresentou níveis de Hg abaixo de 6 $\mu g.g^{-1}$. A concentração mediana de Hg no cordão umbilical (7.5 ng/g)

estava próxima à mediana de Hg na placenta. A análise de Hg no cabelo do RN mostrou que 92% apresentavam concentrações <6 $\mu g.g^{-1}$. Os coeficientes de correlação entre variáveis maternas e dos RN estão sumarizados no anexo 1.

As avaliações do desenvolvimento neuropsicomotor das crianças aos seis meses são apresentadas nos anexo 1, 2, 4 e 5. A maioria das crianças (74%) apresentou desenvolvimento normal e 26% atraso desenvolvimental em ou mais setores da EDGA: 1% apresentou atraso motor, 9% déficit na linguagem, e 16% tiveram atraso em dois ou mais setores (7% motor, linguagem, adaptativo, e 9% motor, linguagem). Após a identificação (e remoção) de variáveis redundantes, foi aplicada uma Análise de Correspondência para identificar grupos de variáveis associadas. A associação (co-ocorrência) é caracterizada pela posição similar nos dois planos dimensionais apresentados na Fig. 1, anexo 1 (Marques *et al.*, 2007a).

A exposição pós-natal ao Hg derivada da amamentação e das VCT é ilustrada na tabela 1, anexo 5. Houve uma grande evolução nos perfis de desenvolvimento quando as crianças estavam mais velhas. Comparando com as avaliações dos seis meses, o mesmo número de crianças permaneceu com QD abaixo de 75% em três anos, mas os centis elevaram-se (anexo 5). A relação entre PC e QD não foi significantemente correlacionada (Tabela 3, anexo 4).

Apresentamos os QD (aos 6 meses) relacionando-os com a dose de timerosal ao nascimento nos anexos 2, 3, 4, e 5; não houve correlação significativa. Porém, os QD foram significativamente correlacionados com duração do aleitamento materno (anexo 5). Os resultados das análises de regressão múltipla são apresentados nas Tabelas 3 a 5, anexo 4. Os modelos demonstraram que os atrasos desenvolvimentais foram transitórios.

Este estudo extraiu informações da assimetria associada com mudanças nas

concentrações de Hg no cabelo materno e infantil em amostragens específicas: nascimento (exposição fetal), seis meses de aleitamento materno exclusivo, 36 meses (desmame) e 60 meses (pré-escola). A distribuição do Hg no cabelo em lactentes seguiu um padrão diferente de suas mães (Fig. 1 a 4, anexo 5).

Mudanças metabólicas pós-parto, desenvolvimento infantil e dietas de transição e, provavelmente, Hg das VCT contribuem para a assimetria das mudanças do Hg no cabelo entre mães e crianças. É importante notar que a exposição à VCT a partir da primeira vacina tomada ao nascimento (0 dia) é a maior e a mais desafiadora das doses. Neste momento a pequena massa corporal dos neonatos tem um impacto equivalente ao dobro das doses (da DTP, hep B) aos seis e doze meses de idade. A estimativa da exposição ao Hg do leite materno só foi possível até o sexto mês, quando as mães foram cuidadosamente monitoradas e psicologicamente apoiadas a manter o aleitamento materno exclusivo. Contudo, não houve associação significante entre o Hg do cabelo e a duração do aleitamento, tanto para as mães quanto para as crianças. A maior média de Hg no cabelo aos seis meses coincidiu tanto com a exposição ao Hg, ao leite materno, quanto com o pesado cronograma vacinal. Além disso, a distribuição de Hg em lactentes seguiu um padrão diferente de suas mães (figura 2, anexo 5).

Após seis meses, crianças em aleitamento exclusivo receberam uma carga de imunização (cinco tipos de vacinas; até 150 µg de Hg) que mostrou um incremento das concentrações de Hg superiores ao nascimento, aos três e cinco anos de idade. As concentrações de Hg em suas respectivas mães foram mais altas no parto. A freqüência de consumo de peixes é apresentada e discutida nos anexo 1, 4 e 5.

Usando o Hg total no cabelo como marcador de exposição pós-natal ao Hg orgânico (Hg inorgânico e MeHg do leite materno; EtHg do timerosal) nós estudamos

sua associação com a escala de Gesell mensurada aos seis meses, três e cinco anos de idade. O Hg no cabelo aos seis meses respondeu aos eventos relacionados à exposição ao Hg e amamentação.

A maioria dos atrasos desenvolvimentais observados aos seis meses foram superados com o crescimento; aos cinco anos 87% das crianças apresentavam QD adequados (>85). O tempo de amamentação e o Hg no cabelo foram, cada um, significantemente correlacionados com QD, mas de maneiras opostas: o tempo de amamentação foi positiva e significantemente associado com o QD aos cinco anos; as concentrações de Hg no cabelo foram negativa e significativamente correlacionadas com QD aos 6 meses (r=-0.3329; p=0.0022) e cinco anos (r=-0.8029; p=0.0106), mas não aos 36 meses (r=-0.1722; p=0.1218). O resultado dos QD aos 5 anos dependeu do QD aos 3 anos, que por sua vez, foi influenciado pelas variáveis do desenvolvimento e de exposição ao Hg. O QD aos 6 meses foi significantemente influenciado pela exposição pré-natal (Hg no cabelo materno e infantil) e pós-natal aos seis meses.

Enquanto as mães apresentaram as mais altas concentrações de Hg no cabelo à época do parto, as crianças apresentaram os mais altos valores aos seis meses, coincidindo com período em que o calendário vacinal com VCT é mais intenso. Depois disso, a tendência de diminuição no cabelo da criança coincidiu tanto com o período do desmame quanto com o período de vacinação menos freqüente (cinco anos). A amamentação estendida (até 36 meses) não foi significantemente associada com o Hg no cabelo materno ou infantil. Porém, foi observada associação significante (Spearman's r) entre as concentrações de Hg no cabelo da mãe e da criança ao nascimento (r = 0.3534; P = 0.001), seis meses (r = 0.4793; P < 0.0001), três anos (r = 0.0122; P = 0.012) e cinco anos (r = 0.0357; P = 0.005). Mudanças metabólicas maternas no pós-parto, desenvolvimento infantil, dietas de transição e provável Hg

de VCT contribuem para a assimetria das mudanças entre mães e crianças.

É importante notar que a exposição ao Hg a partir da primeira VCT recebida ao nascimento (0 dia) é a maior e a mais desafiadora das doses. Neste momento a pequena massa corporal dos neonatos recebe um impacto equivalente ao dobro das doses (DTP e hep B) aos seis e 12 meses. A estimativa da exposição ao Hg do leite materno só foi possível até 6 meses, quando mães foram cuidadosamente monitoradas e psicologicamente apoiadas para manter-se em aleitamento materno exclusivo. Na verdade, a maioria das mães (66%) amamentou por 12 meses e algumas relataram amamentar até 60 meses (figura 1, anexo 5).

Não encontramos associação estatisticamente significativa entre o Hg no cabelo (mãe e filho) e a duração do aleitamento. A média de Hg no cabelo aos seis meses coincidiu tanto com a exposição ao Hg do leite materno quanto com o pesado cronograma de vacinação. A distribuição do Hg no cabelo de lactentes seguiu um padrão diferente de suas mães (figura 2, anexo 5). Houve uma grande melhoria nos QD quando as crianças estavam mais velhas. Comparando com as medidas dos seis meses, o mesmo número de crianças permaneceu abaixo de 75% aos três anos, mas os centis subiram. Nenhuma correlação significativa foi observada entre Hg no cabelo da mãe (exposição pré-natal) e QD aos seis meses. Entretanto, a exposição pós-natal (Hg no cabelo da criança) foi significantemente correlacionada com os QD aos seis meses ($r=-0.3329$; $P=0.0022$) e 60 meses ($r=-0.8029$; $P=0.0106$), mas não aos 36 meses ($r=-0.1722$; $P=0.1218$).

Entre os seis e 36 meses, não houve somente mudanças nas práticas de alimentação (desmame) associadas às mudanças na exposição ao Hg, mas também nas doses e intervalos de vacinação.

Nós investigamos o crescimento do PC e sua associação com os QD. O

percentual de aumento do PC e QD não foram significantemente correlacionados (Tabela 1 e 3, anexo 4). O mecanismo de crescimento somático que rege o PC foi independente do neurodesenvolvimento funcional. O crescimento cerebral durante diferentes períodos de alimentação (aleitamento materno exclusivo e desmame) e a intensidade da exposição ao EtHg não foram significativamente correlacionadas. Atrasos no neurodesenvolvimento ocorreram independentemente da exposição pré - natal ao Hg ou do percentual de aumento pós – natal do PC, mas foram influenciados pela amamentação e concentrações Hg no cabelo.

No anexo 3 e 6 apresentamos a escala de Gesell (aos seis meses) em função da dose de timerosal ao nascimento e não houve correlação significativa. No entanto, os QD foram significativamente correlacionados com a duração da amamentação (figura 5, anexo 6). Correlações positivas e significantes foram observadas para o QD motor (r= 0.3118; P=0.0044), linguagem (r= 0.3093; P=0.0047), QD adaptativo (r= 0.3246; P=0.0029), QD pessoal social (r= 0.3272; P= 0.0027) e para o QD total (r=0.3479; P=0.0014).

Aos seis meses o QD foi significantemente influenciado pelo pré-natal (Hg no cabelo materno e do RN) e exposição pós-natal aos seis meses. Neste modelo as interações de variáveis relacionadas às concentrações de Hg ao nascimento (pré-natal) e aos cinco anos, bem como a duração da amamentação, idade gestacional e peso ao nascer, não foram afetados significativamente pelo QD total. Além disso, também é possível inferir que os níveis de Hg no cabelo aos seis meses podem responder a eventos relacionados à exposição ao Hg, ou seja, aleitamento materno e VCT (anexos 4 e 5).

6 DISCUSSÃO

Assumindo que o cabelo é o melhor integrador da exposição passada ao MeHg, um dos achados deste estudo é que o marcador de consumo de peixe (Hg no cabelo materno) foi significantemente correlacionado com Hg no cabelo da criança coletado no nascimento. Essa significante correlação permaneceu até os seis meses. Contudo, o atraso motor observado em crianças com altos níveis de Hg no cabelo não indicaram uma relação dose-resposta: os mais altos valores foram encontrados em crianças do grupo considerado normal. Na figura 1, anexo 1, os mais altos níveis de renda e educação estão posicionados no grupo #2. Esses achados sugerem a ocorrência de algumas condições protetoras que não estão presentes em famílias desprivilegiadas. A situação de pobreza e baixo nível educacional da família provavelmente requerem suporte para o desenvolvimento ideal da mãe e filho. A média de Hg de 7.4 $\mu g/g^{-1}$ no cabelo dessas mulheres é mais baixa que os valores relatados para mulheres ribeirinhas (8.3–9.4 $\mu g/g^{-1}$) de outros estudos apresentados e discutidos no anexo 1.

A amostra de mulheres acompanhadas neste estudo, não é homogênea, nem cultural nem socioeconomicamente. Entretanto, como não houve insultos pré-natais severos que pudessem distorcer os padrões iniciais do neurodesenvolvimento, os atrasos observados aos seis meses estavam dentro do esperado para a população brasileira (Paine e Pasquali, 1983). Mesmo em populações homogêneas há variações nos resultados neurocomportamentais. Dodge *et al.* (1975) discutiram as limitações de confiabilidade de uma única função como avaliadora de um insulto sobre o

desenvolvimento normal. Quando se levam em consideração as avaliações dos três e cinco anos, observam-se atrasos no desenvolvimento não correlacionados com parâmetros socioeconômicos ou culturais. Estudos associando Hg e resultados neurodesenvolvimentais de crianças algumas vezes não fazem referência ao status do aleitamento materno. Por causa do papel fundamental da amamentação no desenvolvimento neuropsicomotor é importante ressaltar esses estudos.

Componentes específicos como os ácidos graxos poliinsaturados da série Omega 3 (PUFA), presentes no leite materno, mas não em fórmulas, que são essenciais para o desenvolvimento e organização neuronal tem explicado escores cognitivos e desenvolvimentais favoráveis ao aleitamento materno (Agostoni *et al.*, 2001). Por isso, quando a amamentação é considerada em estudos de exposição ao Hg, há inequívocos benefícios para o desenvolvimento das crianças amamentadas (Grandjean *et al.*, 1994; Jensen *et al.*, 2005; Marques *et al.*; 2007a).

Em estudos neurodesenvolvimentais com crianças durante o período de amamentação, freqüentemente não levam em conta a exposição iatrogênica das vacinas. Redwood *et al.* (2001) revisou exposição ao Hg devido ao timerosal (EtHg). O timerosal contribui com até 25 $\mu g/g^{-1}$ por dose; 12.5-40% dependendo da vacina. Durante o esquema de imunização, crianças podem receber vacinas ao nascimento (12.5 $\mu g/g^{-1}$), um (12,5 $\mu g/g^{-1}$), dois (62.5 $\mu g/g^{-1}$), quatro (50 $\mu g/g^{-1}$) e seis (62.5 $\mu g/g^{-1}$) meses que podem expô-la a um total de 207.5 $\mu g/g^{-1}$ de EtHg durante o primeiro semestre de vida (Marques *et al.*, 2007b; 2007c).

O impacto causado pelo EtHg injetado num organismo imaturo é um ponto importante a se considerar. Especula-se que a remoção do timerosal das vacinas não produziria mais que 50% de redução na exposição de Hg infância (Bigham e Copes, 2005). Esta redução só poderia ocorrer no leite humano com concentrações de Hg

superiores ao valor médio (figura 1, anexo 2) e mais freqüentemente com fórmulas, que geralmente contêm concentrações de Hg mais elevadas (Dórea e Donangelo, 2006). Além do mais, este cenário assume uma discutível equivalência de um bolus (Hg injetado) versus a integração (de seis meses) do Hg no leite ingerido e não contempla os princípios da neurotoxicidade do Hg: a forma química, a dose, a via de administração, os fatores atenuantes de neurotoxicidade do aleitamento e os fatores neurotóxicos do período perinatal (peso ao nascer/prematuridade).

As esperadas implicações metabólicas dessas rotas de exposição e formas químicas de Hg extremamente diferentes (EtHg injetado vs Hg ingerido no leite), bem como as doses de Hg são difíceis de decifrar. Temos a alteração nas interações fisiológicas de uma dose diária de Hg intrínseco auto – ajustada (inorgânico e MeHg) na amamentação contra o impacto total de uma dose extrínseca de EtHg. No que se refere à amamentação, o Hg do leite ocorre num contexto de benefícios comprovados em uma ampla gama de circunstâncias atenuantes da neurotoxicidade. Neste estudo, nós observamos um aumento relativo do Hg no cabelo. Este achado reforça uma ligação entre o EtHg do timerosal e aumento de Hg no cabelo, sustentando à utilização do Hg no cabelo de crianças vacinadas como um fator de confundimento da exposição ao Hg de fontes maternas.

A possível associação entre VCT e autismo está ganhando atenção científica. Mutter *et al.* (2004, 2005) discutiram a epidemiologia e o mecanismo das DN associadas ao Hg e sugerem que efeitos do MeHg encontrado em peixes são menos tóxicos quando comparados a fontes iatrogênicas de Hg. É necessário, novamente, advertir que estudos epidemiológicos relacionando autismo e VCT não têm levado em consideração o aleitamento materno. Aliás, há um estudo sugerindo que o início do desmame precoce pode contribuir para a etiologia do autismo (Tanoue e Oda, 1989).

Os perfis de distribuição das concentrações de Hg no cabelo materno e infantil durante os primeiros cinco anos revelaram diferenças no tipo de exposição. A cronologia da amostragem de Hg no cabelo da criança correspondeu aos eventos associados à dieta (que incluiu amamentação) e calendário de imunização. Nós encontramos uma clara tendência do Hg no cabelo da criança coincidindo com o calendário vacinal, mas não podemos provar uma relação causa-efeito. A ocorrência do EtHg nas VCT e de dietas de transição do desmame podem ter contribuído para o padrão de mudanças do Hg no cabelo infantil. A média nos níveis de Hg no cabelo infantil aos 5 anos é equivalente à materna, refletindo provavelmente exposição às mesmas fontes dietéticas de Hg. Uma vez que os lactentes foram expostos a doses mais altas de EtHg durante os primeiros seis meses, o aumento nos níveis de Hg no cabelo da criança pode ser resultado das taxas mais elevadas de transferência do EtHg no cabelo, em comparação com períodos subseqüentes da amostragem.

A flutuação do Hg no cabelo materno é uma questão importante, com implicações para políticas de saúde pública. Além disso, nós identificamos a entrada de outras fontes orgânicas de Hg. Contudo, nossa limitada capacidade analítica para determinar o EtHg no cabelo derivado das VCT é uma importante limitação na interpretação desses aspectos dos resultados.

Uma variedade de fatores intervenientes associados à imaturidade infantil e ao tempo de exposição complica a avaliação dos efeitos da exposição precoce ao Hg sobre neurodesenvolvimento. Os efeitos no SNC respondem a fatores relacionados ao hospedeiro (susceptibilidade genética, estado nutricional e condição do SNC), a exposição (dose, freqüência e duração) e a formas químicas específicas de Hg (inorgânicos e orgânicos). Essas interações governam a reatividade química, a biotransformação e, eventualmente, a toxicocinética e toxicodinâmica do EtHg em

lactentes. Ainda que não possamos ter certeza se o Hg no cabelo das crianças veio das vacinas ou de fontes maternas, estudos em animais já mostraram que o EtHg foi incorporado ao crescimento do cabelo de forma semelhante ao MeHg (Fang e Fallin, 1973; 1976; Zareba, 2007).

Um problema comum frente aos efeitos tóxicos do Hg ou à susceptibilidade em níveis subclínicos é a dificuldade de reconstruir com precisão respostas às fontes após a exposição. Isto é especialmente o caso com lactentes e acompanham mudanças nas dietas integradas com fontes de Hg (que podem incluir EtHg extrínseco), como no presente estudo. Além disso, existem as considerações éticas relacionadas com a amamentação e vacinação que limitam comparações de grupo (sem tais características), tornando estudos de exposição particularmente difíceis de realizar.

A associação transitória entre dosagem de EtHg das VCT e DN observados baseia-se no pressuposto que: a) a amamentação estava igualmente presente durante um período mínimo de seis meses em todas as crianças e exerceu um efeito protetor do SNC; b) durante o aleitamento materno exclusivo todas as vacinas foram igualmente administradas a todas as crianças; c) a exposição a todas as formas de Hg podem ter como resultado que só a formas orgânicas podem ser encontradas no cabelo (e incluir o EtHg). Nós usamos Hg total no cabelo como um substituto para as três possíveis formas de exposição ao Hg: do leite (Hg inorgânico e MeHg) e imunização (EtHg das VCT). Nossos modelos de regressão foram bem sucedidos em diferenciar os efeitos negativos da exposição ao Hg dos resultados do neurodesenvolvimento (talvez relacionados com a amamentação).

Usando a análise dos componentes principais (PCA) foi possível identificar hierarquias e posições de variáveis inter-relacionadas que identificaram riscos variáveis de exposição associados com escalas de DN aos seis meses. Deste modo,

nossos resultados podem explicar o aparente conflito relatado por Thompson *et al.* (2007): seu modelo estatístico foi insuficiente para discriminar resultados contrastantes de danos e benefícios no mesmo sistema neurológico, levando à associações inconsistentes entre a exposição ao timerosal das vacinas e os testes neuropsicológicos.

Embora não haja evidência de associação entre autismo e VCT, permanecem as questões relativas a outras DN. Andrews *et al.* (2004) estudaram crianças inglesas retrospectivamente no que se refere à DTP/DT recebida em três e quatro meses: foram observados resultados significativos para o aumento dos riscos de espasmos com o aumento das doses de VCT. Também foram observadas associações negativas significantes para atrasos desenvolvimentais inespecíficos, atraso geral e déficit de atenção em relação à exposição VCT.

Os estudos retrospectivos de crianças norte-americanas mostraram resultados diferentes (Geier e Geier, 2003; 2006; Verstraeten *et al.*; 2003). Estes estudos foram baseados em dados projetados para eventos pós-vacinais e coletados no sistema de notificação de efeitos adversos do governo norte-americano (*Vaccine Adverse Effects Reporting System-VAERS*) e no Sistema de Ligação dos Dados Vacinais (*Vaccine Data Link System-VDS*) e utilizaram estratégias diferentes de investigação.

Quando os efeitos protetores do aleitamento materno estão ausentes, insuficientes ou diminuídos no período do desenvolvimento, os riscos de atrasos ou DN são aumentados. Portanto, é surpreendente que estudos sobre VCT associando efeitos neurodesenvolvimentais e práticas de aleitamento materno não tenham sido realizados. Na verdade, apenas Heron e Golding (2004) ajustaram diversas variáveis, incluindo amamentação em seu estudo epidemiológico. Após este ajustamento, eles interpretaram os resultados como ausência de associação entre VCT e DN. Até agora,

estudos epidemiológicos retrospectivos (de países que suprimiram a utilização de VCT em crianças) têm fornecido pistas de possíveis relações entre VCT e DN. Estas pistas devem ser seguidas com estudos que sejam direcionados para fatores de modificação relacionados a práticas de alimentação materna (exposição pré-natal e dieta) e infantil, especialmente nos países que continuam a utilizar timerosal.

A imunização conferida pelas VCT está no centro do controle e prevenção das doenças infecciosas (salvando vidas e evitando sofrimento desnecessário). Esta proteção imprescindível é reforçada quando a criança é amamentada. O aleitamento materno tem demonstrado um efeito imuno-modulador (Pabst *et al.*, 1997) que confere significativamente níveis mais altos de anticorpos da DTP ao lactente do que para aquelas crianças que são alimentadas com fórmula (Hahn-Zoric *et al.*, 1990; Silfverdal *et al.*, 2007). Os estímulos imunológicos moderados das vacinas podem causar febre e respostas anoréticas, assim, o aleitamento materno protege contra ingestão de energia diminuída, essencial para o desenvolvimento infantil (Lopez-Alarcon *et al.*, 2007). A amamentação parece aliviar o trauma da dor causada pelas inoculações (Efe e Ozer, 2007).

Incertezas sempre devem beneficiar as crianças. Países ricos (geralmente com baixas taxas de amamentação), que podem pagar vacinas livres de timerosal optaram pela retirada VCT. Nos países que utilizam VCT, a abordagem do “benefício da dúvida” deve incluir práticas protetoras do SNC contra DN transitórias associadas com exposição pós-natal ao Hg. Neste contexto a estratégia de apoio ao aleitamento materno exclusivo deveria acontecer junto com a vacinação infantil.

Ademais, nossos resultados mostram que atrasos no desenvolvimento neuropsicomotor podem estar associados às iniqüidades em saúde e situação socioeconômica desprivilegiada. Cory-Slechta (2005) discutiu o baixo status

socioeconômico como um fator de risco para resultados adversos à saúde e disfunções comportamentais tanto em adultos quanto em crianças. Baixo status socioeconômico está associado com altos níveis de retardo mental, desordens do aprendizado e déficits de atenção e linguagem em crianças. Rice (2005) argumentou que a mais alta incidência de doença e disfunções que acompanham baixo status socioeconômico é devido ao alto estresse ambiental vivenciado por tais populações: uma injúria crônica associada a dificuldades econômicas resultando em prolongada elevação dos níveis de cortisol.

7 CONCLUSÕES

1. A dose e via parenteral da exposição ao etilmercúrio derivado das vacinas modulam o aumento relativo do mercúrio no cabelo de crianças amamentadas aos seis meses de idade.
2. Mudanças nos metabolismo materno pós-parto, desenvolvimento infantil e dietas de transição, e o mercúrio das vacinas conservadas com timerosal contribuem para a assimetria das mudanças nas concentrações de mercúrio entre mães e crianças.
3. Em crianças amamentadas, diferenças na exposição inicial ao etilmercúrio das vacinas conservadas com timerosal, não podem predizer atrasos clínicos do neurodesenvolvimento aos seis meses. Nós acreditamos que o aleitamento materno foi importante para a proteção do SNC das crianças frente aos desafios da exposição precoce ao mercúrio.
4. As desordens neurodesenvolvimentais em crianças imunizadas com vacinas conservadas com timerosal são sensíveis à duração da amamentação e exposição ao mercúrio (que inclui fontes maternas). Portanto, controlar a amamentação é um aspecto importante nos estudos epidemiológicos sobre vacinas conservadas com timerosal e desordens neurodesenvolvimentais.
5. Por fim, sugerimos que o aleitamento materno exclusivo deve ser parte complementar de uma estratégia preventiva contra possíveis atrasos transitórios do neurodesenvolvimento associados com exposição pós-natal ao mercúrio nas quais as vacinas conservadas com timerosal representam uma carga adicional.

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Maternal mercury exposure and neuro-motor development in breastfed infants from Porto Velho (Amazon), Brazil

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Abstract

Fish is an important item in the diet of Amazonians, and per se is their best single source of essential nutrients. Rapid urbanization and migration are bringing changes in dietary habits of Amazonians. Exposure to fish-Hg during pregnancy and lactation were studied in 100 women and newborns from Porto Velho. Tissue-Hg concentrations and neurodevelopment (Gesell Developmental Schedules) were assessed at birth and at 6 months in exclusively breastfed infants. Maternal mean frequency of fish consumption was low (<2 meals/week; range 0–>7 meals/week) compared to Amazonian standards. Women consuming <2 fish meals/week showed less median hair-Hg ($3.5\,\mu g\,g^{-1}$) than women that consumed $\geqslant 2$ fish meals/week (5.7 μg g). Median total Hg in maternal hair ($5.4\,\mu g\,g^{-1}$) was higher than in newborns ($1.6\,\mu g\,g^{-1}$). Significant correlation was observed between maternal hair-Hg and infant hair-Hg at birth ($r = 0.353$; $p<0.01$) and at six months ($r = 0.510$; $p<0.01$). Placenta-Hg was also significantly correlated to maternal hair-Hg ($r = 0.321$; $p<0.01$), newborn hair-Hg ($r = 0.219$; $p<0.05$), maternal blood-Hg ($r = 0.250$; $p<0.01$) and to umbilical cord-Hg ($r = 0.857$; $p<0.01$). Most infants (74%) had normal Gesell Schedules but among the 26% showing neuro-motor development delays only six (7%) had multiple (motor, language, and adaptative) delays. The infants with multiple delays were born from mothers with range of hair-Hg comparable to mothers of normally developed infants. Coincidentally, mothers of infants with multiple delays also showed the lowest range of income and level of education. Fish consumption, income, and level of education varied greatly among these breastfeeding urban mothers. It seems that development delays of exclusively breastfed infants are a component of the health inequalities that accompanies socioeconomic disadvantages.

Keywords: Amazon; Fish consumption; Neuro-motor development; Breastfeeding; Hair-Hg

Introduction

Mercury is listed among the most toxic substances in industrialized countries. One-third of emissions are estimated to originate from natural sources and the

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other two-thirds from anthropogenic sources (Patrick, 2002). Environmental Hg is found in various chemical forms: elemental Hg, inorganic Hg, and organic Hg (ethyl-, methyl-, alkyl-, or phenyl-Hg). The chemistry of Hg modulates its toxicity and metabolism. While inorganic Hg acts mainly on the kidneys, volatile metallic Hg and especially methylmercury (MeHg) primarily affect the central nervous system (CNS). The vulnerability of the developing human brain is the most important window for harmful Hg compounds (Castoldi et al., 2003). Dietary intake of fish and seafood products is the main source of MeHg exposure. Fish MeHg is easily absorbed by gastrointestinal tract and rapidly enters the blood stream. It is distributed throughout the body within 3–4 days. It is estimated that 5% of dietary MeHg is found in the blood and 10% in the brain, with a half-life ranging between 45 and 70 days (Castoldi et al., 2003).

Fish is an important item in the diet of Amazonians, and per se is their best source of essential nutrients. Fish is a fundamental complement for native Amazonians on protein-poor starchy food diets (70–80% of dietary energy from cassava); its protein content is well digested and has a high biological value (Dorea, 2004). Furthermore, besides its nutrient content, fish flesh can enhance absorption of Zn and Fe (Dorea, 2004). According to Inhamuns and Franco (2001), Amazonian fish contain omega-3 polyunsaturated fatty acids (PUFA; decosahexanoic [22:6] acid and eicosapentaenoic [20:5] acid) essential for infant neurodevelopment. Decosahexanoic acid is an essential component of nervous system cell membranes that is delivered to the fetus and post-natally into milk. However, fish is also a bioconcentrator of natural MeHg. Fortunately, Amazonian fish are, on the other hand, a good source of selenium (Dorea et al., 1998), known to counteract the toxic effects of Hg; significant correlations between Hg and Se in hair were reported in Amazonians (Vasconcellos et al., 2000; Campos et al., 2002).

A legitimate concern arising from the large amounts of metallic Hg (used to extract gold) discarded in the Amazonian environment prompted fish-Hg studies that were summarized by Dorea (2003). Amazon fish bioconcentrate MeHg originated from naturally occurring Hg in the rainforest (Barbosa et al., 2003). It is now known that Amazon soils are rich in Hg and those constitute a natural source of Hg for methylation (Fadini and Jardim, 2001; Bastos et al., 2006). However, while deforestation (along with agricultural projects) and alluvial-gold extraction have brought about vast changes in the environment of West-Amazonia, urban development has brought changes in the traditional lifestyles of human populations.

Fish consumption is part of the cultural adaptation of indigenous "*ribeirinhos*" (riverine populations). Amazonian women (especially Rio Negro *ribeirinhos*) depending heavily on fish consumption have showed hair-Hg that is among the highest of the world (Barbosa et al., 1998). Despite this no neurological problems involving fish consumption or other neurotoxic substance in foods like cassava products have been described (Dorea, 2004). Neuro-behavioral tests suggested that riverine children of East Amazonia presented alterations associated with high hair-Hg concentrations (Grandjean et al., 1999), but a recent study reported no significant difference (Tavares et al., 2005). In the French-Guyanese Amazon, Cordier et al. (2002) reported no major neurologic signs in Amerindian children of three different levels of fish-Hg exposure (regions with high, intermediate and low fish consumption). Nevertheless, but they observed that after adjusting for some potential confounders, there were dose-dependent (regions) effects, which were increased deep tendon reflexes, poor coordination of the legs, and decreased performance in the Stanford-Binet Copying score.

Tissue accumulation and toxicity are not equivalent in the case of Hg; MeHg accumulation is greater in the kidney than in the brain, but the brain appears to be the key target (Cory-Slechta, 2005). It is a point of fact that the CNS formation and development during pregnancy and lactation can be affected by multiple causes, ranging from maternal nutrition to neurotoxic-substance exposure. In this specific time window, Hg exposure can affect neurobehavioral functions. Mild exposure may result in delayed symptoms (not observed at birth), such as difficulty in walking and talking, and persistence of abnormal perinatal reflexes (World Health Organization (WHO), 1990; Myers and Davidson, 1998).

Our objectives were: (a) to study fish-Hg exposure of urban Amazonian mothers; (b) to associate maternal exposure (hair-Hg) with tissue-Hg and factors relevant to neuro-motor development of breastfed infants; and (c) to examine the association between generated dimensions of infant neurodevelopment and maternal socioeconomic and Hg exposure features.

Materials and methods

Porto Velho is the capital of the state of Rondonia (West Amazonia). Until the 1960s it was a traditional Amazonian city but after agricultural projects in the southwest region of the state followed the opening of roads, there was also a great influx of prospectors for exploring alluvial gold along the banks of the Rio Madeira basin. Since then, Porto Velho has experienced significant demographic changes with people coming from many other Brazilian regions.

The research protocol was approved by the Ethics Committee of Studies for Humans of the Universidade Federal de Rondonia. Pregnant mothers were introduced to the study and invited to participate by a nurse

during their routine visits to the Pre-natal Clinics of three hospitals of Porto Velho: Hospital de Base, Hospital Panamericano and Hospital Regina Pacis. Potential participants received plain-language information about the study and a written consent form was presented and signed by the volunteering mother; the written consent stated that participation was voluntary, their confidentiality was assured and that they could withdraw from the study at any time. Mothers were selected among those in good health, reporting no illness or complaints at the time of the study and who were willing to breast feed. Excluding factors were occupational exposure to toxic chemicals and hereditary neurological illnesses. One hundred mothers between the ages of 15 and 45 years were recruited among those that manifested the intention of exclusively breastfeeding up to 6 months of age.

For each mother a complete clinical evaluation was obtained from medical records and at the time of the first interview a questionnaire was applied to assess socioeconomic and educational status. The questionnaire also evaluated food habits, frequency of fish consumption, and intention of breastfeeding. While at the maternity wards, we collected samples of cord blood, placenta and hair from mothers and respective infants (fetal hair). Maternal blood (4.5 mL), placenta (three different aliquots) and umbilical cord sample (5–10 g) were stored in nitric-acid clean vessels, refrigerated and taken to the laboratory and frozen at −20 °C until analysis. Hair strands were cut from the occipital region and placed in plastic bags, with the root end stapled on a paper sheet.

The newborns were clinically examined with special attention to vitality, perinatal reflexes, maturity, and congenital malformations; weight, length, head circumference, and Apgar scores were recorded. Anthropometric data at birth (weight, length and head circumference) were compared with data tabulated by US National Center for Health Statistics—(NCHS) after its adaptation by the WHO for world-wide use (World Health Organization (WHO) Group on the Growth Reference Protocol, 1998; Kuczmarski et al., 2000). The software for calculating pediatric anthropometry (ANTHRO 1.02, 1999) from the Centers for Diseases Control and Prevention was used.

At 6 months of age, only 86 of the 100 original mother–infant pairs reported for the programmed clinical and neurobehavioral examination when hair samples were collected again. Five mothers had moved out of the state, five did not report and could not be found, and four babies had died within the first month. Because two mothers developed gestational diabetes and two others developed pre-eclamptic toxemia, we used only data from 82 mother–infant pairs. Children received the full immunization scheme in accordance with Brazilian vaccination program.

The infants' development assessment was conducted at the age of 6 months by trained professionals using the Gesell Developmental Schedules (Gesell, 2003; Gesell and Amatruda, 2000). The Gesell Schedules included all reactions (voluntary, spontaneous or learned) and reflexes. We also evaluated postural reactions, hand pressure, locomotion and coordination, constructive ability (which is influenced by motor development), visible and audible communication, individual reactions regarding people and stimulations (depending mainly on the temperament of the child and the surroundings). The results were expressed as developmental scores for the Motor Skills, Language Development, Adaptive Behavior, and Personal Social behaviors.

Hg determination

Sample preparation and Hg determination were done according to routine procedures previously established at the Universidade Federal do Rio de Janeiro (Bastos et al., 1998). We followed routine procedures of the laboratory after adaptation of analytical protocol used for Hg determination in previous studies analyzing blood, hair and fish-flesh matrices (Bastos et al., 1998; Malm et al., 1989, 1998). Briefly, the hair samples were comminuted with stainless steel scissors, weighed, and digested before analysis. Blood samples were digested with concentrated HNO_3 (3 mL) and $KMnO_4$ (5%; 6 mL) using a microwave oven system for 35 min (CEM-Coorporation, MDS 2000, Matthews, NC, USA). Placenta and umbilical cord samples were weighed and digested with HNO_3:H_2SO_4 (1:1; 5mL) and $KMnO_4$ (5%; 4 mL) using a digestion block at 80 °C for 1 h (Tecnal Ltd., Piracicaba, São Paulo, Brazil). Human hair samples were washed with EDTA 0.01%, dried in an oven at 50 °C, weighed and digested with 5 mL of HNO_3:H_2SO_4 (1:1) and 4 mL of 5% $KMnO_4$ using a digestion block at 80 °C for 40 min. The determination of total Hg in the digested samples was done by cold vapor atomic absorption spectrometry with a flow injection system-FIMS (CV-AAS, Perkin-Elmer—FIMS 400, Ueberlingen, Germany). All glassware used in the analytical protocol was washed clean, rinsed with 5% EDTA and double distilled, and left to rest in 5% HNO_3 overnight. Then it was rinsed again in double-distilled water, and dried at 100 °C for 12 h. Precision and accuracy of Hg determinations were assured by the use of internal standards, use of triplicate analyses of samples and certified reference materials (IAEA-085 and 086, Vienna, Austria) with recoveries of 92%.

Statistical analysis

A multivariate correspondence model was used to analyze maternal factors (tissue Hg, socio-economic

status) that might affect the infant's social and adaptive abilities, gross motor ability or the fine motor ability; this step was done with statistical software (Statsoft, 2002, Tulsa, AZ, USA). Variables used for the analysis are listed in Table 1. Afterwards, correlation analysis was used to compare the tissue-Hg concentrations between mothers and respective neonates (SPSS for Windows 14.0, 2005, Chicago, USA).

Results

Descriptive data of mothers and infants are presented in Tables 1–5. The demographics summarized in Table 1 show a wide range of socioeconomic status (SES). Most of the 100 mothers were enrolled from a public hospital ($n = 61$, Hospital de Base) while 39% were from corporate middle-class hospitals ($n = 13$, Hospital Panamericano; $n = 26$, Hospital Regina Pacis); a substantial proportion (39%) of these mothers were primiparas. The median income was US$125, and 64% did not have indoor plumbing, thus indicating that the majority of mothers were socioeconomically underprivileged. Irrespective of income and education, 57% reported that they consumed fish up to once a week, 4% reported more than 7 fish servings a week and only 5% reported not consuming fish (Table 1). Anthropometric data of mothers and infants are shown in Table 2. Nine newborns were below and two were above NCHS reference curves for weight. After 6 months of exclusive breastfeeding four children were overweight and only three boys presented short length.

Mercury exposure data are presented in Tables 3–5. Tissue Hg concentrations as a function of reported

Table 2. Biodata of the 82 mother–infant pairs

	Minimum	Median	Maximum
Maternal data			
Pre-natal			
Age (years)	15	22	40
Weight(kg)	43	55	68.5
Height (m)	1.51	1.61	1.7
BMI (kg m^{-2})	17.40	21.19	26.56
Gestational age (w)	36	39.5	43
Pregnancy, n[a]	1	2	8
At the end of pregnancy			
Weight(kg)	53	69	85
BMI (kg m^{-2})	22.35	26.53	32.39
Infant data			
Birth			
Weight (g)	2200	3200	4370
Length(cm)	46	50	55
Head circumference(cm)	30	34	39
Six months			
Weight (g)	6110	7000	8500
Length(cm)	61	68	73
Head circumference (cm)	40	43	45

[a]Primiparas = 39.

Table 1. Socioeconomic characteristics of the 100 mothers enrolled in the study

Characteristics	Minimum	Median	Maximum	Percentage
Mother education (years)	0	8	18	
Income (US$)/m	16.67	125.00	1250.00	
Type of home				
Owned				57
Rental				14
Living with relatives				29
Persons/household	2	5	14	
No electricity				20
Water supply				
Running water				36
Well				61
Local rivers				3
Fish-eating habits				
Fish meals (week)	0	1	14	
0–1/week				57
2–7/week				43
Fish-eating sources				
Market fish				53
Local rivers				42
No fish				5

Table 3. Maternal income, education and tissue Hg concentrations as a function of reported frequency of fish consumption

Fish servings/w	*N*	Blood [Hg] ($\mu g\,L^{-1}$)	Hair [Hg] ($\mu g\,g^{-1}$)	Umbilical cord ($\mu g\,g^{-1}$)	Placenta ($\mu g\,g^{-1}$)	Income (US$)	Education (y)
0–1	57	0.6 (0.01–10)[a]	3.5 (0.2–28.7)	8.1 (0.53–58.1)	8 (0.7–50.4)	125 (50–1250)	8 (0–18)
$\geqslant 2$	43	0.5 (0.01–10)	5.7 (0.16–62.4)	6.5 (0.1–43.7)	7.9 (0.2–56.3)	125 (17–1250)	8 (4–17)

[a]Median (Min−Max); w = week; y = year.

Table 4. Total Hg concentrations in tissues of mothers and infants

	Minimum	Median	Maximum
Mothers			
Umbilical cord ($ng\,g^{-1}$)	0.12	7.44	43.74
Placenta ($ng\,g^{-1}$)	0.37	8.10	56.28
Blood ($\mu g\,L^{-1}$)	0.01	0.55	9.97
Hair ($\mu g\,g^{-1}$)	0.39	5.40	62.43
Infant's hair Hg ($\mu g\,g^{-1}$)			
Fetal	0.05	1.59	19.65
Six months	0.02	1.81	32.95

frequency of fish consumption are shown in Table 3. In this study, maternal fish consumption (hair-Hg) did not depend on monthly income or years of education; mean income and education were very close between the groups. The maternal hair-Hg concentrations varied widely (0.2–62.4 $\mu g\,g^{-1}$) reflecting extremes of reported fish consumption. Indeed mothers that reported low fish-consumption (<2 servings a week) showed a lower median hair-Hg concentrations (3.5 $\mu g\,g^{-1}$) than the other group (5.7 $\mu g\,g^{-1}$). Most of the maternal hair-Hg concentrations (57%) were below 6 $\mu g\,g$; 34% of hair samples showed Hg concentrations between 6 and 15 $\mu g\,g^{-1}$ and 9% showed hair-Hg greater than 15 $\mu g\,g^{-1}$. The statistics of Hg concentrations in mothers and infants are shown in Table 4. The median umbilical cord-Hg concentration (7.5 $ng\,g^{-1}$) was close to the median placenta-Hg concentration. Fetal hair-Hg of the 82 newborns showed that 92% were <6 $\mu g\,g$; 7% were between 6 and 15 $\mu g\,g^{-1}$ and only 1% was >15 $\mu g\,g^{-1}$. Correlation coefficients among maternal and infant Hg-variables are summarized as follows: significant non-parametric correlations (Spermans) were observed between maternal hair-Hg and infant hair-Hg at birth ($r = 0.353$; $p < 0.01$) and at 6 months ($r = 0.510$; $p < 0.01$). Placenta-Hg was also significantly correlated with maternal hair-Hg ($r = 0.321$; $p < 0.01$), newborn hair-Hg ($r = 0.219$; $p < 0.05$), maternal blood-Hg ($r = 0.250$; $p < 0.05$), and with umbilical cord-Hg ($r = 0.857$; $p < 0.01$).

The assessment of neuro-motor development of breastfed 6-month-old infants is summarized in Table 5. Most infants (74%) showed normal schedules and 21 children (26%) exhibited developmental delay in one or more features of the Gesell Schedules: 1% showed motor impairment, 9% had language deficits, and 16% had multiple impairments (7% motor, language, adaptative, and 9% motor, language). There were no children who presented developmental delay in the personal social behavior. There were no children who presented developmental delay in the social ability. The 26% of infants showing neuromotor delays showed higher median hair-Hg values; these infants were born from mothers that had median hair-Hg concentrations also higher than mothers of normally developed infants. However, infants with multiple delays were born from mothers that also showed the lowest median income. The infants with higher median fetal hair-Hg showed even higher median hair-Hg at 6 months (Table 5).

Multivariate analysis was performed to identify groups of related variables. After identification (and removal) of redundant variables by Cluster Analysis, Correspondence Analysis was applied to identify groups of associated variables. Association (co-occurrence) is characterized by similar position in the two-dimensional (2D) plane depicted in Fig. 1. The first dimension is characterized by the infant groups which showed the most delayed development (adaptive, motor and language) and the greatest hair-Hg concentrations (group #1 and group #3). In contrast with a group of normal children from high income families and more educated mothers (group #2). The second dimension is mainly characterized by the highly exposed neonates (group #1, with high hair-Hg) in contrast with another group of infants with mild delay in Gessel scores (group #4).

Discussion

Assuming that hair strands are the best integrator of past MeHg exposure, the main finding of this study is that the marker of fish consumption (maternal hair-Hg) significantly correlated with fetal hair-Hg; this significant association lasted until 6 months of lactation. Notably, delay in neuromotor development observed in infants with higher median hair-Hg did not indicate a dose response relationship: the highest hair-Hg values were found in the normal infant group (Table 5).

Table 5. Frequency distribution of neurodevelopment (Gesell Schedules) of infants at 6 months of age and summary of corresponding markers of Hg exposure and socioeconomic status

Gesell Scale	*N* [%]	Infant hair Hg, 0 m, ($\mu g\,g^{-1}$)	Infant hair Hg, 6 m, ($\mu g\,g^{-1}$)	Mother hair Hg ($\mu g\,g^{-1}$)	Income (US$)	Mother education (y)
Normal	61 [74]	1.4 (0.05–9.3)	1.6 (0.01–33)	4.28 (0.39–62.43)	145.8 (16.7–1250)	8 (4–18)
Motor (M)	1 [1]	0.62	1.7	10.56	375	11
Language (L)	7 [9]	1.59 (0.3–3.5)	2.8 (0.9–26.9)	7.29 (1.30–28.73)	145.8 (50–525)	8 (5–16)
M and L	7 [9]	2.3 (0.6–7.4)	5.6 (1.2–15.1)	10.34 (2.14–12.48)	104.16 (70–125)	8 (4–11)
M, L and A[a]	6 [7]	6.4 (2.3–19.7)	6.9 (0.4–18.1)	5.74 (3.32–20.77)	104.16 (83.3–208.3)	6.5 (6–11)
Overall	82 [100]	1.6 (0.05–19.7)	1.8 (0.02–33)	5.40 (0.39–62.43)	125 (16.7–1250)	8 (0–18)

Median (Min–Max); m = month; y = year.
[a]A = Adaptive.

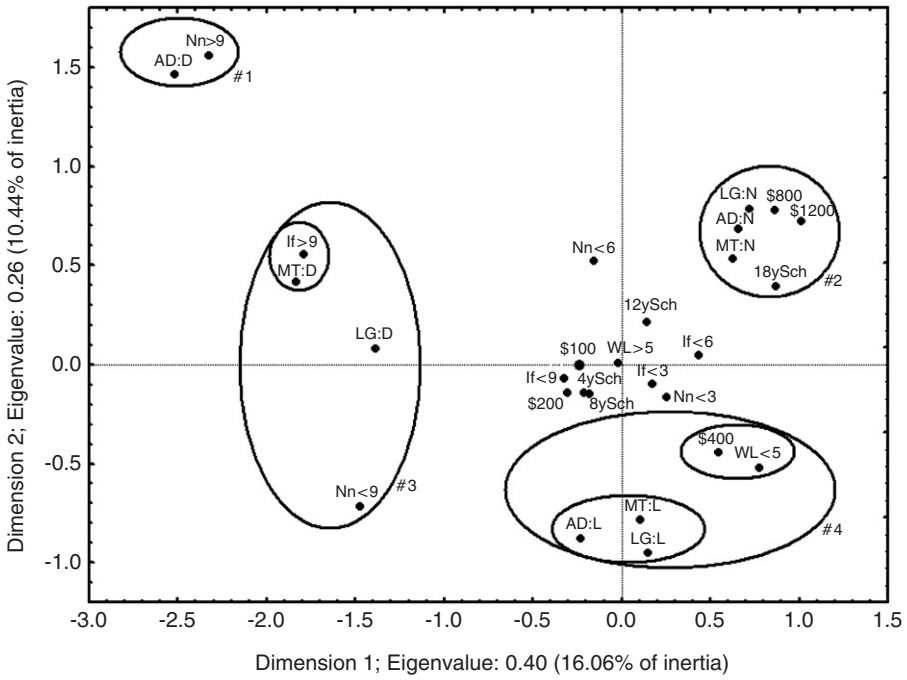

Fig. 1. Multivariate analysis (anthropometrics parameter, performance in Gesell schedule and socioeconomics status). MT—Motor, LG—Language, AD—Adaptive, N—Normal, D—Delay, L—Borderline, If—infant Hg-hair, Nn—newborn Hg-hair, ySch—mother education in year, $—income, WL—weight-for-length percentile.

In Fig. 1, the highest income and educational levels were clustered in group #2. These finding suggests the occurrence of some protective conditions which were not present in underprivileged families. Poorer and less educated families more probably lack mother–fetus pairs ideal development support.

The mean hair-Hg of these mothers ($7.4\,\mu g\,g^{-1}$) is lower than reported values for *ribeirinho* mothers ($8.3–9.4\,\mu g\,g^{-1}$) of the Rio Tapajós (Pinheiro et al., 2005), breastfeeding mothers ($14.3\,\mu g\,g^{-1}$) of the Rio Madeira (Barbosa et al., 1997) and the Rio Negro (Dorea et al., 2003). In the *ribeirinho* women of the Rio Negro consuming at least one fish-based meal a day median hair-Hg ($18.3\,\mu g\,g^{-1}$) was much higher than that of women in the present study (Dorea et al., 2003). In *ribeirinho* women of the Rio Madeira, the mean breastmilk-Hg concentration was $5.8\,ng\,g^{-1}$ and infant hair-Hg was $9.8\,\mu g\,g^{-1}$ (Barbosa et al., 1997). There were indications that MeHg transfer was higher during pregnancy than during breastfeeding. Nevertheless, there are no reports of clinical signs of neuropathologies in that population associated with fish consumption. A recent study of urban mothers from Paramaribo (Surinamese Amazon) showed a much lower concentration ($0.8\,\mu g\,g^{-1}$) of hair-Hg (Mohan et al., 2005). In non-traditional urban pregnant-women of Alta Floresta (southern Amazonia) the mean hair-Hg concentrations was also $1.2\,\mu g\,g^{-1}$ (range, $0.05–8.2\,\mu g\,g^{-1}$).

This population is formed by non-fish eaters that migrated from the South of Brazil during the gold rush (Hacon et al., 2000).

The studied sample of Porto Velho women is not homogenous, neither culturally or socioeconomically. Therefore, because there were no severe prenatal insults that could distort early pattern of neurodevelopment, the observed delays were within expected rates for Brazilians (Paine and Pasquali, 1983). Even in homogenous populations there are variations in neurobehavioral outcomes. Dodge et al. (1975) have discussed the limitations of the reliability of a single function as the evaluator of an insult on normal development. In our case there were only six cases of retardation of all milestones.

Regarding prenatal Hg exposure, our results (Gesell Schedules) are consistent with the absence of neurodevelopmental abnormalities in early childhood reported in Peruvian mothers (Pacific coast) with comparable mean hair-Hg levels ($8.3\,\mu g\,g^{-1}$; range $1.2–30\,\mu g\,g^{-1}$) during pregnancy (Marsh et al., 1995). A larger longitudinal study with 708 Seychellois infants also reported that in utero exposure to MeHg (from a maternal fish diet) caused no adverse outcomes in neurological and psychological development at 6 months of age (Myers et al., 1995). In that study maternal hair-Hg ranged from 0.5 to $26.7\,\mu g\,g^{-1}$ (Myers et al., 1995). Neurodevelopmental and Hg-associated studies of infants and young children sometimes do not make references to breastfeeding status. Because of the fundamental role of breastfeeding and neuromotor development it is important to emphasize such studies. Only 6% of the breastfed infants in the present study showed adaptive behavior delays. Specific components (PUFA) present in breast milk, but not in infant formulas, which are essential for neuronal development and organization have accounted for cognitive and developmental scores in favor of breastfeeding (Agostoni et al., 2001). Therefore, when breastfeeding is considered in maternal-Hg contamination studies, there are clearly benefits for the neurodevelopment of the breastfed infants (Grandjean et al., 1994; Jensen et al., 2005).

Although we collected fetal hair at birth, infant exposure to maternal Hg (consumed as fish-MeHg) during pregnancy is better assessed with maternal hair-Hg. Indeed, maternal hair-Hg was significantly correlated with fetal hair-Hg, which is in agreement with other Amazonian studies of women with high (Barbosa and Dórea, 1998) and low (Mohan et al., 2005) fish consumption. Lindow et al. (2003) also found significant correlation between maternal hair-Hg and fetal hair-Hg in a group of mothers predominantly exposed to dental amalgam-Hg. There are several drawbacks in fetal hair-Hg as a reliable indicator of intra-uterine exposure. Not all infants are born with hair; hair starts to grow at the 18–20th week of development at the frontal and the parietal regions and is likely to fall by the time of birth. Also, occipital hair is likely to grow at the time of birth and to fall after 12 weeks. Furthermore, hair density, length and texture are dependent upon factors such as race and sex. Fetal hair-Hg in the present study was higher ($2.4\,\mu g\,g^{-1}$) than for newborns ($1.6\,\mu g\,g^{-1}$) of urban Surinamese mothers (Mohan et al., 2005).

Studies involving non-occupational maternal Hg exposure (fish consumption) during pregnancy and infant neuro-motor development are complicate to design because of the confounding factors. This is further complicated when mothers are exposed to neurotoxic substances other than MeHg present in fish (Stewart et al., 2003). Nevertheless, maternal fish intake during pregnancy was associated with higher developmental scores for language comprehension, and social activities (Daniels et al., 2004). A recent study by Oken et al. (2005) in low fish-eating American mothers showed that the mean visual recognition memory was higher at six months in infants born to mothers that ate >2 fish servings/week but had less than 1.2 of hair-Hg during pregnancy, clearly suggesting an interaction between positive effects of fish eating and negative effects of Hg contamination. Although the multivariate model adjusted for breastfeeding duration, Oken et al. (2005) failed to address the organic Hg effects currently debated in vaccinated infants.

Fetal Hg exposure is exclusively derived from maternal contamination. However, during breastfeeding, iatrogenic Hg exposure (vaccines) is frequent but has not been taken into consideration in infant neurodevelopmental studies. Redwood et al. (2001) reviewed Hg exposure due to Thimerosal (Thiomersal, ethylmercurithiosalicate-TMS), a preservative found in many infant vaccines. TMS contains 49.6% ethyl mercury-EtHg (by weight) contributing 25 μg of EtHg per dose; 12.5–40% depending on the vaccine (Redwood et al., 2001). During an immunization schedule, infants may receive vaccines at birth (12.5 μgEtHg), two (62.5 μgEtHg), four (50 μgEtHg) and six (62.5 μgEtHg) months that could expose them to a total of 207.5 μgEtHg during the first 6 months of life. The possible association of vaccinations (containing TMS) and autism spectrum disorders (ASD) is gaining scientific attention. Mutter et al. (2004, 2005) discussed the epidemiology and mechanism of Hg-associated developmental (behavioral) disorders and suggested that effects of MeHg found in fish are less toxic compared to iatrogenic sources of Hg. They argued that the vaccine situation resembles the epidemic of acrodynia in the last century which affected up to 1 of 500 infants. After removing teething powder, which contained Hg as calomel (Hg_2Cl_2), acrodynia disappeared. They also argued that in 1953 immunizations with TMS-containing vaccines preceded the onset of acrodynia in several cases. It should be noticed that epidemiological studies relating ASD and vaccine-TMS have not considered

breastfeeding. However, there is one study indicating that early weaning could contribute to the etiology of autism (Tanoue and Oda, 1989).

Mercury in the umbilical cord is not frequently assessed, but one such study (Murata et al., 2004) reported that maternal hair-Hg concentration was significantly correlated with the MeHg in the umbilical cord obtained from 49 newborns. This is closely in line with our present study. Besides easier sampling and processing, umbilical cord may be collected 1 week after birth. A retrospective study (babies born between 1950 and 1965) of the Minamata crisis in Japan showed that the 24 patients diagnosed with Minamata disease had umbilical cord Hg concentrations of $1.63\,\mu g\,g^{-1}$ (Akagi et al., 1998). This is higher than values that we (in the present study) and others (Daniels et al., 2004) found in low fish eating mothers.

Although fish consumption has been monitored for riverine populations this is the first study reporting fish consumption of urban Amazonian mothers. In Amazonian populations hair-Hg is a surrogate of fish consumption of adults (Dorea et al., 2005a) and children (Dorea et al., 2005b). The Brazilian Amazon population showing elevated hair-Hg exposure is mainly indigenous and *ribeirinho*. The importance of subsistence fishing for *ribeirinho* communities has shown to be proportional to their distance from urban centers (Alves et al., 2006). In remote communities of the Rio Madeira subsistence on fish is also well characterized by mean hair-Hg concentrations $>16\,\mu g\,g^{-1}$. We recently showed that these communities may consume fish twice a day (Bastos et al., 2005). In the present study, fish consumption of the Porto Velho mothers is greatly decreased probably as a result of urbanization and migratory fluxes from other parts of the country. However, hair-Hg is much higher than in Paramaribo women (Mohan et al., 2005). This study also shows that neuro-motor development is not associated with fish consumption but could be placed with health inequalities and social deprivation. Indeed, Cory-Slechta (2005) discussed low SES itself as a known risk factor for adverse health outcomes and behavioral dysfunctions both in adults and children. Low SES is associated with higher levels of mental retardation, learning disorders and language and attention deficits in children. Rice (2005) argued that the higher incidence of disease and dysfunction accompanying low SES is due to the greater environmental stresses experienced by such populations: a chronic strain associated with persistent economic hardships resulting in protracted elevation of cortisol levels.

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ORIGINAL PAPER

Hair mercury in breast-fed infants exposed to thimerosal-preserved vaccines

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Abstract Because of uncertainties associated with a possible rise in neuro-developmental deficits among vaccinated children, thimerosal-preserved vaccines have not been used since 2004 in the USA (with the exception of thimerosal-containing influenza vaccines which are routinely recommended for administration to pregnant women and children), and the EU but are widely produced and used in other countries. We investigated the impact of thimerosal on the total Hg in hair of 82 breast-fed infants during the first 6 months of life. The infants received three doses of the hepatitis-B vaccine (at birth, 1 and 6 months) and three DTP (diphtheria, tetanus, and pertussis) doses at 2, 4 and 6 months, according to the immunization schedule recommended by the Ministry of Health of Brazil. The thimerosal in vaccines provided an ethylmercury (EtHg) exposure of 25 μgHg at birth, 30, 60 and 120 days, and 50 μgHg at 180 days. The exposure to vaccine-EtHg represents 80% of that expected from total breast milk-Hg in the first month but only 40% of the expected exposure integrated in the 6 months of breastfeeding. However, the Hg exposure corrected for body weight at the day of immunization was much higher from thimerosal- EtHg (5.7 to 11.3 μgHg/kg b.w.) than from breastfeeding (0.266 μgHg/kg b.w.). While mothers showed a relative decrease (−57%) in total hair-Hg during the 6 months lactation there was substantial increase in the infant's hair-Hg (446%). We speculate that dose and parenteral mode of thimerosal-EtHg exposure modulated the relative increase in hair-Hg of breast-fed infants at 6 months of age.

Keywords Thiomersal · Ethyl-mercury · Methyl-mercury · Breastfeeding · Immunization

Abbreviations

EtHg	ethyl-mercury
MeHg	methyl-mercury
DTP	difteria tetanus and pertussis
PUFA	polyunsatured fatty acids
DHA	docosahexaenoic acid
EPA	eicosopentanoic acid
GSH	glutathione

Introduction

Great strides in the control of epidemics have been made possible by the effective production and safe use of vaccines. The elimination of polio, measles and other scourges are the hallmark of successful immunization programs. However, in the earlier days of vaccine production there were several tragic incidents that caused death in vaccinated children [1]. To reduce the risk of bacterial contamination, many countries require a preservative in vaccines. The antiseptic agent of choice to prevent bacterial growth is sodium ethylmercurithiosalicylate (thiomersal), also known as thimerosal (USA). Thimerosal contains 49.6% Hg by weight and has been used since the 1930s at the preservative level

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in vaccines. Some vaccines contain thimerosal at a concentration of 0.01%; thus a vaccine dose of 0.5 ml contains 50 μg of thimerosal or approximately 25 μg of Hg [1].

The amount of EtHg received from vaccinations by infants up to 6 months old is an additional exposure to Hg frequently found in human milk and formulas [10]. However, because the vaccine-Hg dose (extrinsic EtHg), unlike intrinsic milk-Hg, is not part of a food, it bypasses the barrier and detoxifying entero-hepatic system, and not being physiologically processed, it may instead migrate to the brain. The uncertainties about the safety of thimerosal-preserved vaccines and controversy about whether EtHg might be associated with neurodevelopmental disorders (including autistic spectrum disorder) are debatable issues that have involved both scientists and society [32]. Because Hg toxic effects are manifested long after the exposure, it is necessary to have reliable markers of Hg exposure. However, depending on the elapsed time, one of the difficulties in studying neurotoxic effect of vaccine-thimerosal is that post-exposure to vaccine-EtHg may not leave Hg traces to be measured.

The organic Hg form of thimerosal is ethyl-Hg which, like methyl-Hg (MeHg), can also bind to protein matrices. In vitro studies showed that the neuronal and glial cell toxicity of MeHg and EtHg are both mediated by glutathione depletion [15]. Although chemically and toxicologically related, ethyl-Hg and methyl-Hg have differences in metabolism. Compared to EtHg, MeHg binds more avidly to cysteine-groups and is more slowly metabolized. Thus, thimerosal-derived EtHg half-life in the blood of infant monkeys was 2.1 and 8.6 days after exposure (i.m. injection), significantly shorter than the 21.5 days elimination half-life of MeHg exposure by gavage [6]. Additionally, it was observed that the concentration of inorganic mercury in the brains of thimerosal-EtHg exposed monkeys was more than double that found in the MeHg exposed monkeys, and it was observed that the inorganic mercury in the brains of the thimerosal-EtHg exposed monkeys showed no significant decline 120 days after exposure. Pichichero et al. [26] estimated that half-life of EtHg in human infants is 6 days compared with 40 to 50 days for MeHg. Furthermore, concentrations of Hg were low in urine after vaccination, but were high in stools of thimerosal-exposed infants. Conversion to inorganic Hg is expected to yield high urine-Hg; thus, stool Hg elimination is a feature also found in MeHg metabolism. In such a scenario, the neurotoxic effects of thimerosal-EtHg evaluated by post-exposure markers such as hair-Hg can be compromised.

Stajich et al. [31] studied thimerosal-EtHg metabolism in preterm human infants by measuring total Hg levels in blood before and after the hepatitis-B vaccination. A significant increase in Hg was seen in both preterm and term infants. Additionally, preterm infants had greater than tenfold higher mean Hg levels at the baseline reading compared with term infants. Although this difference was not statistically significant, Stajich et al. [31] speculated that it might indicate that preterm infants may not be able to metabolize Hg efficiently. Indeed we have shown that larger babies, as measured by liver weight, had significantly higher mean liver Cu concentrations, an indicator of metallothionein [16], than smaller babies [9]. Either immaturity of the liver could be responsible for less metallothionein to bind inorganic Hg or a difference in body composition of infants with less body mass could metabolize EtHg at a slower rate [31].

There are no studies that measured hair-Hg in infants at the time of vaccination, but Redwood et al. [29] developed models that predicted increases in hair-Hg concentrations after a full immunization schedule within the first 6 months of life. They assumed that neonates up to 6 months of age have low Hg excretion due to immature hepatic function, low bile production, and insufficient glutathione which binds the Hg.

Substances that accumulate in the body should be looked at specifically. In the case of Hg, not only the chemical form but also the exposure route, as well as the modifying effect of changing metabolism (body weight with accompanying blood volume and organ maturity) are critical for newborns. Therefore, we compared the exposure to injected EtHg in vaccines with the expected exposure to breast-milk Hg and examined the hair-Hg of breastfeeding mother-infant pairs after the recommended immunization schedule of the first 6 months.

Materials and methods

The research protocol to study the effects of Hg contamination of urban (Porto Velho, Brazil) mothers on neurodevelopment of pre-school children was approved by the Ethics Committee of Studies for Humans of the Universidade Federal de Rondonia and details appeared elsewhere [21]. In this study we used data from our previous study which was not collected with the purpose of evaluating the impact of immunization on hair-Hg, since when the research project started we were not aware of the EtHg issue in pediatric vaccines. Briefly, pregnant mothers were introduced to the study and invited to participate during their routine visits to the pre-natal clinics of three hospitals in Porto Velho (Hospital de Base, Hospital Panamericano and Hospital Regina Pacis). Only Hospital de Base is a state-run facility that receives mostly poor mothers.

Plain-language information about the study was presented and a written consent form signed by the volunteering mother. The written consent stated that participation was voluntary with assured confidentiality and the right to withdraw from the study at any time. Potential participants

were selected among mothers in good health, reporting no illness or complaints at the time of the study and who were willing to breast feed and adhere to the post-natal attendance of the pediatric clinic for the regular immunization program. One hundred mothers between the ages of 15 and 45 years were recruited.

At birth the infants were clinically examined with special attention to vitality, perinatal reflexes, maturity, and congenital malformations; weight, length, head circumference, and Apgar scores were recorded. Anthropometric data (weight, length, and head circumference) were recorded and samples of hair from the mothers and infants (fetal hair) were collected. Mothers followed the immunization schedule recommended by the Ministry of Health of Brazil and returned at 30, 60, 120 and 180 days. Only 86 mother-infant pairs reported for the programmed clinical and immunization at 6 months of age when infants were weighed and measured for length, and hair-samples were again collected from mothers and infants. Because Hospital de Base is a public health facility, only the babies born at this hospital (66%) received the hepatitis-B vaccine within the first day postpartum. Babies born at the Hospital Panamericano and the Hospital Regina Pacis received the hepatitis-B vaccine immediately after the mother's discharge (2–4 days postpartum). At this time the mothers were taken under our supervision to a state-run clinic where vaccines are distributed free.

Estimated exposure to Hg from vaccines (injected thimerosal) and breastfeeding

Differences in infants' weight at birth and at 6 months were used to estimate daily weight gain and integrated gain at 30, 60, 120 and 180 days. As stated by manufacturers, vaccines contained 0.01% Thimerosal; the Hg concentration of the doses delivered through vaccines was 25 μgHg/0.5 mL for hepatitis-B (Korea Green Cross Corporation, Kiheung-Eup Yougin-Goon Kiyunggi-Do, Korea; Euvax B injectable, LG Life Sciences, Jeonbuk-Do, Korea) and difteria, tuberculosis and pertussis-DTP (Triple Antigen, Serum Institute of India Ltd., India; Vacina Tríplice, Instituto Butanta, São Paulo, Brazil).

We used the data of breast milk-Hg concentrations (adapted from Dorea [8]) with the same approach as Bigham and Copes [4] to estimate Hg exposure during breastfeeding: infant mean weight × mean daily breast milk consumption (140 mL/kg) × number of days × mean total Hg concentration in breast milk (1.9 μg/L).

Hair Hg determinations

Hair sample preparation and Hg determination were done according to routine procedures previously established at the Universidade Federal do Rio de Janeiro [3]. We followed routine laboratory procedures after adapting the analytical protocol used for Hg determination in previous studies analyzing blood, hair and fish-flesh matrices [3, 19, 20]. Briefly, the hair samples were comminuted with stainless steel scissors, weighed and digested before analysis. Blood samples were digested with concentrated HNO_3 (3 mL) and $KMnO_4$ (5%, 6 mL) using a microwave oven system for 35 min (CEM-Corporation, MDS 2000, Matthews, North Carolina, USA). Placenta and umbilical cord samples were weighed and digested with HNO_3: H_2SO_4 (1:1, 5 mL) and $KMnO_4$ (5%, 4 mL) using a digestion block at 80°C for 1 h (Tecnal Ltd., Piracicaba, São Paulo, Brazil). Human hair samples were washed with EDTA 0.01%, dried in an oven at 50°C, weighed and digested with 5 mL of HNO_3:H2SO_4 (1:1) and 4 mL of 5% $KMnO_4$ using a digestion block at 80°C for 40 min. The determination of total Hg in the digested samples was done by cold vapor atomic absorption spectrometry with a flow injection system-FIMS (CV-AAS, Perkin-Elmer-FIMS 400, Ueberlingen, Germany). Glassware used in the analytical protocol was washed clean, rinsed with 5% EDTA and double distilled, and left to rest in 5% HNO_3 overnight. Then it was rinsed again in double distilled water, and dried at 100°C for 12 h. Precision and accuracy of Hg determinations were assured by the use of internal standards, use of triplicate analyses of samples and certified reference materials (IAEA-085 and 086, Vienna-Austria) with recoveries of 92%.

Statistical analysis and data summarization (mean, SD) were performed using SAS software (SAS Institute, Cary, NC, USA). For the statistical test, $p<0.05$ was considered significant.

Results

The anthropometry and the hair-Hg concentrations of infants and mothers are shown in Table 1, while the immunization schedule and exposure to the injected thimerosal as well as the expected exposure to total Hg in breast milk are shown in Table 2.

The mean birth weight was slightly higher for girls, but there was a relatively higher weight gain for boys at 6 months. Every child received a cumulative dose of 150 μg/Hg (as Thimerosal-EtHg) distributed in five boluses during the first 6 months of life. An adaptation of world data on human milk-Hg concentrations [8] showed that the median mean Hg concentration is 1.9 ngHg/mL (Fig. 1). The estimated total Hg intake integrated over the first 180 days of breastfeeding showed a cumulative exposure of 290 μg of total-Hg (Fig. 2). Comparatively, the integrated EtHg exposure in the first month was 80% of that from

Table 1 Mean (and SD) of anthropometry, hair-Hg concentrations and Gesell scores of infants

	Girls	Boys
N	38	44
Birth weight, g	3,177.50 (450.8)	3,281.25 (393.4)
Weight at 180 d	7,012.63 (508.7)	7,010.23 (417.3)
Weight gain, g	3,835.13 (434.2)	3,728.98 (382.8)
Weight gain/d	21.31 (2.4)	20.72 (2.1)
Hair [Hg], μg/g		
Birth	2.58 (3.7)	2.32 (2.4)
180 days	4.14 (5.9)	3.9 (5.3)
Mean increase at 6 m	1.55 (5.8)	1.27 (5.3)
Relative increase, %	398.28 (541.9)	487.08 (1,515.0)
Maternal hair [Hg] μg/g		
Delivery, 0 days	7.14 (6.8)	7.55 (10.2)
180 days	2.97 (3.1)	3.38 (4.4)
Mean decrease at 6 m	4.17 (4.0)	4.16 (6.2)
Relative decrease, %	39.94 (16.2)	45.50 (23.1)

breast milk but declined to 40% at 180 days (Fig. 2). At two months the infants increased body weight by 60% but received an integrated EtHg challenge threefold higher than in the perinatal days (Table 2).

Per unit of body weight the amount of EtHg/kg b.w. given to newborn babies (before hospital discharge) was higher than at any other time (Fig. 3), even at 6 months when infants had increased body weight by more than 100%. The corrected exposure to thimerosal (EtHg) and breast-milk Hg per unit of body weight is shown in Fig. 3. Considering a milk volume transfer of 140 mL/kg b.w., and considering the median breast-milk Hg concentration of 1.9 ngHg/mL (Fig. 1), the exposure to breast-milk Hg was

Table 2 Infant immunization schedule, type of vaccine, and Hg intake during the first 6 months

Age, days	Vaccine		Body weight, g	Breast milk[b] Hg intake, μg
	Type[a]	μgHg/dose		
0	Hp-B	25.0	3,233.17	0
30	Hp-B	25.0	3,862.87	30.83
60	DTP	25.0	4,492.56	44.65
90	–		5,067	
120	DTP	25.0	5,751.95	91.80
150	–		6,327	
180	DTP+ Hp-B	50.0	7,011.34	111.90
Total[c]		150.0		279.18

[a] Hp-B: Hepatitis B (assumed 0.01%Thimerosal/dose, Korea Green Cross Corporation, Kiheung-Eup Yougin-Goon Kiyunggi-Do, Korea; Euvax B injectable, 0.01%Thimerosal, LG Life Sciences, Jeonbuk-do, Korea); DTP (Serum Institute of India Ltd; Vacina Tríplice, 0.01%Thimerosal/dose; Instituto Butanta, São Paulo, Brazil)

[b] Integrated total Hg intake (estimated from Dorea [8])

[c] Total exposure of the respective Hg chemical forms

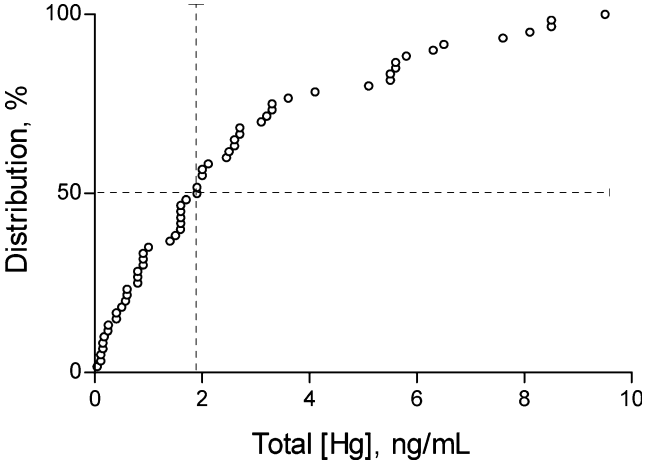

Fig. 1 Distribution of mean total mercury concentrations in studies of different parts of the world (adapted from Dorea [8])

0.266 μgHg/kg b.w. at any time after the first immunization at 0 days. Excluding the small volumes of milk intake at birth (day 0 of first vaccine), this corresponds to a small percentage (4.7 to 8.1%) of the EtHg doses injected between 30 and 180 days.

Because of the variability in hair-Hg concentrations, the relative changes from 0 days (birth) to 180 days were calculated and shown in Fig. 4. While mothers had a mean decrease of 40% and 45%, their girls and boys had, respectively, increased hair-Hg concentrations by as much as 398.3% and 487.1%.

Another notable feature is the time of exposure to EtHg shown in Fig. 5. A distinct pattern is shown as a function of the immunization policy regarding the dispensation of vaccines. The infants that received the first vaccine within the first 24 hours after birth were all from the only public hospital in Porto Velho (Hospital de Base). Because the other two hospitals (Panamericano and Regina Pacis) are privately owned and operated, the infants are vaccinated in

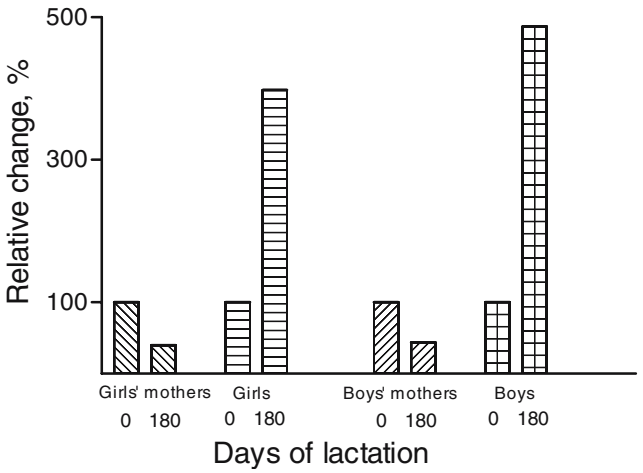

Fig. 2 Estimation of the integrated total-Hg intake during the first 180 days of breastfeeding

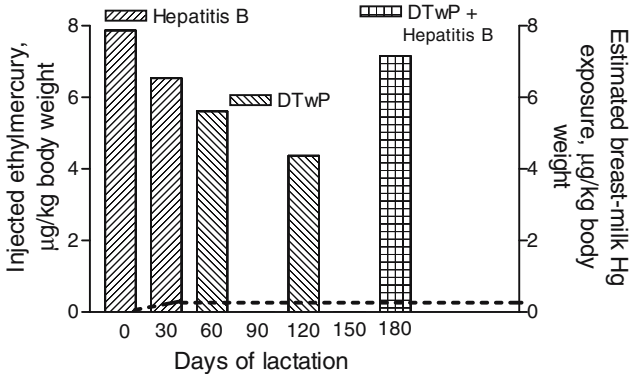

Fig. 3 Infants' vaccine schedule and injected ethyl-Hg (as Hg), and estimated total Hg in breast milk (– adapted from Dorea [8]) during the first 180 days of life

immunization clinics run by the Ministry of Health after the hospital discharge.

Discussion

Unlike breast-milk intake, there are no body-weight dosing adjustments for vaccines; therefore, neonates and young infants (up to 60 days) with less blood volume are exposed to more injected EtHg than older infants from 120 to 180 days (Table 2). Over the first 6 months, the estimated accrual of total Hg exposure from breast milk was higher than the five doses of injected EtHg (Table 2). However, differing from parenteral EtHg in vaccines, breast-fed infants were exposed to enteral Hg (intrinsic to the breast milk matrix) amortized over 6 months. Because the mean frequency of feeds/day is 5.5 [30], the milk-Hg exposure in the breastfed infant occurs in several small daily suckling episodes. This type of exposure amounts to only 4.7%–8.1% of the vaccine-Hg bolus (per kg/b.w.) taken on the day of the immunization. Furthermore, the ingested milk-Hg is hindered by all gastrointestinal barriers, metabolic and detoxifying mechanisms (albeit immature) attendant to enteral feeding. Coupled with that, another attenuating factor is the tendency of human milk Hg to decrease during initial lactation [8], i.e., from day four to 6 weeks after delivery [5]. Additionally, central to the toxic effects of Hg during early life is the unique chemical composition of human milk with its specific neurotoxic attenuating factors.

Neurodevelopment is rapid in the postnatal period and substantial amounts of long-chain polyunsatured fatty acids (PUFAs) are critical to neurite growth and proper brain development. The beneficial PUFAs are docosahexaenoic acid (DHA=22:6n-3) and eicosopentanoic acid (EPA=20:5n-3). DHA is an essential component of nervous-system cell-membranes and is transported across the placenta and across the mammary gland into milk. During the critical window of early life, significant amounts of PUFAs are provided via breast milk and then avidly incorporated into membranes throughout the infant's nervous system [22]. Indeed, post-natal DHA is positively correlated with visual and language development in breast-fed infants [14].

Another mitigating factor against Hg-induced neurotoxicity is the intracellular defense provided by glutathione (GSH). Mercury has a high affinity for thiol (sulfhydryl groups), the thiol-containing antioxidant of GSH [15]. According to Cai and Sauve [7], thimerosal is a poorly membrane-permeable hydrophilic-molecule that oxidizes SH groups with a high affinity. It reduces S–S bonds of molecules such as GSH. Therefore, the potential protective effect of GSH against Hg toxicity can be compromised in situations limiting cysteine nutrition. Indeed, James et al. [15] showed that thimerosal-induced cytotoxicity was associated with depletion of intracellular GSH. Because brain cells cannot synthesize cysteine, (the rate limiting amino acid for GSH synthesis) it is dependent on the liver to synthesize and export cysteine to the brain for intracellular glutathione synthesis [15]. Human milk contains a high amount of bioavailable cysteine and differences between breast-fed and formula-fed infants have been reported with respect to cysteine concentrations and its metabolites [23].

The safety of thimerosal vaccines is assumed on the basis of the faster metabolism of EtHg compared to MeHg [6]. While we do not know if the infants' hair-Hg in this study was derived from the vaccine's thimerosal, it can be stated that there was an asymmetric change in infants' hair-Hg compared to the mothers' hair in the same period. We

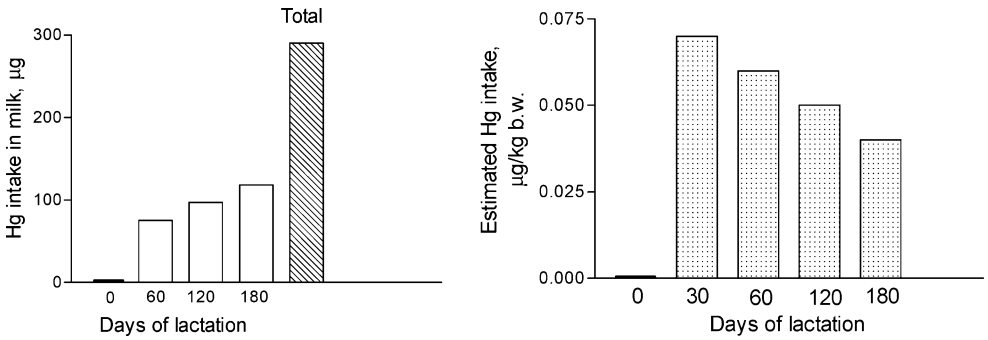

Fig. 4 Relative hair-Hg changes (%) from birth (0 days) to 180 days in mothers according to infant gender

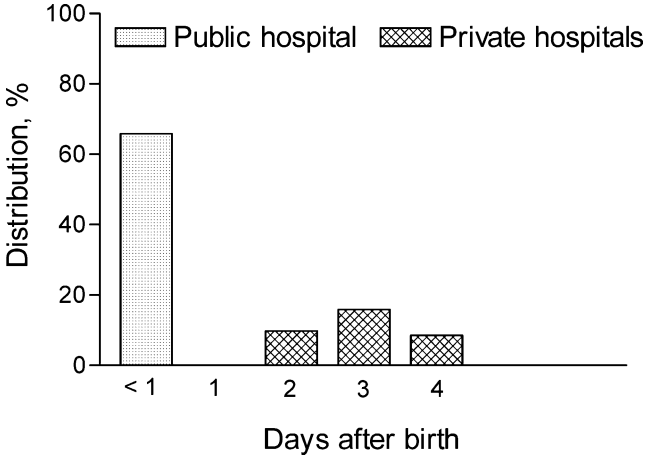

Fig. 5 Percent distribution of the time-after-birth interval of the first vaccine (hepatitis B)

have reported that fish-eating riverine mothers showed higher hair-Hg concentrations than their respective breast-fed infants [2]. In line with these observations it has been shown that maternal hair-Hg concentration was higher than fetal hair-Hg [28]. Recently, Lindow et al. [17] reported higher fetal hair-Hg concentrations in babies of mothers exposed to dental amalgam restoration either before or during pregnancy; they showed maternal/fetal Hg ratios>1 in most samples (66%). However, contrary to these studies, Mohan et al. [24] reported that 80% of newborns had higher hair-Hg than their mothers. Nevertheless, the mean increase in hair-Hg concentrations we found at 6 months is compatible with the levels predicted by Redwood et al. [29].

Holmes et al. [12] describe that mothers from autistic children received more mercury during pregnancy than mothers from healthy controls and also hair-Hg differences between autistic and control infants at the time of their first haircut (11–24 months). They speculated that hair excretion patterns among autistic infants were significantly reduced relative to controls. Furthermore, they claimed that hair-Hg in control infants were significantly correlated to Hg exposure through prenatal maternal dental amalgam fillings, but that correlations were absent in the autistic group. Contrary to that, older (4–7 years old) autistic children showed higher hair-Hg concentrations than age-matched controls [11]. Hair-Hg association with behavioral disorders has also been extended to interactions with zinc deficiency [13]. However, the assessment of hair-Hg concentrations after immunizations containing thimerosal (EtHg) is incomplete without speciation of the Hg forms. Nevertheless, our total hair-Hg results are in line with previous work. Barbosa and Dorea [2] estimated that mean concentration of Hg in breast milk of "riverine" women (living near rivers, high fish eaters) with higher hair-Hg (14.3 ppm) would give an exposure to breast-fed infants (mean hair Hg, 9.8 ppm) of 0.64 μg/kg body weight. In the present study the urban mothers are low fish eaters and show much lower mean hair-Hg concentration (7.5 ppm).

The neurotoxicity of administered MeHg is well known and seems to be higher than EtHg, the Hg metabolite of thimerosal [18]. There is limited information on human metabolism of EtHg and even less information is available for infant metabolism of thimerosal. Because there are no known studies of intrinsically incorporated EtHg in food matrices, comparing parenteral vaccine-EtHg to the breast milk-Hg (intrinsic-Hg) metabolism is challenging. The decomposition rate of EtHg is higher than MeHg because of the molecule size. Qvarnstrom et al. [27] reported that the carbon-Hg bond in $C_2H_5Hg^+$ is less stable than that in CH_2Hg^+. In addition, the efficiency of the blood-brain barrier is directly proportional to the size of the organic radical [18].

Infant Hg exposure in early life can only happen through milk diets [10]. However, given the variation (24 to 67 h postpartum) of the onset of lactation [25], for many infants a high exposure to parenteral EtHg (25 μg) preceded the much lower (Fig. 2) enteral total-Hg (intrinsic to the human milk matrix). A second point is the impact caused by injected EtHg in an immature organism. It has been speculated that the removal of thimerosal from vaccines would produce no more than a 50% reduction of Hg exposure in infancy [4]. This reduction could only happen with human milk Hg concentrations greater than the median value (Fig. 1) and more frequently with formulas, which usually contain higher Hg concentrations [10]. Furthermore, this scenario assumes a debatable equivalency of a bolus (injected Hg) *versus* the integration (over 6 months) of ingested milk-Hg; it does not contemplate the tenets of Hg neurotoxicity: chemical form, dose, route of administration, attenuating neurotoxic factors of breast feeding, and enhancing neurotoxic factors of the perinatal period (birth weight/prematurity).

The expected metabolic consequences of such extremely different exposure routes and Hg chemical forms (injected EtHg *versus* ingested milk-Hg) as well as Hg dose are difficult to disentangle; we have the changing physiological interactions of a self-adjusted daily dose of intrinsic Hg (inorganic and MeHg) in breastfeeding against the full impact of an extrinsic dose of EtHg. With regard to breastfeeding, milk-Hg occurs in a context of proven benefits in a broad array of neurotoxic mitigating factors. In this study, we observed a relative increase in hair-Hg. This finding reinforces a mechanistic connection between thimerosal-EtHg and hair-Hg increase, supporting the use of hair-Hg in vaccinated children as a confounder of neurobehavioral maternal-Hg contamination.

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REGULAR ARTICLE

Time of perinatal immunization, thimerosal exposure and neurodevelopment at 6 months in breastfed infants

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Abstract

Aim: Brazilian newborns immunized with hepatitis-B (thimerosal containing vaccine, TCV) receive the first dose within 24 h if delivered in public hospitals, but at a later time if born in private hospitals. We compared neurodevelopment (ND) in infants born in a state hospital (immunized within 24 h) and in privately run hospitals (immunized 2–4 days postnatally).

Methods: We used the Gesell Developmental Schedules in 82 healthy exclusively breastfed infants at 6 months to assess motor skills, language development, comprehension capacity and social skills.

Results: Compared to the group immunized 2–4 days after hospital discharge, the group immunized within 24 h showed no significant difference in ND delays. Despite the variation in gestational age (range 36–42 weeks) and TCV-ethylmercury (EtHg) dose (5.7–11.3 μgHg/kg b.w.) at birth, time of exposure to TCV showed no significant association with ND. Gesell Developmental Score was not significantly correlated with total parenteral EtHg/unit of body mass neither with the relative increase in hair-Hg (as an additional challenge to prenatal Hg exposure).

Conclusion: In breastfed infants, differences in early exposure to TCV-EtHg cannot portend clinical neurodevelopment delays at 6 months. We speculate that breastfeeding remains a significant strategy to improve central nervous system protection of infants facing early exposure challenges.

INTRODUCTION

Despite the universal use of thimerosal-containing vaccines (TCV) in immunization of children, our knowledge of the toxicokinetics (TK) and toxicodynamics (TD) of their Hg metabolite (ethylmercury–EtHg) is derived mostly from methylmercury (MeHg) studies. The mechanistic significance of thimerosal neurotoxicity through EtHg is unquestionable, but its toxicological significance is the result of direct demonstration in animal and theoretical models. Our ability to understand the safety of TCV-EtHg is still unsatisfactory; although there is no proven direct association of neurodevelopmental disorders in children immunized with TCV-EtHg, its plausibility has been shown by neurotoxic disasters caused by organic Hg compounds (1). However, there are no studies pointing to neurologic disorders being an unambiguous result of TCV-EtHg; rather, the associative studies have so far emerged amid intense scientific debate.

Rodier (2) and many others have discussed the vulnerability of the young brain to neurotoxic substances over the early neonatal period. Compared to other organs, the infant brain takes an unusually long period to form. Besides the blood–brain barrier (BBB), which is not fully developed until the middle of the first year of life, neuron proliferation and migration also continue in the postnatal period. Additionally, myelin production, along with development of receptors and transmitter systems, is part of the great postnatal activity of the central nervous system (CNS). Organic Hg compounds are among the neurotoxic substances with effects that depend on the neuron structure at time of exposure (2). Coincidently, during the first 6 months, Brazilian infants are impacted by a heavy vaccination schedule: six i.m. injections of TCV (Hepatitis-B at 0, 30 and 180 days; DTP at 60, 120 and 180 days) containing 0.01% of thimerosal as preservative (25 μgHg/dose).

Nursing infants are exposed to Hg from formulas and breast milk (3); however, the extent of exposure during breastfeeding depends on the maternal Hg that diffuses into milk. The amount of Hg in the blood of the nursing mother enters breast milk at a rate (inorganic Hg more than MeHg) that depends on its protein-binding capacity. Oskarsson et al. (4) observed that, compared to organic Hg (MeHg), there was a more efficient transfer of inorganic Hg from blood to breast milk. They estimated that infant exposure from breast milk ranges up to 0.3 μgHg/kg/day; approximately 50% was inorganic Hg. While the breast milk Hg exposure is proportional to milk intake, it is amortized in several sucking events during the day; furthermore, the newborn is not exposed until lactation is established and during this time breastfeeding is acting on the CNS priming. On the other hand, TCV are given immediately after parturition by parenteral route. In our infants, five vaccines are given in the first 6 months, two of them given at the same time (180 days) and providing a double dose of TCV (50 μgHg/dose). Indeed, breastfed vaccinated infants have shown a substantial increase in hair-Hg concentrations during the first 6 months (5).

Guidelines to EtHg use as vaccine preservatives are derived from MeHg toxicity studies. These guidelines from various countries vary from 0.1 to 0.4 μg/kg of MeHg for adults (1). Harry et al. (6) found that in immature mice, when EtHg was injected, either as the chloride or as thimerosal, less of the actual delivered dose reached the brain as compared to an injection of MeHg. They concluded that MeHg does not appear to be a good model for EtHg-containing compounds. Therefore, serum transport of MeHg and EtHg can modulate toxic differences (in the neonate) by regulating the relative levels of free forms transported and available to cross the BBB. Additionally, there are differences in initial weight loss that are more pronounced in breastfed infants. Macdonald et al. (7) identified neonatal median time of maximum weight loss around 2.7 days. Clearly, this can further increase differences in TCV-EtHg exposures.

The inability of the neonatal liver to secrete bile, coupled with an immature renal system, mean that Hg excretion takes longer than in adults. This is particularly relevant for the breastfed infant; because maternal milk usually comes in at between 2–4 days after birth, for breastfed infants this is the time of the lowest expected bowel output (8). Together with lower albumin binding capacity, the immaturity of the transport-protein system in neonates enhances the risk of mercury toxicity. Perinatal immaturity of the CNS and of other physiological functions (detoxifying enzymes, renal and gastrointestinal organs) that interferes with Hg binding and excretion is well established. Additionally, both birth weight (9) and gestational age (10) are positively associated with childhood psychometric intelligence. The TCV-EtHg impact on CNS remains unstudied; gestational age and birth weight are modulators of TK and TD of TCV. The parenteral delivery of TCV-EtHg, albeit in very small quantities, reaches the neonate circulation very quickly and gains unimpeded access to the immature BBB and the blood cerebrospinal fluid barrier (BCSFB). Therefore, the early postnatal days are important windows of opportunity for CNS insults.

Neurodevelopment delay can occur in infants exposed to prenatal maternal neurotoxic substances; its etiology is multifactorial and includes Hg. Given the immaturity of newborns, after an acute parenteral exposure of thimerosal, there is more chance of infants accumulating Hg more readily and excreting it more tardily. When there are differences in time of first exposure there might be additional risk factors; there is, therefore, more likelihood of heightening the risks of untoward neurological effects. Special population groups, such as term neonates, have a variety of constitutional modifying risk factors: (a) parenteral exposure at very early time; (b) sequence of high magnitude EtHg exposures (first days, 1, 2, 4 and 6 months); (c) differences in maturity at birth (gestational ages 36–42 weeks); (d) differences in body mass at birth and (e) additivity of TCV-EtHg to prenatal Hg exposure gradients (especially in fish-eating Amazonian populations).

Because of the complexity of studying neurodevelopment and ethical issues involving study design with TCV we took advantage of an existing research project and analysed recorded data of early and relatively later TCV-EtHg exposure of breastfed infants. Therefore, our primary aim was to compare the effect of time differences in the first exposure to TCV on ND at 6 months. Our secondary aim was to identify modifying factors associated with prenatal Hg exposure.

MATERIALS AND METHODS

The data used in this study were not collected with the purpose of evaluating the impact of immunization on ND. In a parent publication (11) we described the protocol to assess growth and development of children exposed to prenatal background Hg; when the research project started we were not aware of the TCV issue in paediatric vaccines. The parent publication (11) dealt with prenatal Hg exposure; after that, when studying the changes in hair Hg concentrations (5, 11) we realized that because Hospital de Base is a public health facility, only the babies born at this Hospital (66%) received the hepatitis-B vaccine within the first day postpartum. Babies born at the Hospital Panamericano and the Hospital Regina Pacis received the hepatitis-B vaccine immediately after the mother's discharge (2–4 days postpartum). This appeared as an opportunity to study differences in timing of immunization and ND outcomes; additionally, Hospital de Base as a state-run facility receives mostly poor mothers.

The previous publications detailed the characteristics of the population of Porto Velho (West Amazonia); the research protocol was approved by the Ethics Committee of Studies for Humans of the Universidade Federal de Rondonia (11). Briefly, pregnant mothers between the ages of 15 and 45 years were enrolled as volunteers; mothers were in good health, reporting no illness or complaints and were willing to breastfeed. They were introduced to plain-language information about the study, and a written consent form was presented and signed by the volunteering mother. Mothers were selected by a nurse during their routine visits to the Prenatal Clinics (Hospital de Base, Hospital Panamericano and Hospital Regina Pacis). Hospital de Base is a state run facility while Hospital Panamericano and Hospital Regina Pacis are not. Excluding factors were occupational exposure to toxic chemicals and hereditary neurological illnesses.

After birth, the newborns were clinically examined with special attention to vitality, perinatal reflexes, maturity and congenital malformations; weight, length, head circumference and Apgar scores were recorded. While in the maternity wards, we collected samples of hair from mothers and respective infants (fetal hair); the hair sampling was repeated at 6 months. Hair strands were cut from the occipital region and placed in plastic bags, with the root end stapled on a paper sheet. These samples were analysed by routine laboratory procedures described in previous publications (5,11).

The mothers were closely monitored by nurses to guarantee full support for breastfeeding and pre and postnatal care; immunization scheme followed the Brazilian vaccination program. The first vaccine (Hepatitis B) was taken either before hospital discharge (in Hospital de Base) or a few days latter, and at 30, 60, 120 and 160 days. After hospital discharge the mothers were taken under our supervision to a state-run clinic where vaccines are distributed free.

At 6 months of age, only 82 of the 100 original mother–infant pairs reported for the programmed clinical and neurobehavioural examination when infants were weighed and measured for length, and hair samples were collected again (5,11).

The neurodevelopment tests were conducted by trained professionals using the Gesell Developmental Schedules (12,13). These tests included all reactions (voluntary, spontaneous or learned) and reflexes. We also evaluated postural reactions, hand pressure, locomotion and coordination, constructive ability (which is influenced by motor development), visible and audible communication, individual reactions regarding people and stimulations (depending mainly on the temperament of the child and the surroundings). The results were expressed as developmental scores for the Adaptive Ability, Language and Motor deficiencies (gross motor ability), and Social/Emotional ability.

Estimated exposure to EtHg from vaccines (injected thimerosal):

Integrated weight gains at 30, 60, 120 and 180 days were estimated from differences in infants' weight at birth and at 6 months. Differences in infants' weight at birth and at 6 months were used to estimate daily weight gain and integrated gain at 30, 60, 120 and 180 days. As stated by manufacturers, vaccines contained 0.01% thimerosal; the Hg concentration of the doses delivered through vaccines was 25 μgHg/0.5 mL for hepatitis-B (Korea Green Cross Corporation, Kiheung-Eup Yougin-Goon Kiyunggi-Do, Korea; Euvax B injectable, LG Life Sciences, Jeonbuk-Do, Korea) and diphtheria, tetanus and pertussis-DTP (Triple Antigen, Serum Institute of India Ltd., India; Vacina Tríplice, Instituto Butanta, São Paulo, Brazil). The exposure to EtHg derived from vaccines was based on the current national immunization program of the Ministry of Health of Brazil.

Statistical analysis

Statistical analysis for testing differences between groups was done using the Statistica (StatSoft, Inc., v.6.0) statistical package. One-way analysis of variance was used with Tukey's procedure. The Shapiro-Wilk test of normality was applied and data transformed when required. For the association between variables Pearson's correlation was calculated and tested for significance; we accepted a value of <0.05 as statistically significant.

RESULTS

Parameters and outcome differences due to timing of the first dose of TCV (hepatitis-B) are shown in Table S1; no significant differences were observed in neurodevelopmental scores at 6 months. The doses of Hg derived from injected thimerosal varied greatly as a function of birth weight. Although they may seem comparable between groups, actually because the estimation was based on birth weight, the infants that took the vaccine at a later time (mean of 3 days) were probably underestimated due to obligatory neonatal weight loss. As a function of body mass, the mean TCV-EtHg perinatal dose was equivalent to the two vaccine (third dose of hepatitis-B and third dose of DTP) doses (50 μgHg) at 6 months when infants had double their body weight. Despite differences in socio-economic status between women delivering at the public hospital and at private run facilities, no significant differences were observed in infant anthropometry (and relative TCV dose) at birth or at 6 months.

Because of variation in body mass gain during the first 6 months there was an asymmetry of TCV-EtHg exposure at time of immunization; this is illustrated by percentage distributions in Figure S1 (a, b and c). During this period (six shots, 150 μgHg) there was considerable variation in individual thimerosal exposure as a function of body mass. The exposure to TCV-EtHg on the first and last vaccination day (birth and 180 days) exceeds the recommended tolerable daily intake of MeHg for adults. The amount of Hg per unit of integrated body-mass gained over 4 months (Fig. S1c) shows how high the immunized infant can be exposed to EtHg. Despite a wide variation in birth weight, the correlation of first dose of EtHg with Gesell scores was not statistically significant (Fig. S2a).

Continuous variables were analysed as single linear correlations; Pearson correlations were not statistically significant and are illustrated in the scatter plots of Figures S2 and S3. The hair-Hg representing antenatal (fetal hair-Hg at 0 day) and postnatal (hair-Hg of 180 days) Hg exposure events are plotted as percent change during the study period (Fig. S3). The percentage increase in hair-Hg concentrations was not significantly correlated with Gesell scores (Fig. S2a); it should also be noted that that the change in hair-Hg was not dependent of changes in body mass (Fig. S2b). Indeed, Gesell scores at 6 months (Fig. S2c) were independent of body mass increase.

DISCUSSION

The main finding of this work is that differences in time of the first exposure to TCV (hepatitis-B) did not predispose infants to clinical neurodevelopment delays at 6 months. Furthermore, percent change in hair-Hg (prenatal to 6 months hair-Hg, after the additional load of 150 μgHg) was not significantly correlated with neurodevelopment delays of these breastfed infants. The effect-modifiers of NDD risk considered in this study [(a) time of exposure of first EtHg challenge, (b) sensitivity due to immaturity (birth weight/gestational length) and (c) prenatal Hg exposure] could have been overruled by breastfeeding.

The maturation of the infant brain is a process that extends over a long period; during this time, brain fine structure and functioning are susceptible to neurotoxic substances. Mercury is one of such substance and the earlier the exposure the higher the risk of adverse effects. We realize that the relatively small amount of thimerosal in vaccines is unlikely to cause overt clinical neurological alterations (at least in non-susceptible populations). However, given the time differences (0 day versus 3 days), maturation gradients related to gestational age (36–42 weeks) and attendant birth weight, these results shed some light on the uncertainties raised

by theoretical models. Furthermore, because TCV-EtHg is quickly excreted through stools (14) possible differences due to bile production, bowel movements that could coincide with differences in time of immunization did not seem to affect neurological landmarks.

Thimerosal has been used in vaccines since the 1930s without dispute but since then breastfeeding rates have fallen considerably. While the adverse effects of TCV-immunization on children have not been satisfactorily demonstrated, the absence and/or limited duration of breastfeeding has been amply demonstrated to negatively affect neurodevelopment in short- and long-term studies. It is recognized that the consequences of early exposure to mercury can be subtle and may be evident after a prolonged latency (15–17). Therefore, a causal relationship between EtHg (from vaccines) exposure and an adverse neurological effect is difficult to establish in the relatively short time of 6 months, especially when infants had the benefit of breastfeeding. Nevertheless, the lack of a linear correlation between neurodevelopment delays with parameters related to gestational age/birth weight (Fig. S2) indicates that either infant sensitivity to small doses of injected thimerosal lies beyond these clinical tests or may manifest itself at a later time.

The Gesell developmental quotient seems adequate in gauging neurodevelopmental changes; it was able to detect differences in mean gross motor development at 1 year of age between term-infants fed formula with alpha-linolenic acid (18). The 6-month old breastfed infants in this study were not susceptible to identifiable effects caused by TCV-EtHg. It is plausible that estimates of the actual neurotoxic risk from TCV-EtHg exposure could be overruled by the CNS protective functions of breastfeeding. It is worth noting that the small number of observations did not provide the necessary material for multivariate analysis that could adjust important confounding factors. Therefore, suboptimal neural development due to TCV, if present in the more susceptible individuals, could only be detected in much larger epidemiological studies. Indeed, subtle NDD which amortized against the broad spectrum of the Brazilian population could reach significant numbers. Indeed, the large number of infants born annually in Brazil (3.7 million) has national DTP coverage of 90% in 72% of the Brazilian districts (19).

We can predict outcomes of positive effects of breastfeeding on neuromotor development, but can only indicate the possibility that NDD may occur as a result of TCV-EtHg. Furthermore, breastfeeding has been shown to have an immunomodulating effect (20) and to significantly increase antibody levels of DTP to a greater degree than in those that are formula fed (21). Although it is possible that untoward events in neurodevelopment may not be detected at 6 months, it is also possible that breastfeeding could act as a modifying-risk factor. At this young age, few cognitive skills are available to the infant; the basic neuronal constitution that modulates motor and perception involves time-critical processes. Therefore, adaptive functions or acquisition of skills that might be compromised by neurotoxic insults may take much longer than 6 months, to manifest themselves. Indeed we had a small number of individuals with a considerable overdose of thimerosal-EtHg (Fig. S1a). In such individuals, subtle neurodevelopment may lag behind but impairments could be detected only in large epidemiological studies.

Although postnatal exposure to toxic metals can occur as a result of milk feedings (3), exposure to EtHg occurs only through immunization with TCV. However, contrary to formula feeding, breastfeeding carries an established advantage of CNS-priming substances and maternal stimuli that aid infant neurodevelopment; together these neuroprotective effects of breastfeeding have a lifelong benefit on CNS integrity and general health status. Studies of perinatal events related to neurodevelopment and breastfeeding are rare. Radzyminski (22) has noted that intact and functioning CNS is crucial for breastfeeding behaviour. His study compared breastfeeding behaviours and neurobehaviours at birth and at 24 h of age in neonates of mothers who received epidural analgesia during labor and controls; the higher the infant scored in relation to neurobehavioural functioning, the higher the infant scored on breastfeeding behaviours.

The NDD effects of thimerosal depend on the interplay between TK and TD of EtHg. In infants, these features have not been sufficiently studied (particularly in neonates with a wide range of birth weight and maturation–gestational age); in the neonate, the fast surge of serum thimerosal could be a factor regulating the relative levels of free forms of EtHg available to cross the BBB. On the other hand, the TD of thimerosal have been based on animal (mostly small rodent) models due to ethical difficulties in conducting such studies in humans. The lack of even observational studies, like the present, has made our understanding of the TK and TD of thimerosal depends on expert opinion (23). Therefore, it is reassuring to have the opportunity to observe no clinical neurodevelopment delay as a result of early immunization timing. However, it is important to emphasize that breastfeeding could have played a role in overruling subtle effects that such early impact of EtHg might have caused. Therefore, it is recommendable that breastfeeding should be considered as a frontline procedure to minimize uncertainties related to neurotoxic effects of early TCV-EtHg exposure.

CONCLUSION

This paper draws on information derived from a singular situation in which breastfed infants, opportunistically divided into early (<1 day) and late (mean of 3 days) thimerosal-EtHg exposure groups, did not show untowards effects on developmental landmarks at 6 months.

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Supplementary material

The following supplementary material is available for this article:

Table S1 Mean (range) of anthropometry, pre and perinatal Hg exposure, and Gesell Scores of infants
Figure S1 Percent distribution of Thimerosal-EtHg/ unit of body weight at birth (a–first dose of hepatite-B, 25 μgHg) and at 180 days (b–third dose of hepatite-B and third dose of DTP, 50 μgHg); (c) percent distribution of Thimerosal-EtHg (second dose of hepatite-B, first and second dose of DTP, 75 μgHg)/weight gain between first and last vaccines.
Figure S2 Plot illustrating correlation between Gesell scores and (a) dose of injected TCV-EtHg as function of body mass ($r = -0.1724$; $p = 0.1214$), (b) between relative change in hair-Hg at 6 months ($r = 0.0753$; $p = 0.5037$) and (c) relative weight gain at 6 months ($r = -0.1185$; $p = 0.2892$).
Figure S3 Plot illustrating correlation between change in hair-Hg at 6 months and respective change in body mass ($r = -0.0287$; $p = 0.7988$).

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Table S1. Mean (range) of anthropometry, pre- and perinatal Hg exposure, and Gesell Scores of infants.

Schedule	Within 24 hours	48 to 96 hours
N	53	29
Birth weight, kg	3.2 (2.2-4.4)	3.3 (2.5-4.1)
Gestational age, d	39.4 (36-43)	39.2 (37-41)
Prenatal Hg exposure		
Fetal hair [Hg], µg/g	2.1 (0.1-12.9)	3.0 (0.1-19.7)
Maternal hair [Hg] µg/g	7.1 (0.4-62.4)	7.8 (0.6-30.5)
Perinatal Hg exposure		
Thimerosal, µgHg/kg[1]	8.0 (5.7-11.3)	7.7 (6.1-10.0)[2]
Outcome at six months		
Body weight, kg	7.0 (6.1-8.5)	7.0 (6.4-8.0)
Thimerosal, µgHg/kg	7.2 (5.9-8.2)	7.2 (6.3-8.0)
Gesell Scores, quotient		
Motor	76.0 (0-100)	72.5 (0-100)
Language	71.0 (0-100)	62.0 (0-100)
Adaptive ability	81.4 (0-100)	83.2 (20-100)
Social	85.6 (70-100)	87.0 (70-100)
General	78.5 (18-100)	76.2 (23-100)

Mean differences were not statistically significant.
[1]Estimated from birth weight (5,11).
[2]Likely to be underestimated because of obligatory perinatal weight loss (approximately 10%).

Table S1. Mean (range) of anthropometry, pre- and perinatal Hg exposure, and Gesell Scores of infants.

Figure S1. Percent distribution of Thimerosal-EtHg/ unit of body weight at birth (a – first dose of hepatite-B, 25 µgHg) and at 180 days (b - third dose of hepatite-B and third dose of DTP, 50 µgHg); (c) percent distribution of Thimerosal-EtHg (second dose of hepatite-B, first and second dose of DTP, 75 µgHg)/weight gain between first and last vaccines.

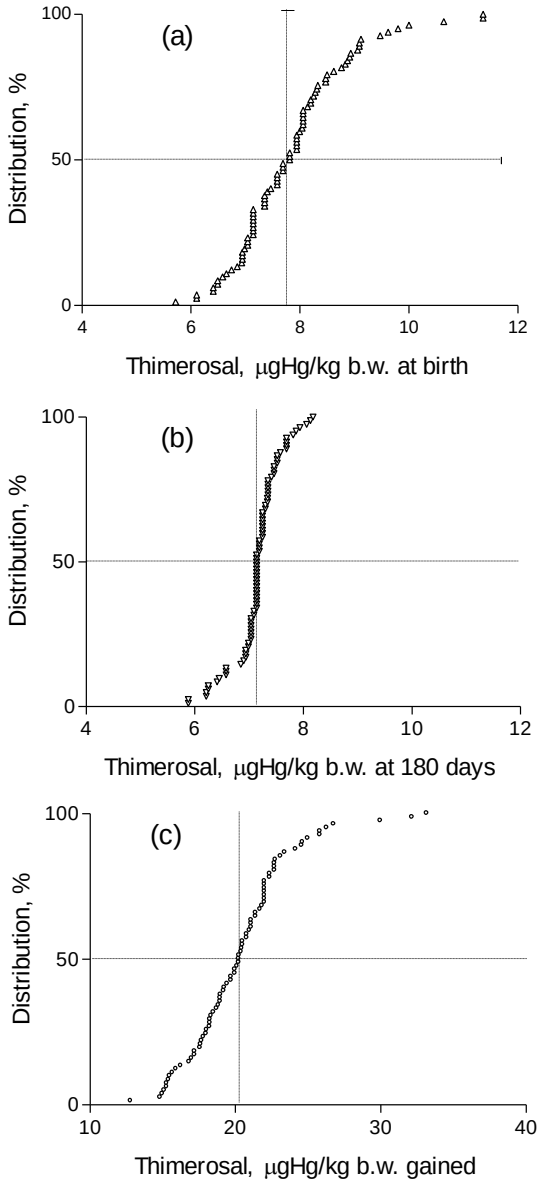


Figure S2. Plot illustrating correlation between Gesell scores and (a) dose of injected TCV-EtHg as function of body mass (r=-0.1724; P=0.1214), (b) between relative change in hair-Hg at six months (r=0.0753; P=0.5037) and (c) relative weight gain at six months (r=-0.1185; P=0.2892).

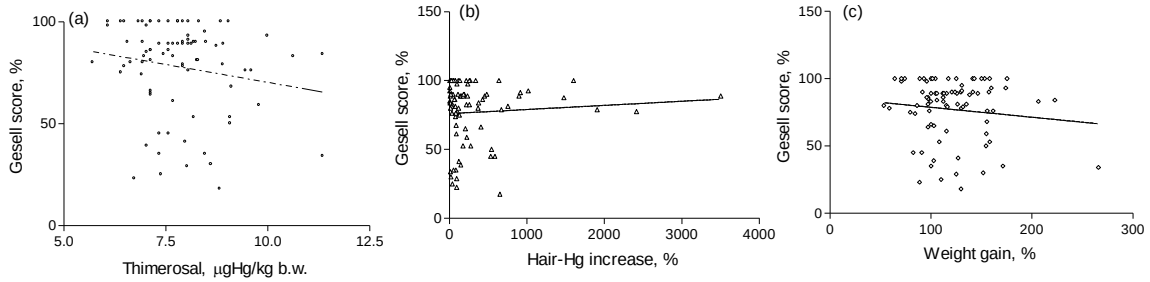
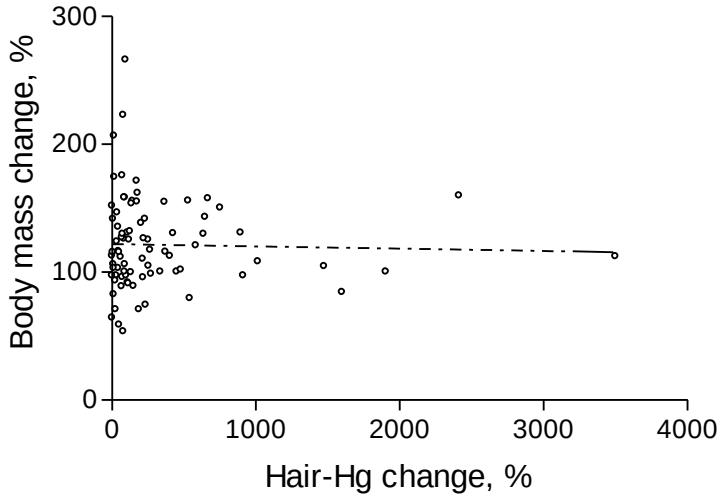


Figure S3. Plot illustrating correlation between change in hair-Hg at six months and respective change in body mass (r=-0.0287; P=0.7988).



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Principal component analysis and discrimination of variables associated with pre- and post-natal exposure to mercury

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Abstract

The variance of variables associated with neurodevelopment at 180 days, pre-natal variables (Hg in placenta, blood and hair) and post-natal Hg exposure (including Thimerosal-containing vaccines, TCV) were examined in 82 exclusively breastfed infants using principal component analysis (PCA). This multivariate method was applied to identify hierarchy and sets of interrelated variables. The PCA yielded a two-factor solution, explaining 92% of variance and summarizing most of the relevant information in the dataset matrix: the first component represented birth weight and vaccine (first doses of Hepatitis B and DTP) variability and explained 57% of variance; the second component represented a gradient of neurodevelopment (Gesell scores) and explained 35% of variance. The third component explained only 3% of the remaining 8% variance. Beside CNS priming by breastfeeding, infant development (birth weight) and time of immunization with TCV should be considered in epidemiological studies. PCA can classify sets of variables related to vaccination and neuromotor development schedules, clearly discriminating between earlier and later TCV exposures of exclusively breastfed infants. In conclusion, the incommensurable concept of the chance of toxic risk caused by TCV-EtHg exposure against the proven benefit of immunization is in no way disputed here. However, infant neurodevelopmental (ND) disorders linked to Thimerosal-Hg stands in need of proof, but PCA points to the possibility of identifying exposure risk variables associated with ND schedules.

Keywords: Thimerosal; Ethylmercury; Methylmercury; Vaccines; Breastfeeding; Neurodevelopment; Infants

Introduction

Mercury's most widely recognized effects are neurological; the developing central nervous system (CNS) of fetus, infants and young children are vulnerable to these effects. Fetal exposure to methylmercury (MeHg, from fish consumption) is thought to lower intelligence and alter behavior. The Harvard Center for Risk Analysis panel quantified the impact of chronic MeHg exposure on cognitive development (Cohen et al., 2005) and concluded that pre-natal MeHg exposure sufficient to increase maternal hair-Hg by 1 μg/g at parturition decreases intelligence quotient by 0.7 points (Cohen et al., 2005). However, neurological effects of post-natal

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Hg exposure are more difficult to evaluate. In infants, Hg exposure occurs through milk feeding (inorganic Hg and MeHg) and during immunization with Thimerosal containing vaccines (TCV), which metabolize into ethyl mercury (EtHg).

During the 1990s, there were several reports claiming that immunization with TCV could increase autism and other neurodevelopmental disorders (NDD). The preponderance of epidemiologic evidence does not support an association between TCV and autistic disorders (Parker et al., 2004), but biological plausibility is well defended (Mutter et al., 2005). Therefore, the epidemiological interest in NDD (including autism) and association with TCV continues to be studied with conflicting results (Verstraeten et al., 2003; Geier and Geier, 2004, 2006a, b, c; Heron et al., 2004; Andrews et al., 2004).

While risk of pre-natal Hg exposure has prompted issues of fish consumption advisories, efforts to decrease children's exposure to Hg have included a ban on the use of TCV by industrialized countries; as a precautionary measure, the USA and EU countries have shifted from TCV to Thimerosal-free vaccines. Although the WHO considers TCV safe for use in underdeveloped countries, uncertainty about the possibility of links between TCV and NDD will continue; banning immunization of children with TCV was recognition by advanced nations that NDD have a tendency to occur.

There are many studies showing that fetal exposure to neurotoxicants is overruled by breastfeeding. Furthermore, the link between NDD and TCV, or more often, its absence as seen in some epidemiological studies involves a failure to properly address the weight of compromising circumstances related to breastfeeding absence, insufficient quantity or duration. The immunized infant is exposed to EtHg in Thimerosal by a parenteral route which is different from exposure to Hg (organic and inorganic) in human milk. While Hg exposure in breast milk is buffered by the enterohepatic barrier, the regulatory mechanisms of the gastrointestinal tract are bypassed by injected Thimerosal.

The first 6 months of life are marked by significant qualitative (organ maturity, priming of the nervous system, function of enzymes) and quantitative (tissue differentiation, organ and body growth) changes. Therefore, this relatively short period of life is impacted by a high load of EtHg from immunizations; the smaller body mass and blood volume of neonates are important modulators of the toxicokinetics and toxicodynamics of Thimerosal, especially at times of more vulnerability of the blood–brain barrier (BBB). Newborns and infants requiring immunization (in Brazil and many countries) are still exposed to EtHg through TCV. Although the use of TCV is considered safe, uncertainties related to sub-clinical NDD in population studies await statistical evaluation (Magos and Clarkson, 2006). Indeed, studies that ignored colinearity or the many confounders (related to pre- and post-natal exposure and infant development) have produced rather incongruent results such as a negative correlation between Thimerosal dose and risk of neurological disorders (Andrews et al., 2004; Heron et al., 2004).

PCA is a simple multivariate method useful to extract relevant information from complex datasets. It is the objective of the present study (a continuation of part I, Marques et al., 2007a) to use powerful PCA to discriminate variables associated with neurodevelopment outcomes, pre- and post-natal Hg exposure and it is expected that one can learn about the main factors that influence variation in neurodevelopment of normal breastfed children.

Materials and methods

As part of the study protocol to assess growth and development of infants exposed to pre-natal background maternal-Hg load, we took advantage of the study already published (Marques et al., 2007a) to focus on variable interactions. During the review process of the parent publication (Marques et al., 2007a), it was brought to our attention that the possible post-natal exposure include the vaccines given to infants which were the type preserved in Thimerosal. We investigated the origin of the vaccines and were informed that all vaccines in the Brazilian immunization program contained Thimerosal within the permitted limits (0.01%). In order to evaluate metabolic aspects related to our main marker of organic Hg exposure (hair Hg concentrations) in infants, we addressed these issues in a second publication (Marques et al., 2007b).

The parent publication (Marques et al., 2007a) dealing with pre-natal Hg exposure described in details the characteristics of the population of Porto Velho (State of Rondônia, West Amazonia). Mothers between the ages of 15 and 45 years were selected by a nurse during their routine visits to the Pre-natal Clinics (Hospital de Base, Hospital Panamericano and Hospital Regina Pacis), among those in good health, reporting no illness or complaints. Excluding factors were occupational exposure to toxic chemicals and hereditary neurological illnesses. Pregnant mothers who were willing to breastfeed were introduced to the study's plain-language information, and a written consent form was presented and signed by the volunteer mother. Confidentiality was assured as well as freedom to withdraw from the study at any time. The mothers were closely monitored by nurses to guarantee full support of breastfeeding and pre- and post-natal care including the full immunization schedules recommended by the Brazilian immunization program.

After birth, the newborns were clinically examined with special attention to vitality, perinatal reflexes, maturity, and congenital malformations; weight, length, head circumference, and Apgar scores were recorded. While on the maternity wards, we collected samples of placenta, blood and hair from mothers and respective infants (fetal hair); the hair sampling was repeated at 6 months. Hair strands were cut from the occipital region and placed in plastic bags, with the root end stapled on a paper sheet. Sample preparation and Hg determinations in hair and blood were done according to routine procedures (Marques et al., 2007a).

After hospital discharge the mothers and the children were monitored for the programmed post-natal visits and immunization scheme in accordance with the Brazilian vaccination program. At 6 months of age, mother–infant pairs reported for the programmed clinical and neurobehavioral examination when hair samples were collected again. Infants were again weighed and measured for length and hair-samples were again collected from mothers and infants. Integrated weight gains at 30, 60 and 120 days were estimated from differences between infants' weight measured at birth and at 6 months. In this study we used only datasets of 82 mother–infant pairs of the initial 100 enrollment; due to various causes we had drop outs and incomplete data in 18 pairs (Marques et al., 2007a).

The neurodevelopment tests were conducted by trained professionals using the Gesell Developmental Schedules (Gesell, 2003; Gesell and Amatruda, 2000). These tests included all reactions (voluntary, spontaneous or learned) and reflexes. We also evaluated postural reactions, hand pressure, locomotion and coordination, constructive ability (which is influenced by motor development), visible and audible communication, individual reactions regarding people and stimulations (depending mainly on the temperament of the child and the surroundings); using appropriate measurements we compare the child developmental stage with that expected for the respective age (qualitative and quantitative). The results (as percentages) were expressed as developmental quotients (DQ) for the Adaptive Behavior, Language Development, Gross Motor Skills and Personal/Social Behaviors.

Estimated exposure to injected Thimerosal-Hg (EtHg) from vaccines: Mothers followed the immunization schedule recommended by the Ministry of Health of Brazil. The first dose of Hepatitis B vaccine was taken by neonates before hospital discharge and the subsequent two doses (at 30 and 180 days) as well as the three doses of DTP at 60, 120 and 180 days (Table 1). Only the babies born at the state-run hospital (Hospital de Base, 66%) received the Hepatitis B vaccine within the first day postpartum. Babies born at the Hospital Panamericano and the Hospital Regina Pacis received the Hepatitis B vaccine immediately after the mother's discharge (2–4 days postpartum). At this time, the mothers were taken under our supervision to a state-run clinic where immunizations are done.

Table 1. Infant anthropometry, neurodevelopment schedules and markers of prenatal Hg exposure

Characteristics	Mean ± S.D.
Infant anthropometry	
Weight at birth (g)	3233.17 ± 421.56
Length at birth (cm)	49.77 ± 2.43
Head circumference at birth (cm)	33.88 ± 2.29
Weight at 6 month (g)	7011.34 ± 458.98
Length at 6 month (cm)	67.60 ± 2.59
Head circumference at 6 month (cm)	42.58 ± 1.08
Pre-natal Hg exposure	
Maternal blood Hg (μg/L)	6.03 ± 14.07
Placenta Hg (ng/g)	10.75 ± 9.82
Umbilical cord Hg (ng/g)	10.54 ± 9.93
Maternal hair Hg at delivery (μg/g)	7.36 ± 8.73
Infant hair Hg at birth (μg/g)	2.44 ± 3.04
Infant hair Hg at 6 month (μg/g)	3.84 ± 5.55
Maternal hair Hg 6 month (μg/g)	3.19 ± 3.85
Neurodevelopmental schedules	
Motor DQ	74.82 ± 31.95
Language DQ	67.87 ± 36.23
Adaptive DQ	82.01 ± 19.62
Personal social DQ	86.10 ± 10.21
Total DQ	77.70 ± 22.24

DQ, development quotient.

The calculated exposure to EtHg was estimated as Thimerosal-Hg derived from vaccines used in the current national immunization program of the Ministry of Health of Brazil. The Hg concentration of the doses delivered through vaccines (containing 0.01% Thimerosal as stated by manufacturers) was 25 μg/0.5 mL for Hepatitis B (Korea Green Cross Corporation, Kiheung-Eup Yougin-Goon Kiyunggi-Do, Korea; Euvax B injectable, LG Life Sciences, Jeonbuk-Do, Korea) and for DTP (Triple Antigen, Serum Institute of India Ltd., India; Vacina Tríplice, Instituto Butantã, São Paulo, Brazil).

Statistical analysis

A principal component analysis (PCA) model was used for the measured variables to gain information with fewer variables. This mathematical model calculates new variables (principal components) that account for the variability in the data and enables the study of covariances or correlations between variables. Thus, the principal components are linear combinations of the original variables. The combination of variables that explains the greatest amount of variability is the first

principal component. The subsequent ones (second and third principal component) describe the maximum amount of remaining variability; they must be independent of the first principal component. For this multivariate model we used the approach discussed by others (Ludwing and Reynolds, 1988; Reyment and Jöreskog, 1996).

The main steps of the analysis were:

(i) *Transformation of the data:* PCA standardize the original matrix assuming means as zero and standard deviations as one. The purpose of this step is to derive a small number of linear combinations (principal components) that retain as much of the information in the original variables as possible; this allows many variables to be summarized in a few, jointly uncorrelated principal components. The result is considered good if we obtain a high proportion of the total variance explained by a few principal components. Commensurate with the analysis done in principal components analysis, we also treated the variables as active and supplementary variables. The supplementary variables can be projected onto the vector subspace generated by the factors.

We used the STATISTICA package (StatSoft, Inc., Data Analysis Software System, version 6, www.statsoft.com, 2001, Tulsa, OK 74104, USA) on the following variables: (1) birth weight, (2) length at birth, (3) head circumference at birth, (4) maternal blood-Hg concentration at delivery, (5) Placenta-Hg concentration, (6) umbilical cord-Hg concentration, (7) maternal hair-Hg concentration at delivery, (8) infant hair-Hg concentration at birth, (9) infant hair-Hg concentration at 6 months, (10) maternal hair-Hg concentration at 6 months, (11) infant weight at 6 months, (12) infant length at 6 months, (13) head circumference at 6 months, (14) motor DQ at 6 months, (15) language DQ at 6 months, (16) adaptative DQ at 6 months, (17) personal and social DQ at 6 months, (18) total DQ at 6 months, (19) Thimerosal-Hg (Hepatitis B 1st dose)/kg b.w. at birth, (20) Thimerosal-Hg (Hepatitis B 2nd dose)/kg b.w. at 30 days, (21) Thimerosal-Hg (DTP 1st dose)/kg b.w. at 60 days, (22) Thimerosal-Hg (DTP 2nd dose)/kg b.w. at 120 days, (23) Thimerosal-Hg (3rd dose of Hepatitis B and DTP)/kg b.w. at 180 days, (24) integrated mean Thimerosal-Hg/kg b.w. at 180 days; we did not use varimax rotation or any other additional factor analysis.

(ii) *Construction of two-dimensional graph* (Fig. 1): This step organizes the variables on the appropriate vector basis allowing visual inspection of the data in a two-dimensional score plot that reveals patterns hidden in the dataset. To visualize the separation of groups, Fig. 1 shows score plots of the first two PCs and the values of the PC loadings. Plots of loadings (PC1 and PC2) on these data allow us to recognize groups of samples with similar behavior and the existing association among the original variables.

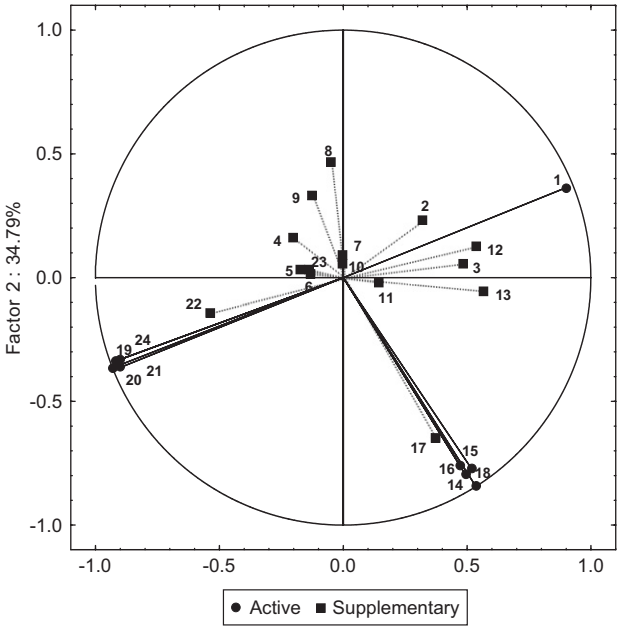

Fig. 1. Scatter plot of loadings of PCA (principal component analysis) dataset. The identities of the responses and the neurodevelopment variables are indicated by the plot labels.

Results

The summary, as mean and S.D., of the chosen variables is shown in Tables 1 and 2. These variables include pre- and post-natal Hg exposure, infant anthropometry and neurodevelopment scores (Table 1); prenatal Hg exposure is represented by hair-Hg of mother and infants at birth, Hg concentrations in placenta and umbilical cord. Post-natal Hg exposure is represented by infant hair-Hg at 6 months and immunization doses of TCV-Hg (as EtHg) during the first 6 months (Table 2). Higher Thimerosal-Hg dose per unit of body mass is shown at birth (when newborns had the smallest body mass) and 6 months (when they were injected with two doses of TCV – third shot of both Hepatitis B and DTP).

The main component analysis was used to explore the data matrix order; this can help determine the weight (importance) of parameters in the total variability through vector size and loads and respective percentual contributions. The result of PCA gives two significant principal components (eigenvalues >1), which explain 92% of the variation in the data (57% and 35%, respectively). The classification of loads (Tables 3–5) points to the post-natal Hg exposure (immunization

Table 2. Infant immunization schedule, type of vaccine, and Thimerosal-Hg/dose (EtHg) during the first 6 months

Age (days)	Type[a]	μgHg/dose[b]	Infant weight (kg)[c,d]	μgHg/kg[d]
0	Hp-B	25.0	3.23 (0.42)	7.87 (1.09)
30	Hp-B	25.0	3.86 (0.35)	6.53 (0.61)
60	DTP	25.0	4.49 (0.31)	5.59 (0.38)
120	DTP	25.0	5.75 (0.32)	4.37 (0.23)
180	DTP + Hp-B	50.0	7.01 (0.46)	7.16 (0.44)
Total		150.0		6.30 (0.45)

[a]Hp-B: Hepatitis B (Korea Green Cross Corporation, Kiheung-Eup Yougin-Goon Kiyunggi-Do, Korea; Euvax B injectable, LG Life Sciences, Jeonbuk-do, Korea); DTP (Serum Institute of India Ltd.; Vacina Tríplice, Instituto Butanta, São Paulo, Brazil). These vaccines contained 0.01% Thimerosal/dose as stated by manufactures.
[b]Vaccine dose: 0.5 mL; Hg mass is approximately 50% of the Thimerosal.
[c]Estimated from Marques et al. (2007a).
[d]Mean (and standard deviation).

Table 3. Principal component loads indicating variability percentages of measured parameters (factor-variable correlations (factor loadings), based on active and supplementary variables[a])

	Factor 1	Factor 2	Factor 3
1. Weight at birth	0.898249	0.362923	0.064494
14. Index of motor development	0.493417	−0.796391	0.139435
15. Index of language development	0.517388	−0.771637	0.235206
16. Index of adaptative development	0.470206	−0.757382	−0.440593
18. Index of general development	0.534355	−0.841893	0.049363
19. TCV-Hg (Hepatitis B 1st dose, birth)/kg b.w.	−0.897585	−0.361978	−0.057133
20. TCV-Hg (Hepatitis B 2nd dose, 30 days)/kg b.w.	−0.930730	−0.364520	0.000802
21. TCV-Hg (DTP 1st dose, 60 days)/kg b.w.	−0.902208	−0.331506	0.075336
24. Total Thimerosal-Hg/kg b.w. at 180 days	−0.917511	−0.336700	0.054712
2. Length at birth[a]	0.319748	0.229549	−0.096108
3. Head circumference at birth[a]	0.484365	0.053901	−0.012174
4. Maternal blood-Hg at delivery[a]	−0.204777	0.163233	0.338478
5. Placenta-Hg[a]	−0.174918	0.030322	−0.219712
6. Umbilical cord-Hg[a]	−0.132638	0.015406	−0.069268
7. Maternal hair-Hg at delivery[a]	−0.001043	0.088459	−0.046659
8. Infant hair-Hg at birth[a]	−0.051069	0.468469	0.092115
9. Infant hair-Hg at 6 months[a]	−0.126927	0.329956	0.120974
10. Maternal hair-Hg at 6 months[a]	−0.001800	0.055173	0.043098
11. Infant weight at 6 months[a]	0.142328	−0.019707	−0.245993
12. Infant length at 6 months[a]	0.537043	0.124353	−0.134842
13. Head circumference at 6 months[a]	0.568066	−0.055300	−0.132429
17. Index of personal and social development[a]	0.372902	−0.650683	0.005994
22. TCV-Hg (DTP 2nd dose-120 days)/kg b.w.[a]	−0.537471	−0.140750	0.209801
23. TCV-Hg (Hepatitis B and DTP – 180 days)/kg b.w.[a]	−0.138998	0.030978	0.242526

TCV: thimerosal-containing vaccines; DTP: diphtheria, tetanus, pertussis.
[a]Supplementary variables are illustrated in Fig. 1 (Eigenvalues <1).

Table 4. Eigenvalues of correlation matrix and related statistics of active variables only – individual and cumulative percent contributions

	Eigenvalue	% Total	Cumulative	Cumulative
1	5.152354	57.24837	5.152354	57.2484
2	3.130954	34.78837	8.283307	92.0367
3	0.287416	3.19351	8.570724	95.2303

schedules of newborns and infants): two vaccines within the first month (0 and 30 days) and the high Thimerosal-Hg dose (in the third dose of Hepatitis B and DTP) at 180 days. The analyzed variables and respective loads are listed in Table 3; the first component (57% of variance) is accounted for by variables with the highest loads: (1) birth weight (0.90), (19) Thimerosal-Hg (Hepatitis B 1st dose)/kg b.w. at birth (−0.90), (20) Thimerosal-Hg (Hepatitis B 2nd dose)/b.w. at 30 days

Table 5. Discrimination of variables with percent contributions

	Factor 1	Factor 2	Factor 3
1. Birth weight	0.156598	0.042068	0.014472
14. Index of motor development	0.047252	0.202571	0.067645
15. Index of language development at 6 months	0.051955	0.190173	0.192479
16. Index of adaptative development	0.042911	0.183212	0.675405
18. Index of general development	0.055419	0.226380	0.008478
19. TCV-Hg (Hepatitis B 1st dose, birth)/kg b.w.	0.156367	0.041849	0.011357
20. TCV-Hg (Hepatitis B 2nd dose, 30 days)/kg b.w.	0.168129	0.042439	0.000002
21. TCV-Hg (DTP 1st dose, 60 days)/kg b.w.	0.157982	0.035100	0.019747
24. Total Thimerosal-Hg/kg b.w. at 180 days	0.163387	0.036208	0.010415

TCV: thimerosal containing vaccines; DTP: diphtheria, tetanus, pertussis.

(−0.93), (21) Thimerosal-Hg (DTP 1st dose)/kg b.w. at 60 days (−0.90), (24) mean Thimerosal-Hg/kg b.w. at 180 days (−0.92). The second component, which explained 35% of the variation, is accounted for by the following variables: (14) motor DQ (−0.80), (15) language DQ (−0.78), (16) total DQ (−0.84), (18) adaptative DQ (−0.76). This can be interpreted as a gradient of variability among variables; positive signs mean that variables increase together while a negative sign means that as one increases the other decreases. It should also be conceptualized that the sign of the birth-weight variable is the opposite of those of Thimerosal doses because these are derived from the former (they are reciprocals). The third component explained 3% of the remaining 8% variability. Further details in Table 4 illustrate the percentage of variance explained by components. Components one and two explain 92% of total variance, accounting, respectively, for 57% and 35% of the variation. Table 5 summarizes the factor contribution of the active variables.

Most of the variation is explained by two-dimensional scattergram (Fig. 1); plotting the values for the first two principal components of the data points gives the essential features of the multidimensional scatter (PC1 and PC2, whose eigenvalues are >1, represents the main variables). Fig. 1 illustrates the separation of the observational variables: the variables associated with ND schedules and immunizations (as function of body weight) are at opposite quadrant.

Discussion

The main finding of this work is to show that PCA discriminated variability of early vaccine schedule and neurodevelopment outcomes from variables that included pre- and post-natal Hg exposure; immunizations given within 60 days were extracted as the first principal component. Multivariate models involving many variables are often difficult to interpret because plots and lists of loadings can become too overwhelming to comprehend. In order to handle a large number of variables, PCA reorganizes the variables, reducing the dimensions of the original dataset. In other words, without a significant loss of information, this approach describes a shorter list of variables. In this dimension, PCA clearly discriminated the first set of vaccines taken at an earlier time (first and second doses of Hepatitis B at birth and 30 days, and first DTP at 60 days). Although all vaccines contained the same dose of Thimerosal-Hg, the amount of EtHg/kg b.w. given varied as a function of body mass change: newborn babies (within the first days) received doses of TCV-EtHg comparable to those of 6 months old (double the amount of vaccine-Hg in the two shots given at 6 month). Although these doses of Thimerosal (EtHg) per unit of body mass represented the highest TCV exposures, they ended up in different components. However, it should be kept in mind that a point of concern can be raised in respect to birth weight (as well as body mass development) and the given TCV-Hg dose. Birth weight and weight adjusted Hg produced close co-linearity (opposite sign factor-loadings but nearly identical high values – Table 3). For studies intending to use PCA co-linearity, such as this type, it can bias regression models. Nevertheless, it points to the necessity to view the first 6 months in its proper perspective.

Differences in infant growth and maturation (tissue differentiation) between a 1-day-old neonate and a 6-month-old infant (who has doubled its original body mass and already acquired functional abilities) set them apart in terms of the toxicodynamics of Hg exposure.

Infants of this age range are not just small children, but a heterogeneous population with a wide range of physiological, metabolic and anatomical differences. It is essential to consider the dynamic complexity of early post-natal development to understand the spectrum of differentiation that determines the heterogeneity of this group. At one end we have a neonate (“external fetus”) with the highest degree of immaturity and that is defined as “born at term and normal” only by gestational age,

not by markers of immaturity. At the other end, 6-month-old infants are more functional and differentiated (anatomically, biochemically and physiologically). Therefore, there are implications for Thimerosal-EtHg during the first post-natal 24 weeks in term babies (defined as born between 36 and 42 weeks) with such a relatively wide (6 weeks) range of gestational age difference.

During the first 6 months, the fast changes in size, body composition, and organ function dramatically affect pharmacokinetics; this is also likely to affect pharmacodynamics. During this time infants are exposed to Hg in breast milk (Dorea, 2004); such exposure is amortized against a proportional milk intake (as a function of body weight). Any form of breast-milk Hg (inorganic Hg and methyl-Hg, MeHg) is complexed into the milk–protein matrix and its absorption and toxicity are attenuated by entero-hepatic barriers. Concomitantly, CNS is primed by components naturally present in breast milk. Contrary to the enteral breast-milk Hg, the parenteral route of TCV leads to a disproportionate EtHg exposure (per unit of body mass) at different stages of development (highest immediately after birth). The parenteral TCV exposure bypasses the enteral barriers and metabolic defenses, gaining ready access to the systemic venous system and thus reaching the brain within seconds. Magos et al. (1989) showed in rat models that circulation time between Hg absorption site and brain is an important toxicity factor. Indeed, a peak value of mercury appears to be the determinant of toxic damage in population studies (Clarkson and Strain, 2004); depending on post-natal immaturity, this is likely to occur with injected Thimerosal (EtHg).

Stajich et al. (2000) speculated that infants with less body mass could metabolize EtHg at a slower rate than larger infants and that immaturity of the liver could be responsible for less metallothionein available to bind inorganic Hg. However, the reanalysis of their data by Magos (2003) reached the opposite conclusion: "preterm infants handled ethylmercury load more efficiently than term infants." Regardless of these toxicokinetic differences, the vulnerability of the developing nervous system is expected to be higher in the neonatal period than at 180 days. Both Stajich et al. (2000) and Magos (2003) referred specifically to the toxicokinetics of EtHg and by no means referred to its toxicodynamics.

The decision to eliminate Thimerosal in vaccines (claimed as a precautionary measure) by some countries was based on guidelines for MeHg. Although the guidelines for MeHg are not equivalent to guidelines for EtHg (Thimerosal-Hg metabolite), the current assumption is that the health risks from exposure to MeHg and EtHg are the same (NIAID, 2005). This is debatable in the context of the breastfed infant: while breastfed infants are exposed to MeHg (in lower amounts than inorganic Hg) only enterally, TCV-EtHg exposure is a bolus parenteral exposure.

Because we are dealing with exposure to a pure substance by a parenteral route impacting an immature organism, there are specific considerations. The first impact of vaccine-EtHg for some newborns comes within hours of birth (Marques et al., 2007b). Newborns show important differences in body weight and tissue differentiation that affect toxicokinetics. Even in term infants, the blood–brain barrier (BBB) is not complete until after 6 months of age (Adinolfi, 1985). Therefore, the pharmacokinetic and pharmacodynamic of Thimerosal (EtHg) are modified by infant development, thus representing a challenge to infant toxicology. It has been experimentally shown that both genetic and brain maturation are critical determinants of post-natal Thimerosal-related sequelae. Equivalent EtHg dosing and timing of exposure caused behavioral disruption in control mice but not in a strain resistant to autoimmunity (Hornig et al., 2004). However, because only 3% of neurodevelopmental (ND) disabilities are attributed to environmental neurotoxic substances (Grandjean and Landrigan, 2006), a large population study would be required to detect a post-natal Hg exposure effect caused by TCV. Furthermore, in situations of universal exposure such as Hg in vaccines, increased risk of ND disabilities is more complex because of chance of interactions with pre-natal exposure to MeHg in fish eating populations.

It should be stressed that neurodevelopmental variables in this study were obtained from infants whose CNS were primed by exclusive breastfeeding. Breastfeeding has consistently shown efficient CNS priming that could affect the toxicodynamics of TCV-EtHg. The counteractive effects of breastfeeding on NDD have been reported in infants pre-natally exposed to MeHg (Grandjean et al., 1995; Jensen et al., 2005). Breastfeeding predicts faster CNS maturation rates (Hart et al., 2003), higher neurodevelopment scores (Vestergaard et al., 1999), and better intellectual achievements (Victora et al., 2005).

So far, studies that examined NDD effects of vaccine-EtHg exposure have not taken breastfeeding into consideration. One study, however, provides an opportunity to speculate; in 7-year-old Faroese children, breastfeeding was associated with marginally better neuropsychological performance (Jensen et al., 2005). Hair mercury concentration at age of 1 year (adjusted confounder), did not change the positive effects of breastfeeding. These Danish children were born between 1986 and 1987 and probably also received the TCV (125 μgEtHg, three vaccine doses) still in use at that time (Hviid et al., 2003).

The full range of options used in epidemiological studies of infant exposure to TCV (EtHg) and the short- and long-term effects on neurodevelopment should

consider breastfeeding as an important covariate. Multivariate models such as PCA do not establish cause and effect association between variables, but are useful tools to sort them. In our study it was possible to line up vectors that discriminated and classified the immunization schedules (first principal component) and neurodevelopmental scores (second principal component). Because there were no adverse effects of pre-natal Hg exposure in this group of breastfed infants (Marques et al., 2007a), neurodevelopment interaction with early immunization of infants should also consider birth weight (or surrogates of fetal development such as gestational age). This might help devise epidemiological studies with sufficient refinement to include variables that can more precisely account for the complexity of early post-natal development and Hg exposure. There are no cause–effects relations between early Thimerosal-Hg exposure and neurodevelopmental deficits, but the challenge of understanding such complex interactions may benefit from the present findings.

Conclusion

The chance of harm caused by TCV-EtHg exposure against the proven benefit of immunization is in no way argued by these results. Infant NDD linked to Thimerosal-Hg stands in need of proof; however, the present work points to the possibility of identifying modifiable NDD-risk factors associated with infant development and TCV-EtHg dosing.

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Changes in children hair-Hg concentrations during the first 5 years: Maternal, environmental and iatrogenic modifying factors

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Abstract

Children are exposed to Hg from mothers (via placenta and lactation), environment (food), and in many parts of the world by thimerosal-containing vaccines (TCV) during immunization. Neurodevelopment studies based on infant hair-Hg (HHg) have been designed without explicit attention to the factors associated with changes in infant physiology and Hg sources of exposure. A longitudinal study of changes in HHg concentrations from birth to 5 years was done in a sample of children from Porto Velho (Rondonia), Brazilian Amazonia. The study extracted information from the asymmetry associated with maternal and infant HHg changes at specified sampling: birth (fetal exposure), 6 months of exclusive breastfeeding, 36 months (weaning) and 60 months (pre-school). The distribution of HHg in breastfed infants followed a pattern different from their mothers. While mothers had the highest HHg concentrations at childbirth, infants showed the highest HHg values at 6 months after the recommended full schedule (six shots) of immunization with TCV; after that, the downward trend in HHg shown by children coincided with both weaning and less frequent vaccination period (5 years). Extended lactation (up to 36 months) was not significantly associated with HHg of infants or mothers; however, significant association (Spearman's r) between maternal and infant HHg concentration was seen at birth ($r = 0.3534$; $P = 0.001$), 6 months ($r = 0.4793$; $P < 0.0001$), 3 years ($r = 0.0122$; $P = 0.012$) and 5 years ($r = 0.0357$; $P = 0.005$). Maternal postpartum metabolic changes, infant development and transitional diets and possibly Hg from TCV contribute to the asymmetry of HHg changes between mothers and children.

Keywords: Vaccines; Thimerosal; Hair-Hg; Breastfeeding; Fish consumption; Amazon; Neuro-motor development

1. Introduction

Current studies associating Hg exposure and neurodevelopmental disorders (NDD) in children rely on the metal concentrations in hair. Hair is composed of a protein complex formed from amino acids that avidly binds to methyl-Hg (MeHg). The assumption is that the occurrence of NDD results from consuming fish contaminated with MeHg, which avidly binds to hair (Cernichiari et al., 2007). Indeed, epidemiologic studies dealing with MeHg exposure from fish consumption have used HHg concentrations as a reliable indicator. However, during the first years of life, infants can be exposed to Hg in their changing food sources: from breast milk (or formulas) to weaning foods, and at a latter age, from other dietary sources (that might include fish) when eating adult food. Young children can also be exposed to other forms of organic Hg (ethylmercury—EtHg) when immunized with thimerosal (Marques et al., 2007a).

Children of fish-eating populations of the Amazon Basin are exposed to maternal MeHg via placental transfer and breast milk (Barbosa et al., 1998; Barbosa and Dórea, 1998; Marques et al., in press). In this context, the increase in the risk of NDD has been strongly suggested to be associated with MeHg naturally present in fish from the Amazonian rivers (Grandjean et al., 1999). The possibility of

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NDD raised by these studies has relied on HHg concentrations (biomarkers of fish consumption). Indeed, variability in HHg of riverine and Amerindian populations has been correctly assumed to be a function of fish consumption. Barbosa et al. (1995) have shown important differences in urine and HHg concentrations due to occupational exposure (gold miners) and fish consumption for native Amazonians. However, we have recently raised the possibility that, at least for the first 6 months of life, HHg concentrations may be influenced by the heavy immunization (with TCV) schedule of urban populations (Marques et al., 2007a). Indeed, during the infant's first years, susceptibility to modifying factors remains unknown, but physiological and dietary changes (breast milk, weaning and habitual family diets) are likely to influence HHg changes.

Body retention of mercury and its hair uptake depend on dietary and physiological factors. In children, there are drastic physiological changes during the first years that affect the toxicokinetics of Hg. Mercury is taken up from the bloodstream during hair formation. Newborn hair essentially represents Hg exposure during fetal development. During the first 6 months, at least in breastfed infants, the origin of Hg is still maternal, but it is not through the fetal circulation but enteral through breast milk. Enteral exposure to Hg from weaning food has not been explored for fish-eating populations, but it is expected to be different from an adult's diet. However, for children with access to health services, another source of Hg exposure has been unaccounted for in fish-eating populations, that is, immunization with TCV (Marques et al., in press).

Easy collection and preservation make the minimally invasive HHg sampling a method of choice in evaluating MeHg exposure from fish consumption. Indeed, HHg has made it possible to estimate fish consumption more reliably than with conventional dietary recall methods (Richardson and Currie, 1993; Gosselin et al., 2006); it has been conveniently applied in differentiating occupational and environmental (fish intake) exposures (Barbosa et al., 1995) and to overcome difficulties of communication involving cross-cultural studies of Amazonian Amer-Indians (Dorea et al., 2005) and isolated communities of riverine populations (Alves et al., 2006).

Ponce et al. (1998) compared Hg exposure estimates based on self-reported fish intake and measured fish-Hg concentrations and HHg concentrations; they showed bias and random error as components of uncertainty regarding HHg exposure estimates. Indeed, Canuel et al. (2006) demonstrated significant regional differences in HHg kinetics; therefore, generalization of HHg models needs to take into account not only the organic form of Hg and source of exposure but also physiological differences and genetic diversities of individuals within populations.

Both immunization (primary prevention) and breastfeeding (secondary prevention) share desirable health outcomes: prevention of infectious diseases (vaccines) and long-term health CNS and general health indicators warranted by breastfeeding. However, such health-related procedures are also the initial way infants and young children are exposed to Hg (Marques et al., in press). Infant routes of Hg exposure entail a variety of sources and a corresponding chemical species: placenta (MeHg), breast milk (MeHg and inorganic Hg), TCV (EtHg) and weaning foods (MeHg and inorganic Hg); each source of exposure is part of a unique set of events at specific time which influences substantially the toxicokinetics of Hg.

The underlying metabolic pathways attendant on the changing anatomical and physiological functions of early infancy, as well as the interactions among sources (Hg forms, dose and duration) make young children a heterogeneous population in respect to the toxicokinetics of Hg. Additionally, during this time there are special circumstances of intense iatrogenic exposure during the immunization with TCV that still prevails in some countries; these interactions are likely determinants of Hg retention in children' hair. The data collected for this study were conceived for evaluating the neurodevelopment of children exposed to pre-natal exposure Hg from maternal fish consumption (Marques et al., 2007a). When the research project started we were not aware of the TCV issue in pediatric vaccines; this was brought up during the revision process of the parent publication (Marques et al., 2007a). Because we had all records of immunization we could conceptualize the assessment of thimerosal-Hg in the young children. Therefore, we took advantage of our cohort to study longitudinal changes in HHg of mothers and respective children from birth to key ages of 6, 36 and 60 months.

2. Materials and methods

2.1. Sample description

The city of Porto Velho, capital of the state of Rondonia (West Amazonia), has experienced significant demographic changes with people coming from many other Brazilian regions. During the last 30 years, it has changed from a traditional Amazonian city to one under the impact of heavy migration brought by agricultural projects in the south-west region and especially the influx of prospectors seeking alluvial gold along the banks of the Rio Madeira basin. The present population has both traditional families that base their diets on fish and starchy foods and city dwellers with more cosmopolitan food habits. In this changing environment, we investigated the health status of breastfed infants with special reference to food habits and possible Hg exposure due to fish consumption.

The research protocol was approved by the Ethics Committee for Human Studies of the Universidade Federal de Rondonia and details have already been published (Marques et al., 2007a,b).

2.2. Study protocol

Mothers were introduced to the study and invited to participate by a nurse during their routine visits to the Pre-natal Clinics of three hospitals in Porto Velho: Hospital de Base, Hospital Panamericano and Hospital Regina Pacis. One-hundred and sixty potential participants received plain-language information about the study and a written consent form was presented and signed by the 155 that agreed to participate—115 were eligible; the written consent stated that participation was voluntary, their

confidentiality was assured and that they could withdraw from the study at any time. Mothers (between the ages of 15 and 45 years) were selected among those in good health, reporting no illness or complaints at the time of the study and who were willing to breastfeed up to 6 months of age. Excluding factors were occupational exposure to toxic chemicals and hereditary neurological illnesses. While at the maternity wards, we collected samples of hair from mothers and respective infants (fetal hair). Hair strands were cut from the occipital region and placed in plastic bags, with the root end stapled on a paper sheet; newborn hair was sampled in sufficient amount. Hair samples were again taken at the follow-up visits at 6, 36 and 60 months.

2.3. Hair-Hg analysis

Sample preparation and analytical procedures were done according to our standardized protocol for Hg determination in hair as described elsewhere (Marques et al., 2007a). Hair analysis was done on the first centimeter closest to root end. Sample preparation and Hg determination were done according to routine procedures previously established at the Universidade Federal do Rio de Janeiro (Bastos et al., 1998). We followed routine procedures after adaptation of analytical protocol used for Hg determination in previous studies. Briefly, the hair samples were comminuted with stainless steel scissors, weighed, and digested before analysis. Human hair samples were washed with EDTA 0.01%, dried in an oven at 50 °C, weighed and digested with 5 mL of HNO_3:H2SO_4 (1:1) and 4 mL of 5% $KMnO_4$ using a digestion block at 80 °C for 40 min. The determination of total Hg in the digested samples was done by cold vapor atomic absorption spectrometry with a flow injection system-FIMS (CV-AAS, Perkin-Elmer—FIMS 400, Ueberlingen, Germany).

All glassware used in the analytical protocol was washed clean, rinsed with 5% EDTA and double distilled, and left to rest in 5% HNO_3 overnight. Then it was rinsed again in double distilled water, and dried at 100 °C for 12 h. Precision and accuracy of Hg determinations were assured by the use of internal standards, use of triplicate analyses of samples and certified reference materials (IAEA-085 and 086, Vienna, Austria) with recoveries of 92%. The limit of detection for the procedure was determined at <0.01 μg/g and there was hair-Hg determination below the limits.

2.4. Immunization vaccines and schedule

Infants up to 6 months of age received the full immunization scheme recommended by the Brazilian immunization program. After that time mothers were oriented about the importance of immunization and followed recommendations of available pediatric services. Among the vaccines taken by infants during the 5 years of the study some were preserved with 0.01% thimerosal (which metabolizes into EtHg). The Hg concentration of the doses delivered through vaccines was 25 μg Hg/0.5 mL. The immunization schedule with TCV and respective intake of Hg as stated by manufacturers is summarized in Table 1. These were: hepatitis-B (Korea Green Cross Corporation, Kiheung-Eup Yougin-Goon Kiyunggi-Do, Korea; Euvax B injectable, LG Life Sciences, Jeonbuk-Do, Korea), diphtheria, tetanus and pertussis-DTP (Triple Antigen, Serum Institute of India Ltd, India; Vacina Tríplice, Instituto Butanta, São Paulo, Brazil), Hib: Haemophilus influenzae type b (HibTITER®, Lederle-Praxis), Flu: influenza (VAXIGRIP, Pasteur Mérieux Connaught, Sao Paulo, Brazil). Children that were immunized against Hib within the first year (two doses) received the booster dose 6–12 months after the last dose, otherwise received only one dose in the second year of life. The recommendation for the Flu vaccine is to be taken between the ages of 6 and 35 months in two doses of 0.25 mL (1 month apart) and one single dose (0.5 mL) the following year; although the vaccines were taken the ages given represent approximations of the recommended date.

The exposure to EtHg derived from vaccines was based on the current national immunization program of the Ministry of Health of Brazil (Table 1). After 6 months only 20 of the 82 infants were given a fourth dose of DTP between 9 and 12 months. We estimated breast-milk Hg exposure from weight gain up to 6 months, when the infants were exclusively breastfed, and discussed it elsewhere (Marques et al., 2007a).

2.5. Statistical analysis

Of the 100 original enrolled mothers, only complete data of 82 mother–infant pairs could be obtained at the end of the study (Marques et al., in press). The statistical packages contained in Excel and Prism were used for data summarization (means, standard deviation, changes in mean Hg concentrations) and correlation analysis. Repeated measurements analysis was run to test sampling effect on HHg on infant and maternal HHg concentrations; this statistical analysis was performed using SAS release 9.1.3 (SAS Institute Inc., Cary, North Carolina). We accepted a value of <0.05 as statistically significant.

3. Results

A summary of the estimation of Hg forms in each of the exposure media (TCV and breast milk) that can contribute to HHg is shown in Table 1. During the first 6 months, infants received up to six shots of TCV (and were exposed to 150 μg Hg from thimerosal); the TCV corresponded to Hepatitis B and DTP; subsequently the immunization with TCV were for influenza and Hib with a coverage ranging from 2.5 to 25% of the children. Age of children corresponds to recommended vaccination schedules, but only 6-month-olds had the full schedule of immunization and the corresponding EtHg exposure. It is important to note that the exposure to TCV-Hg from the first vaccine taken at birth (0 day) is the highest and the most challenging dose; at this time the small body mass of neonates takes an impact equivalent to the double doses (of DTP and hepatitis B) at 6 and 12 months. The estimation of total Hg exposure from breast milk was possible only up to 6 months when mothers were closely monitored and psychologically supported to keep on with exclusive breastfeeding; indeed, most mothers (66%) breastfed for 12 months and some reported to breastfeed up to 60 months (Fig. 1). However, the effect of lactation on HHg concentrations of mothers (excreting Hg for 60 months) and children (absorbing Hg) over the 36 months is illustrated in Fig. 1: there was no statistically significant association between HHg and length of lactation, either for mothers or for children.

Table 2 summarizes HHg concentrations of children and mothers during the study period. The higher mean HHg at 6 months coincided with both Hg exposure from breast milk and the heavy vaccine schedule. Indeed, the distribution of HHg in infants followed a different pattern of their mothers (Fig. 2). After 6 months, exclusively breastfed infants receiving a full load of immunization (five types of vaccine, 150 μg Hg) showed an expansion of HHg concentrations greater than HHg at birth and at 3 and 5 years of age. HHg in their respective mothers showed the highest concentrations at childbirth, but a similar pattern at 6 months and 3 years later. Frequency of fish consumption (0–1 servings/week vs. compared to 2–7 servings/week) was presented and discussed previously (Marques et al.,

Table 1
Children immunization schedule, type of vaccines, and corresponding EtHg intake (as Hg) during the first 60 months

Age (months)[a]	Vaccine			Breast milk[c]
	% coverage	Type[b]	μg Hg/dose	μg Hg/day
0	100	Hp-B	25.0	0
1	100	Hp-B	25.0	30.83
2	100	DTP	25.0	44.65
3	100	—	—	—
4	100	DTP	25.0	91.80
5	100	—	—	—
6	100	DTP + Hp-B	50.0	111.90
Total Hg injected			150.0	
8	2.4	Hib	25.0	NE
10	8.5	Hib	25.0	NE
12	8.5	Hib + Flu	50.0	NE
15	25.5	DTP	25	NE
24	10	Flu	12.5	NE
36	5.0	Flu	12.5	NE
48	2.4	Flu	25	NE

NE, not estimated.

[a] All 82 infants had a full immunization schedule during the first 6 months; the fourth dose of DTP was taken after the first year only by 25.5%. The Hib and influenza vaccines taken after 6 months were at the recommended age by the specified percentage of the children; the age of immunization after 6 months are approximations based on current recommendations.

[b] Hp-B: Hepatitis B (0.01% thimerosal/dose, Korea Green Cross Corporation, Kiheung-Eup Yougin-Goon Kiyunggi-Do, Korea; Euvax B injectable, 0.01% thimerosal [LG Life Sciences, Jeonbuk-do, Korea]); DTP (Serum Institute of India Ltd; Vacina Tríplice, 0.01% thimerosal/dose [Instituto Butanta, São Paulo, Brazil]); Hib: Haemophilus influenzae type b (HibTITER®, 0.01% thimerosal/dose, Lederle-Praxis); Flu: influenza (VAXIGRIP 0.01% thimerosal/dose, Pasteur Mérieux Connaught, Sao Paulo, Brazil). Children that were immunized against *Haemophilus influenzae* type b (Hib) within the first year (two doses) received the booster dose 6–12 months after the last dose, otherwise received only one dose in the second year of life. The recommendation for the anti-flu vaccine is to be taken between the ages of 6 and 35 months in two doses (1 month apart) of 0.25 mL and one single dose (0.5 mL) the following year; although the vaccines were taken the ages given represent approximations of the recommended date.

[c] Integrated total Hg intake adapted from Marques et al. (2007a); we used the data of breast milk-Hg concentrations (adapted from Dorea (2004)) to estimate Hg exposure during breastfeeding: infant mean weight × mean daily breast milk consumption (140 mL/kg) × number of days × mean total Hg concentration in breast milk (1.9 μg/L).

2007a); in these urban mothers fish consumption depended on the species available at market (season and price are strong determinants).

Fig. 3 can be interpreted as difference in Hg transfer rates between placenta and mammary gland. The profile of ratios of mother:infant HHg clearly indicate that mothers' decreases while infants' increases. However, the association between maternal and infant HHg (Spearman's r) illustrated in Fig. 4 was significant at all times: statistical significant association between maternal and infant HHg concentration was seen at birth ($r = 0.3534$; $P = 0.001$), 6 months ($r = 0.4793$; $P < 0.0001$); Spearman's r values at 3 years ($r = 0.0122$; $P = 0.012$) and 5 years ($r = 0.0357$; $P = 0.005$) although significant were low and indicative of less perfect correlations. Indeed, sampling time was sta-

Table 2
Mean hair-Hg concentrations (SD) in children and respective mothers during the first 5 years

	Children	Mothers
N	82	82
Hair [Hg] μg/g, birth	2.4 (3.0)	7.4 (8.7)
Hair [Hg] μg/g, 6 months	3.8 (5.5)	3.2 (3.8)
Hair [Hg] μg/g, 36 months	2.6 (3.7)	2.8 (3.2)
Hair [Hg] μg/g, 60 months	2.6 (3.5)	2.6 (3.0)

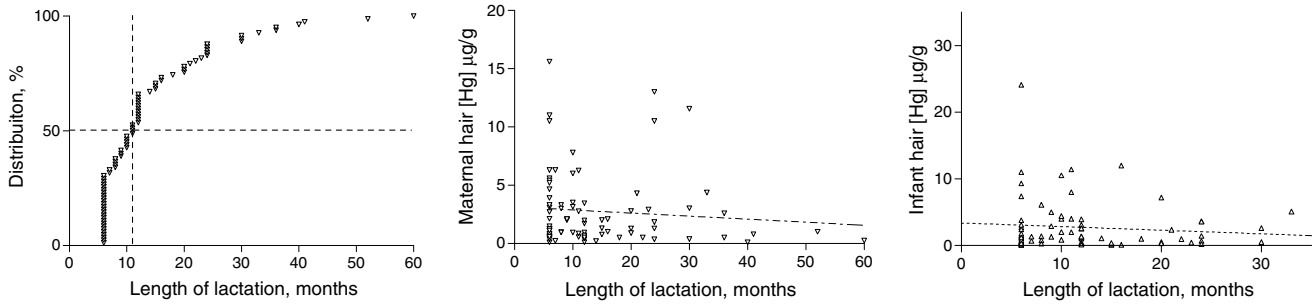

Fig. 1. Length of lactation and its impact on hair-Hg concentrations of mothers and infants.

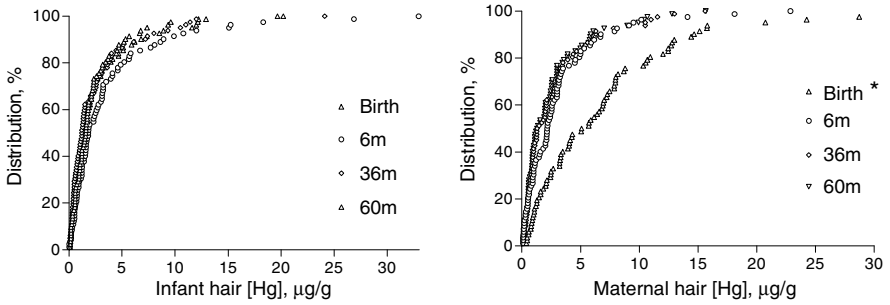

Fig. 2. Frequency distribution of maternal and infant hair-Hg concentrations at specified sampling times; maternal outlier: * = 62 µg Hg/g.

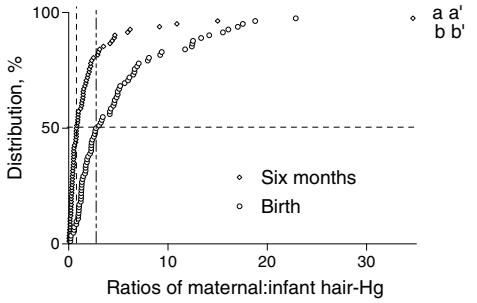

Fig. 3. Frequency distribution of maternal:infant hair-Hg ratios and outliers: $a = 52$, $a' = 158$; $b = 140$, $b' = 151$.

tistically significant for mothers (<0.0001) but not for children (0.0987).

The distribution of HHg in infants followed a pattern different from their mothers. While mothers had the highest concentrations of HHg at birth, breastfed children experience the highest HHg concentrations after the heaviest schedule (six shots) of immunization with TCV. Extended lactation (>36 months) was not significantly associated with changes in HHg of infants or mothers; both maternal and children HHg followed a downward trend.

4. Discussion

The profiles of distribution of maternal and infant HHg concentrations during the first 5 years revealed differences in type of Hg exposure. The chronology of infant hair sampling matched events associated with diet (that included breastfeeding) and immunization schedules. We found a clear trend in infant HHg coinciding with vaccine schedule but cannot prove a cause–effect relationship. The intercurrence of TCV-EtHg and the transitional weaning diets may have contributed to the pattern of infant HHg changes. Different cumulative distribution of HHg (mother:infant) ratios extracted information in response to short-term changes in source and dose of Hg exposure: the effects of the short-term but heavy schedule of immunization with TCV were likely modifying factors.

Based on HHg concentrations, the urban mothers in this study had a much lower consumption of fish than Amazonian "*ribeirinho*" women; we reported before that 57/82 of these mothers ate at most one fish meal a week showed a lower mean hair-Hg concentration than mothers that consumed more than 2 times a week (Marques et al., in press). HHg concentrations were lower than those observed for

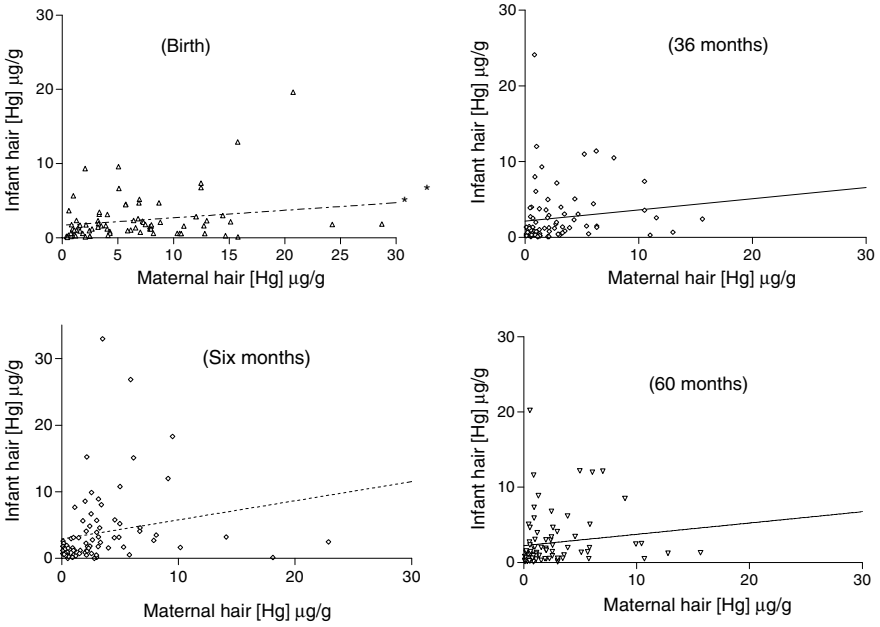

Fig. 4. Scatter plot of correlation of infant and maternal hair-Hg concentrations at specified sampling.

"*ribeirinho*" women of the Rio Madeira in 1991 by Barbosa et al. (1998). Mean HHg concentrations of studies in fish-eating Amazonians have been reported to vary between 6.5 and 34.2 ppm (Barbosa et al., 2001); however, adjusting for inherent HHg variability or long-term studies of HHg changes (within individuals) are rare. Because of HHg variability, the effects of pregnancy and lactation may not impact the mean HHg deposition in Amazonian high fish eaters (Barbosa et al., 2001) but the relative changes (percent adjusted) within mothers show that it decreases during pregnancy (Barbosa et al., 1998); thus, in agreement with the present findings. The percent decline in maternal HHg concentrations observed in this and previous study (Barbosa et al., 1998) may reflect the metabolic changes associated with lactation which includes transfer of Hg to milk.

Events related to pregnancy and lactation accelerate maternal-Hg metabolism (Greenwood et al., 1978), but Hg transfer rates *in utero* (pregnancy) and *ex utero* (breast-feeding) differ depending on the organic (MeHg) or inorganic Hg form (Dorea, 2004). As discussed elsewhere (Dorea, 2004) both Hg forms are equally transferred through milk but MeHg is more readily transferred across the placenta than inorganic Hg. Indeed, inorganic Hg absorption through milk is not a significant source of Hg exposure to breastfed infants (Sandborgh-Englund et al., 2001). But, compared to inorganic Hg, Bjornberg et al. (2005) reported that MeHg seems to contribute more to infant exposure via breast milk; additionally, total Hg in breast milk decreased significantly from day 4 to 6 weeks, remaining unchanged thereafter (Bjornberg et al., 2005). Studies of lactating mothers (after the accidental poisoning in Iraq) showed that the milk-Hg was 5% of blood-Hg but the organic fraction of milk-Hg was only 3% of blood-MeHg (Bakir et al., 1973); this indicates that the placenta plays a greater role in Hg transfer than the mammary gland (Bjornberg et al., 2005). The HHg ratios clearly showed differences in Hg transfer rates between pregnancy and lactation (Fig. 3).

In the present study, the length of breastfeeding does not seem to be a significant determinant of HHg concentrations of mothers or infants, thus showing that the mammary gland is a more effective barrier for Hg transfer than the placenta. Indeed, we had showed that during lactation mothers increased HHg to pre-pregnancy levels (Barbosa et al., 1998). Studies relating length of breastfeeding with HHg concentrations of mothers and infants are rare and results of correlation analysis are not always concurrent. There are differences between populations: infants' HHg and duration of breastfeeding were significant correlated in the Amer-Indians (of eastern Amazonia) but not in the "*ribeirinhos*" of Rio Madeira studied by Barbosa et al. (1998).

The Hg found in hair of breastfed infants comes from both exogenous (breast milk) and endogenous (metabolic turnover of fetal tissues) sources; because of postnatal hair change and growth, the contribution of residual fetal tissue may become negligible at a certain age: nonetheless, HHg in breastfed infants (if immunized with thimerosal-free vaccines) originates from maternal sources. This close association between maternal and children HHg remains longer after breastfeeding: at weaning and at 5 years (Fig. 2). Irrespective of the level of HHg the mother–infant dyad remains in very close association; this indicates that dietary characteristics (type of fish or frequency of consumption) are strong within families (Fig. 2). These observations are in agreement with results obtained with high fish-eating population living at the banks of the Rio Madeira. Barbosa et al. (1998) reported a statistically significant correlation between "*ribeirinho*" mothers and breastfed infants' HHg of older age. This significant correlation was reported by some (in Madrid—Gonzalez et al., 1985) but not by others (Fujita and Takabatake, 1977). Nevertheless, our finding of significant association between maternal and fetal hair is in agreement with studies in other parts of Amazonia (Mohan et al., 2005) and in low fish-eating mothers of other countries (Sikorski et al., 1986; Lindow et al., 2003).

The mean HHg values of our breastfed children are lower than the means reported for 1-year-olds of several Eastern Amazonian communities of the Rio Tapajós (Pinheiro et al., 2007). Although there are no studies of infant HHg (due to breastfeeding and TCV) our mean values (Table 2) are within HHg concentrations predicted from Redwood et al. (2001) TCVl-exposure model. All infants in our study received a substantial load of parenteral EtHg concentrated in the first 6 months; only a small percentage (2.5–25%) of children received an equivalent load (175 μg Hg) distributed over 42 months at a much older age (12–48 months) and larger body weight. The profile of the mother:infant HHg ratios indicates that the breastfed infants' hair retains more Hg than the fetus (Fig. 4); however, since we do not have speciation of Hg chemical forms (EtHg or MeHg), it can not be solely attributed to TCV. Our findings suggest that the significant association between mothers' and infants' HHg at all sampling times reflect the family environmental exposure through fish-Hg.

Neonate metabolic pathways and attendant excretion rates of Hg vary according to stage of development mainly because of differences in gut development and bile secretion as well as source of Hg exposure. The wider distribution (greater variability) of infant HHg at 6 months can be attributed to the fact that in addition to Hg from breast milk, HHg values can be influenced by the EtHg breakdown from TCV, thus increasing organic Hg exposure. Predictive models of hair-Hg concentrations in infants have assumed low or no Hg excretion up to 6 months (Redwood et al., 2001). Therefore, positive Hg balance in infants contrasting with negative balance of mothers can be interpreted from Fig. 4. Nevertheless, additional research is necessary to understand the relationship between infant early development (weight gain and tissue differentiation), and differences in the metabolism of Hg from such distinct sources—Hg intrinsic to breast milk and parenteral EtHg from TCV. The physiological barriers that breast-milk Hg has to cross are bypassed by parenteral-administered

thimerosal-EtHg. This particular aspect of HHg change was discussed in the parent publication (Marques et al., 2007a).

After exclusive (up 6 months) and extended breastfeeding (36 months), infant mean HHg values declined toward the maternal values; this reflects both, decreased Hg exposure from weaning foods and a greatly reduced frequency of TCV-EtHg exposure (Table 1). Overall, the decrease in infant HHg during weaning was relatively less compared to maternal HHg; but the mean HHg of infants at 5 years is equivalent to maternal HHg, thus possibly reflecting exposure to the same dietary Hg sources. Since infants were exposed to higher doses of EtHg during the first 6 months, infant's increase in HHg may result from higher transfer rates of EtHg-to-hair compared to subsequent sampling periods. HHg speciation that could differentiate MeHg from EtHg would be necessary to ascertain the origin of total HHg.

Fluctuation in maternal HHg concentrations has been an unverified event that might occur as a result of altered metabolism during pregnancy. Because the epidemiological evidence suggests a causal link between exposure to fish-Hg and NDD, avoidance of fish consumption during pregnancy and lactation has been warranted for NDD prevention (Ronchetti et al., 2006). The Harvard Center for Risk Analysis panel used maternal HHg to quantify the impact of pre-natal MeHg chronic exposure on infant cognitive development; the panel concluded that MeHg exposure sufficient to increase maternal HHg by 1 μg/g decreases intelligence quotient by 0.7 points (Cohen et al., 2005). Therefore, fluctuation in maternal HHg is an important issue with implications for public health policies.

The strengths of this study include its prospective design and the high rate of retention of mother–infant pairs at key chronological sampling: birth (fetal exposure), 6 months of exclusive breastfeeding, 36 months (weaning) and 60 months (pre-school); additionally we identified input of other organic Hg sources. However, our limitation in analytical capability to determine the TCV-EtHg in hair is an important constraint in interpreting these aspects of the results. A common problem faced by Hg toxic effects or susceptibility at sub-clinical levels is the difficulty of accurately reconstructing responses to sources after the exposure event. This is especially the case with young infants and accompanying changes in diets intertwined with attendant sources of Hg (which might include extrinsic EtHg) such as in the present study; additionally, there are ethical considerations related to breastfeeding and immunization that limit group (without such life-saving features) comparisons, thus making exposure studies uniquely difficult.

5. Conclusion

Unverified changes in maternal and infant HHg are important issues relating this marker to studies of exposure, effects and susceptibility. Maternal postpartum metabolic changes, infant development and transitional diets and possibly Hg from TCV contribute to the asymmetry of HHg changes between mothers and children.

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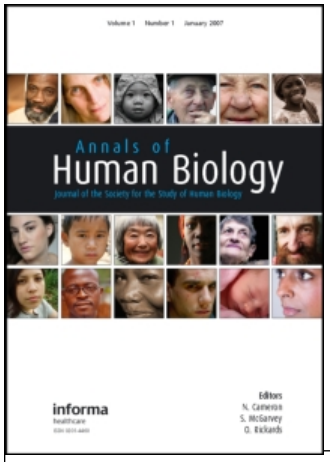

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Maternal fish consumption in the nutrition transition of the Amazon Basin: Growth of exclusively breastfed infants during the first 5 years

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ORIGINAL ARTICLE

Maternal fish consumption in the nutrition transition of the Amazon Basin: Growth of exclusively breastfed infants during the first 5 years

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Abstract

Background: Changes in fish-eating habits due to rapid urbanization in Western Amazon was used as model to investigate whether maternal fish-intake rate impacts on children's weight and height during the first 5 years.

Aim: The study examined the growth of 82 breastfed children, and maternal fish consumption (hair mercury concentrations, HHg) during pregnancy and lactation.

Subjects and methods: Fish consumption in mothers and children was estimated through HHg. The children were measured and weighed at birth and at 6 (exclusive breastfeeding), 36 and 60 months.

Results: Fish consumption rate (HHg) had no significant impact on children's growth at the specified ages ($p = 0.35$). After 6 months of exclusive breastfeeding, children had the highest proportion of *Z*-scores <-1 SD; however, weaning (with extended breastfeeding) had a substantial impact in moving up the attained growth at 3 years. The duration of breastfeeding was significantly correlated with attained *Z*-scores for weight-for-age ($r = 0.26$; $p = 0.02$) and weight-for-height ($r = 0.22$; $p = 0.04$) but not for height-for-age. At 3 years most children had improved *Z*-scores (>-1 SD) for height-for-age (70/82), weight-for-age (74/82) and weight-for-height (74/82). At 5 years, all but one child attained *Z*-scores >-1.

Conclusion: The apparently good nutritional status of subjects is more likely due to a well balanced diet composition than to only one dietary protein source – fish.

Keywords: *Nutrition transition, Amazon, growth, breastfeeding, fish*

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Introduction

The population that inhabits the Amazon Basin shows a wide diversity: Currently, native peoples living traditional lifestyles (in dense forests) contrast with a new urbanization profile. Piperata (2007) has recently described differences between traditional lifestyles and those found among the new Amazonian populations. The more urbanized environment caused by recent Amazonian growth is exemplified in the city of Porto Velho. Gradually replacing riverside communities, the new inhabitants no longer depend on the traditional dominance of fish as the source of animal protein, but instead use markets for their food supply. This fast urbanization has changed the traditional food supply chain and, as a consequence, the typical diet has shifted away from high fish consumption (Dorea 2004) to dependency on industrial or mass processed foods (Piperata 2007). The key challenge in studies of nutrition transition is the ability to characterize which dietary item has substantially changed and its impact on health.

Fish is an abundant natural resource in Amazonian Rivers that is well utilized by riverside populations (Dufour 1991); in traditional communities its consumption is high and this tends to increase the farther these communities are from urban centres (Alves et al. 2006). In the context of traditional riverine diet, the high protein content of fish balances high starchy food consumption (Dorea 2004). Fish are a good source of sulphur amino acids and bioavailable iodine, both crucial to counterbalance cassava goitrogens and low iodine foods produced in iodine-depleted soils of tropical rain forests (Dorea 2004). Indeed, dietary fish enhances absorption of zinc (Garcia-Arias et al. 1993) and iron from plant foods (Layrisse et al. 1990; Gibson and Hotz 2001); dietary fish has been positively associated with women's iodine status (Zollner et al. 2001) and children's ferritin stores (Michaelsen et al. 1995; Gunnarsson et al. 2007). Additionally, Amazonian fish is a good source of selenium (Dorea et al. 1998), known to counteract the toxic effects of Hg. Other essential nutrients, such as zinc, are significantly higher in protein foods like fish when compared to food with low protein content (Terres et al. 2001).

As a specific source of omega-3 polyunsaturated fatty acids (Inhamuns and Bueno Franco 2001), fish is nutritionally important for people living on cassava-dominant diets, especially during breastfeeding (Rocquelin et al. 1998). Tanzanian women with high intakes of freshwater fish (as the only animal lipid source) had milk arachidonic acid (AA) and docosahexaenoic acid (DHA) contents that were well above present recommendations for infant formulae (Muskiet et al. 2006); such findings inspired Muskiet et al. to hypothesize that the 'optimal homeostasis' of AA and DHA is lacking in Western diets and is causing subtle signs of unbalanced maternal glucose homeostasis. Indeed, breast-milk DHA of women from the marine region was higher compared to other regions and twice as high as any reported previously; it was comparable to the amounts found in the milk of women fed fish oil (Ruan et al. 1995).

Fish protein has good biological value (Sikka et al. 1979) that is well utilized by children (Hofvander 1973); porridge containing dried fish has been successfully used to treat undernourished children (Greco et al. 2006). It is not surprising that *per se*, addition of fish powder supported growth just as well as a nutritional supplement fortified with vitamins and minerals (Lartey et al. 1999). The effects of maternal fish consumption on infant growth may start early during pregnancy; Olsen et al. (1993) noticed that the weight and length of the newborn increased with the frequency of seafood meals consumed in pregnancy. After reporting that infant size at birth increased with fish consumption, Thorsdottir et al. (2004) hypothesized that constituents of fish and fish oil might affect birth size. Indeed, increasing fish intake during pregnancy might increase foetal growth rate (Rogers et al. 2004) and cognitive functions (Hibbeln et al. 2007) of British infants.

Fish are complex nutrient-dense foods with exceptional functional characteristics: Increasing maternal fish intake during pregnancy from once a week to 2.5 times per week decreased the risk of eczema at age 1 year by 37%, and the risk of positive skin prick test at age 6 years by 35% (Romieu et al. 2007). Furthermore, regular fish consumption in infancy (before age 1) is associated with a reduced risk of allergic disease and sensitization to food and inhalant allergens during the first 4 years of life (Kull et al. 2006).

One underappreciated problem in dietary surveys is error in recalling meal size. This has been documented in several settings where portions and cultural standards could be favourable; Nelson et al. (1996) showed that small portion sizes tended to be overestimated, and large portion sizes underestimated. That is the reason many studies use intake biomarkers to evaluate the extent of protein misreporting (Kipnis et al. 2002; Subar et al. 2003). Using hair mercury (HHg) we accomplish reduction of intraindividual and interindividual variability; this is particularly important in longitudinal studies subjected to seasonal variations in urban socio-economically limited populations. These variations are amply documented in the epidemiologic literature (Wu et al. 1986). The methylmercury (MeHg) consumed in fish is easily absorbed, binds to protein sulphydril groups, and accumulates preferentially in human hair; the proportion of blood to HHg is estimated at 250 times (Clarkson 2002). This particular characteristic of hair, coupled with its easy collection and preservation, makes HHg a first-choice marker of fish consumption in Amazonian populations (Barbosa et al. 1997, 1998; Dorea et al. 2003); the attendant changes in fish consumption rates for native Amer-Indians have been successfully assessed by HHg determination (Dorea et al. 2005a,b). Mean daily fish consumption rates of subsistence communities from the Rio Tapajós have shown a variation of 115–171 g day^{-1} (Passos et al. 2007); this is in close agreement with 170 g day^{-1} derived from HHg concentrations of Rio Negro mothers (Dorea et al. 2003). It should be noticed that that carnivorous fishes are more likely to have higher mercury content than non-carnivorous fishes (Dorea 2003).

The importance of adequately supplying protein during pregnancy and lactation to ensure full reproductive success is well recognized. Traditionally Amazonians have relied heavily on fish to complement their starch-based diet (Giugliano et al. 1978). Population studies dealing with nutrition transition in the Amazon are rare, and the most recent one has focused on nutritional outcomes (Piperata 2007). In a previous publication we reported mothers as moderate or high fish consumers (Marques et al. 2007); however, our present construct is not typical of a nutrition survey. This study was created to test a selected aspect (shift of fish consumption) in the nutrition transition in the Amazon Basin and its impact on growth of breastfed children.

Materials and methods

Background

The nutrition transition of Porto Velho is typical of many conurbations along the most remote Brazilian frontier. Agricultural projects and a gold rush have changed the face of this Rio Madeira city in the last 30 years, attracting people from different parts of the country. Incomers brought different food habits, and rapid urbanization accompanied social and economic trends that impacted on fish consumption. During the last 30 years the city of Porto Velho, capital of the state of Rondonia (Western Amazonia), has experienced significant demographic changes; as a result of gold prospecting and agricultural development it has changed its traditional Amazonian characteristics. With people coming

from many other Brazilian regions, the present population has both traditional families that base their diets on fish and starchy foods and city dwellers with more cosmopolitan food habits. In this changing environment, we investigated the growth of a sample of urban breastfed infants with special reference to family fish consumption.

The study protocol was approved by the Ethics Committee of Studies for Humans of the Universidade Federal de Rondonia. During routine visits to the pre-natal clinics of three hospitals in Porto Velho pregnant mothers between the ages of 15 and 45 years were initially recruited; the mothers were selected among those in good health, reporting no illness or complaints at the time of the study and who were willing to breastfeed. Excluding factors were occupational exposure to toxic chemicals and hereditary neurological illnesses. Written consent (stating that participation was voluntary and confidentiality was assured) was signed by the participant mother, who could withdraw from the study at any time.

This is part of an ongoing cohort study conceived to study (a) fish Hg exposure of urban Amazonian mothers; (b) to associate maternal tissue Hg with factors relevant to neuro-motor development of breastfed infants; and (c) to examine the association between generated dimensions of infant neurodevelopment and maternal socio-economic and Hg exposure features. A component of the study focusing on the 6-month-old infants has already been published (Marques et al. 2007). Briefly, recruitment of 100 mothers began in 2000; detailed information concerning diet and anthropometry (and infant growth and development) was completed for 82 mother–infant pairs. For each mother a complete clinical evaluation was obtained from medical records. The newborns were clinically examined with special attention to vitality, perinatal reflexes, maturity, and congenital malformations; weight, length, head circumference, and Apgar scores were recorded.

Data collection

We recorded the maternal diet only during the first post-selection visit; this constitutes an assessment of the principal fish consumption characteristics at the time of the study. Participants were interviewed about diet; a questionnaire that could identify frequency and preference of fish was completed. Eighty-two women carried out their intention of breastfeeding exclusively for 6 months, and some extended lactation to 36 months. Exclusive breastfeeding was supported throughout the first 6 months and defined according to the WHO: 'the infant received breast milk only and allowed supplementation of drops and syrups such as vitamins, minerals, and medicines'.

At birth and at ages of 6, 36 and 60 months anthropometric measurements of mothers and infants were taken; weight and height were measured by trained nurses and monitored regularly according to standard procedures. Weight (kg) and height (cm) of mothers were measured at each observation and were used to calculate body mass index (BMI, weight/height^2). Length of newborn babies and of 6-month-old infants was measured in recumbent position with a 0.1 cm stadiometer. At 36 months and 60 months the children were measured and weighed barefoot and dressed in underwear only; standing height was measured to the nearest 0.1 cm and weight was measured to the nearest 0.1 kg with an electronic scale.

Both recumbent length (at birth and 6 months) and standing height (at 36 months and 60 months) were measured for all children. At the same time as recording anthropometry, data were collected on feeding practices, child morbidity, perinatal factors, and socio-economic, demographic and environmental characteristics. *Z*-scores for attained weight-for-age, length-for-age, BMI-for-age and weight-for-length were based on the WHO Child Growth Standards (WHO Multicentre Growth Reference Study Group 2006).

Therefore, the weight-for-height *Z*-scores (WHZ) were calculated using EPI-INFO (version 4.1; Centers for Disease Control and Prevention, Atlanta) and the WHO recommended growth curves. Infant growth rate up to 6 months was estimated as weight gain percentage (IWG%) after Xiong et al. (2007): (infant weight – birth weight)/birth weight × 100. At the specified age, we also collected samples of hair from mothers and respective children during the anthropometric measurements.

Hair Hg determination

Hair sample preparation and analytical procedures were carried out according to our standardized laboratory procedure for Hg determination (Marques et al. 2007). Briefly, the hair samples were comminuted with stainless steel scissors, weighed, and digested before analysis. Human hair samples were washed with EDTA 0.01%, dried in an oven at 50°C and weighed. Samples were then digested using a digestion block at 80°C for 40 min with concentrated HNO_3 (3 mL) and $KMnO_4$ (5%; 6 mL) in a microwave oven system for 35 min (CEM-Coorporation, MDS 2000, Matthews, NC, USA). The determination of total Hg in the digested samples was done by cold vapour atomic absorption spectrometry with a flow injection system FIMS (CV-AAS, Perkin-Elmer, FIMS 400, Ueberlingen, Germany).

All glassware used in the analytical protocol was washed clean, rinsed with 5% EDTA and double distilled, and left to rest in 5% HNO_3 overnight. Then it was rinsed again in double distilled water, and dried at 100°C for 12 h. Precision and accuracy of Hg determinations were assured by the use of internal standards, use of triplicate analyses of samples and certified reference materials (IAEA-085 and 086, Vienna, Austria) with recoveries of 92%. The limit of detection for the procedure was determined at $<0.01\ \mu g\ g^{-1}$ and there was no HHg determination below the limits.

Statistical analysis

The statistical packages, contained in Excel and Prism, were used for data summarization (means, standard deviation, changes in variables) and correlation analysis. The Shapiro–Wilk test of normality was applied and data transformed when required. Statistical analysis to test children's attained growth at 0, 6, 36 and 60 m was carried out using the Statistica (StatSoft, Inc., v.6.0) statistical package. During data analysis, children were classified into two groups on the basis of reported maternal fish consumption (Marques et al. 2007). The General Linear Model of Statistica used a factorial design with repeated measured factors. Pearson's (p) correlation was also used to examine the strength of association between the fish consumption marker (HHg) and anthropometric measures. A value of <0.05 was accepted as statistically significant.

Results

A previous publication has already partially discussed results of maternal fish consumption and infant neurodevelopment at 6 months (Marques et al. 2007); the influence of maternal fish consumption rates on mean HHg concentrations was not statistically significant (Table I). These contemporary urban mothers purchased fish from open markets and food stores.

Only 34 of them showed explicit fish species preference: *Pacu* (*Mylossoma* spp.; 58%), *Tambaqui* (*Colossoma* spp.; 35%), *Jaraqui* (*Semaprochilodus* spp.) and *Jatuarana* (*Brycon* spp.) (33%), *Dourado* (*Brachyplatystoma flavicans*; 30%), *Tucunaré* (*Cichla* spp.) and *Curimatã* (*Prochilodus nigricans*) (28%); other species <30% (*Filhote – Brachyplatystoma filamentosum; Piranha - Serrasalmus* spp., and *Surubim – Pseudoplatystoma* spp.).

Both infant HHg at birth and at 6 months represent Hg transferred through placenta and breast milk, whereas in children at 5 years, HHg concentrations are the result of exposure to fish in the family diet; only during the weaning period or extended lactation are children exposed to both sources, breast milk and family diet. Only at birth and 6 months did HHg concentrations substantially increase in both mothers and infants. Attained *Z*-scores summarized in Table II showed no statistically significant ($p = 0.36$) effect of fish consumption rate (repeated measurement analysis). Also, no statistically significant correlation was noticed between HHg and respective attained *Z*-scores (Figure 1).

Infant characteristics at birth as a function of frequency of maternal fish consumption rates shown in Table III were adapted from a previous publication (Marques et al. 2007). Mean birth weights (3.21 vs 3.29 kg), as well as other variables, were nearly identical. The summary of attained growth and length/height (*Z*-scores) as a function of fish consumption

Table I. Maternal and infant HHg (mean ± SD; values in parentheses are SD) concentrations ($\mu g\,g^{-1}$) as a frequency of fish consumption of mothers.

Fish servings per week	*n*	Birth		6 months		3 years		5 years	
		Mother	Infant	Mother	Infant	Mother	Infant	Mother	Infant
0–1	49	6.02	1.97	2.61	3.09	2.39	2.53	2.23	2.50
		(5.54)	(2.31)	(2.48)	(4.56)	(2.59)	(4.16)	(2.43)	(3.81)
≥2	33	9.35	3.14	4.06	4.96	3.39	2.63	3.10	2.84
		(11.8)	(3.80)	(5.09)	(6.67)	(3.92)	(2.96)	(3.79)	(3.12)
p		0.09	0.08	0.09	0.13	0.21	0.91	0.21	0.66

Table II. Summary (means) of attained growth and length/height (*Z*-scores) as a function of maternal fish consumption; no statistically significant difference between groups ($p = 0.36$).

	Fish servings, groups			
	0–1 per week ($n = 49$)		≥2 per week ($n = 33$)	
	Mean	SD	Mean	SD
Weight-for-age$_{\text{Birth}}$	−0.23673	0.93	−0.02576	0.93
Weight-for-age$_{6\ \text{months}}$	−0.87061	0.69	−0.61364	0.62
Weight-for-age$_{36\ \text{months}}$	0.50041	0.98	0.64061	0.94
Weight-for-age$_{60\ \text{months}}$	0.35653	0.46	0.27636	0.59
Length-for-age$_{\text{Birth}}$	0.20755	0.91	0.20758	1.08
Height-for-age$_{6\ \text{months}}$	0.31224	1.30	0.46909	1.37
Height-for-age$_{36\ \text{months}}$	0.10612	0.97	0.27606	0.91
Height-for-age$_{60\ \text{months}}$	−0.69245	0.65	−0.52788	0.62
Weight-for-length$_{\text{Birth}}$	−0.57	1.15	−0.23	1.15
Weight-for-height$_{6\ \text{months}}$	−1.31	1.17	−1.07	1.31
Weight-for-height$_{36\ \text{months}}$	0.63	1.24	0.70	0.95
Weight-for-height$_{60\ \text{months}}$	1.15	0.74	0.87	0.76

is shown in Table II and the profiles of attained *Z*-scores are shown in Figure 2. The cumulative differences between linear and ponderal *Z*-score outcomes are clear. At birth, most children showed adequate *Z*-scores (>−1) for length-for-age (74/82), weight-for-age (68/82) and weight-for-length (56/82). However, attained growth at 6 months showed remarkable differences; the infants grew much more in height than in weight. After 6 months of exclusive breastfeeding the proportion of infants with attained growth (*Z*-scores <−1) was 14/82, 36/82 and 52/82, respectively, for length-for-age, weight-for-age, and weight-for-length. Most children showed *Z*-scores (>−1) for height-for-age (70/82), weight-for-age (74/82) and weight-for-height (74/82). However, it should be noted that only one child had a *Z*-score <−2 for length-for-age during breastfeeding, but five showed stunting at 36 months. Malnutrition (weight-for-age <−2) was seen in only two breastfed infants. All but one child attained *Z*-scores <−1 at 60 months. Part of the nutritional outcome due to the quality of the weaning diets was due to extended lactation.

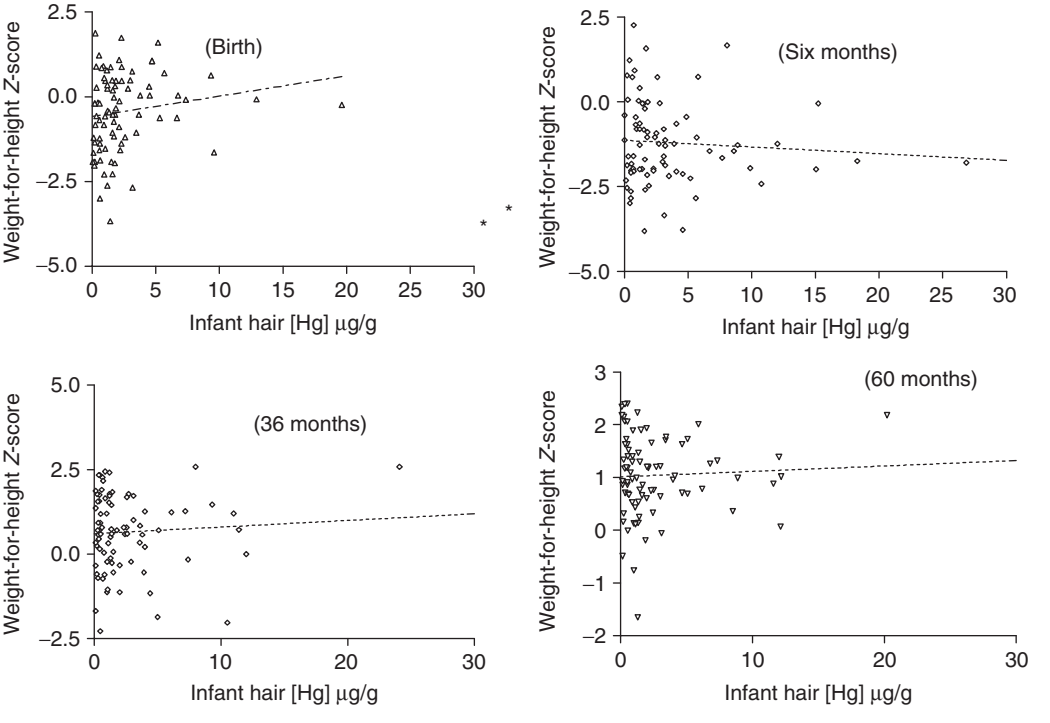

Figure 1. Association between infant HHg and *Z*-scores (weight-for-height) at 6, 36 and 60 months.

Table III. Maternal and infant characteristics (mean ± SD) as a function of reported frequency of fish consumption (adapted from Marques et al. 2007).

Fish servings per week	*n*	Gestation (weeks)	Length at birth (cm)	Infant weight at birth (kg)	Infant weight at 6 months (kg)	IWG%*
0–1	49	39.3 ± 1.3	50.0 ± 1.7	3.21 ± 0.4	6.97 ± 0.5	121 ± 3
≥2	33	39.4 ± 1.4	49.9 ± 1.9	3.29 ± 0.4	7.07 ± 0.5	119 ± 4
p		0.76	0.76	0.41	0.32	0.82

*IWG%: Infant weight gain at 6 months divided by birth weight.

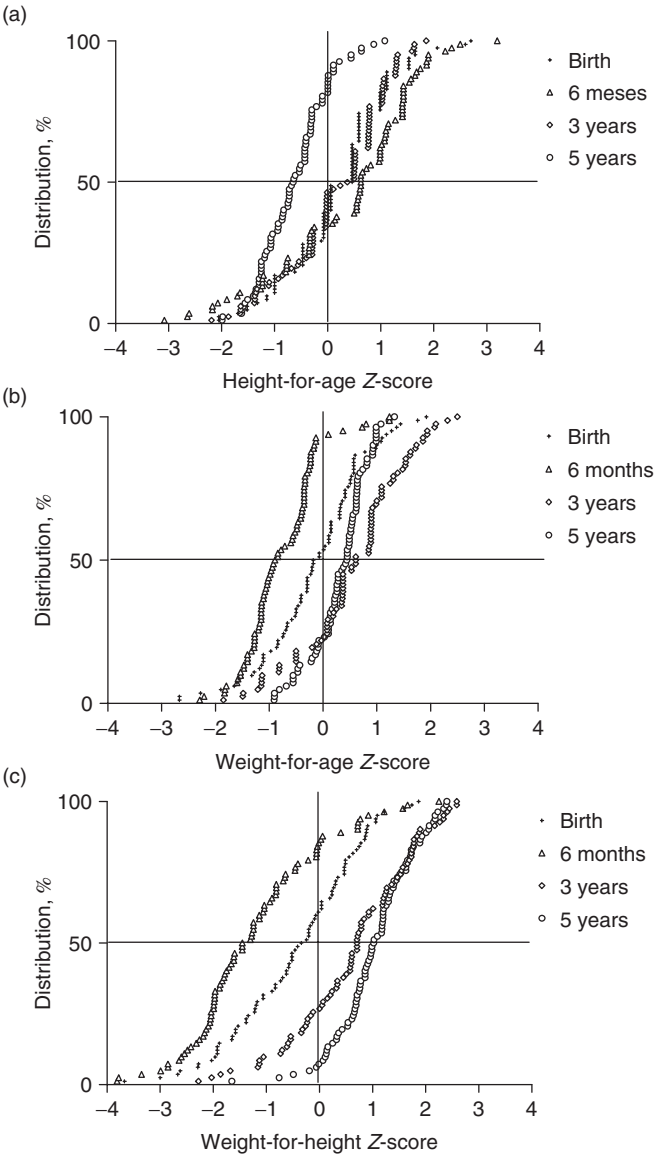

Figure 2. Cumulative frequency of *Z*-scores of attained growth during sampling times.

Exclusive breastfeeding for 6 months was accomplished by all mothers. After that, many mothers extended breastfeeding throughout the first year (Figure 3); 10 extended even further and there was one child still breastfeeding at 5 years. The duration of breastfeeding did seem to interact with the dietary patterns of weaning; there was significant correlation with attained *Z*-scores at 36 months for weight-for-age ($r = 0.26$; $p = 0.02$) and weight-for-length ($r = 0.22$; $p = 0.04$) but not for length-for-age (Figure 3). There was a wide spread in birth weight (range: 2.2–4.3 kg); however, exclusive breastfeeding substantially reduced (by 50%) the range of body mass at 6 months (6.1–8.5 kg). Indeed, the IWG% was inversely proportional to birth weight in a very high and significant correlation ($r = -0.88$; $p = 0.00$) illustrated in Figure 4.

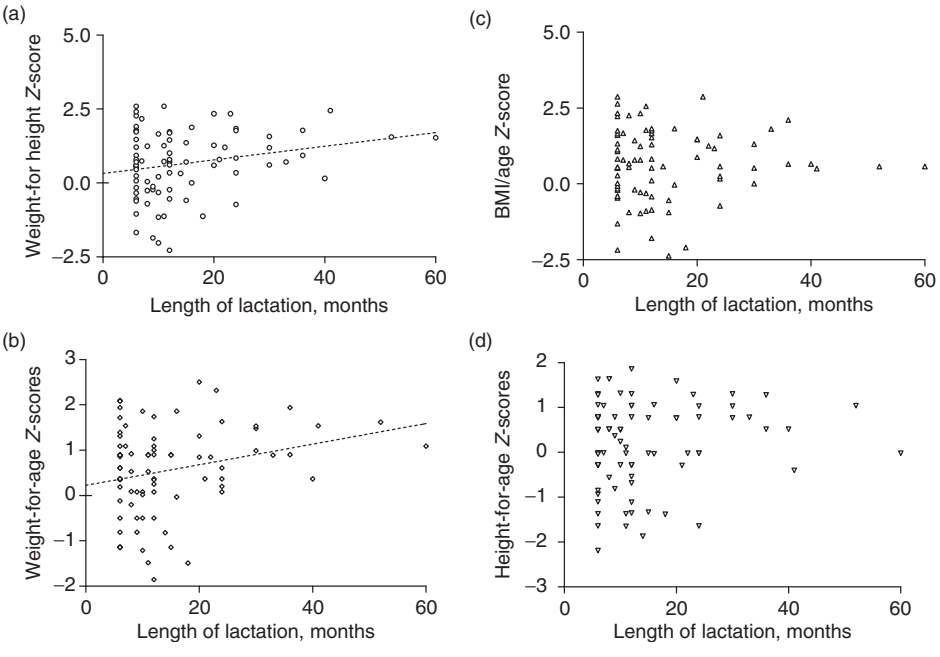

Figure 3. Length of lactation and attained growth.

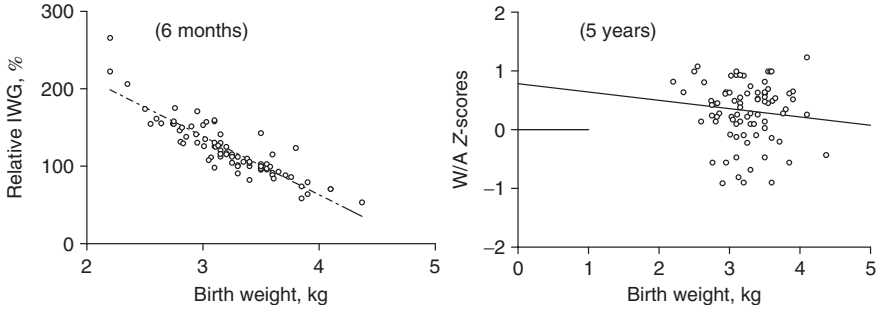

Figure 4. Relative infant weight gain (IWG% – infant weight gain divided by birth weight) at 6 months and weight-for-age *Z*-score (W/A) as a function of birth weight.

The possible taxing of mothers caused by milk demanded to sustain increased infant growth is illustrated in Figure 5; maternal BMI showed no significant association with breastfeeding duration. Mothers with higher BMI at the end of pregnancy had returned to pre-pregnancy levels at 6 months (exclusive breastfeeding) and 3 years (extended breastfeeding). Figure 5 illustrates that the BMI profile is indistinguishable between pre-pregnancy, 6 and 36 months post-natal.

Discussion

Quality of the diet for pregnant and lactating women is a key issue in public health nutrition, especially in poor communities with economic constraints. The mothers of Porto Velho,

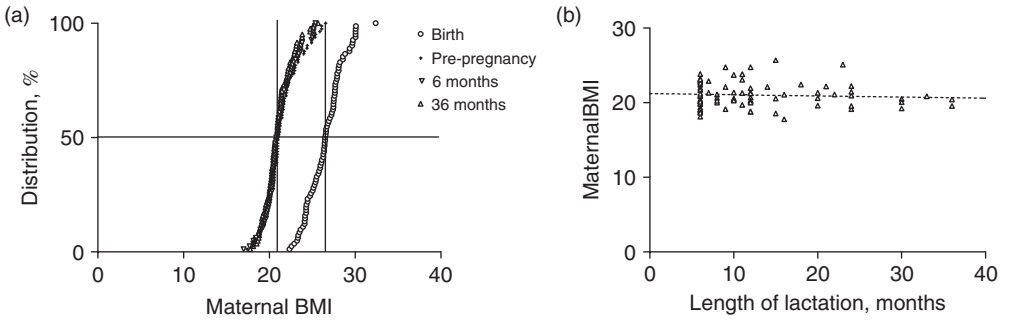

Figure 5. Maternal BMI (body mass index); (a) profile changes during the study; (b) scatter plot as a function of length of lactation.

with respect to levels of dietary fish consumption, showed no significant impact on the attained growth of their young children; this indicates that changes from traditional fish-dominated diet to other animal or vegetal-source food have occurred without consequences for infant development. By examining the longitudinal growth of their children it was revealed that mothers' milk, irrespective of level of fish consumption, could compensate for low birth-weights; furthermore, the length of lactation (beyond 6 months) was an important nutritional supplement measurable at 3 years (Figure 2).

Fish is a differentiator of the survival strategy of some Amazonian villages (Dorea et al. 2005b); it is consumed more in isolated communities (Alves et al. 2006). Early dietary studies of poor Amazonian families showed that they can consume 151 g fish per day comprising 37% of their dietary protein (Giugliano et al. 1978). HHg is a specific and reliable indicator of fish consumption that has been used to study Amazonian populations and successfully employed to quantify the amount of freshwater fish consumed (Dorea et al. 2003). Our study showed that in urban population, no longer following traditional food habits, HHg was higher (although not significantly) in the group reporting higher fish consumption rate. Therefore, HHg can be valuable to understand fish-MeHg exposure as well as to trace beneficial outcomes of fish consumption in Amazonians (Dorea et al. 2005a,b). However, because of concerns for neurodevelopmental disorders due to intrauterine exposure to MeHg, recommendations to reduce fish intake have been made (Cohen et al. 2005). For economically stressed families, especially in regions accustomed to high frequency of fish consumption, such recommendations may not take into account the effect that reducing fish consumption will have on the nutritional status of vulnerable infants and young children. Indeed, Arnold et al. (2005) have questioned whether it is worth curtailing fish Hg exposure because this can tax the consumption of an important source of nutrients; beneficial effects on foetal neurological outcome have been shown as a result of maternal fish consumption (Hibbeln et al. 2007). Additionally, animal-source foods have been less appreciated in studies of economically strained urban families in developing nations. We, therefore, took into consideration the changing habits arising from fast urbanization of an Amazonian city to assess the role of HHg (marker of fish consumption) and outcomes of exclusively breastfed-infant development.

We speculate that dominance of fish consumption, although important for some specific nutrients, was successfully replaced by other animal-food sources. The lack of significant association between HHg and WHZ (especially at 5 years) indicates that difference in protein quality (fish vs other sources) was not a determinant of attained growth in these children. This is in agreement with our studies in Amer-Indian children inhabiting the banks

of the Xingu-basin Rivers in Eastern Amazonia (Dorea et al. 2005a); in these subsistence-level communities of indigenous people a clear change in dominance of fish consumption between the tribes (Kayabi and Munduruku) was not associated with infant growth; other factors like infectious diseases, may influence the health of subjects.

Infant weight at birth (intrauterine growth rate) is directly influenced by maternal constitutional (size) and environmental factors (nutrition). Weight and linear growth of breastfed infants are influenced by human-milk output and nutritional profile (Fornes and Dorea 1995); although genetic factors may be significant, infant postnatal growth is also greatly influenced by illnesses. In the case of exclusive breastfeeding, weight gain is indirectly a result of breastfeeding protection against the nutritional consequences of illness; by decreasing the number of days with gastrointestinal and respiratory tract illnesses (Launer et al. 1990), infant food efficiency is optimized with growth maximization.

Infant growth is the key to assessing nutritional status; while cross-sectional growth data are useful to identify infants whose weight and length outcomes are considered substandard they are limited in assessing the dynamics of breastfeeding in support of the growing child. In this context monitoring infant growth to understand true growth potential is better when based on longitudinal data (Xiong et al. 2007). Several papers have drawn attention to the supposition that infants born with low birth weight may remain lighter and shorter (Xiong et al. 2007); however, in our study, breastfeeding showed a growth rate inversely proportional to birth weight. Overall, our results showed an accelerated rate of length gain in the first few months. These results are in agreement with others (Cole et al. 2002), showing that prolonged and exclusive breastfeeding accelerated weight and length gain in early weeks. Infants in our study showed an apparent increase in thinness at 3 years but no detectable deficit at 5 years.

The infants with lower birth weight – or small size – reflected poor intrauterine growth (or shorter gestational age); however, in the present study, they grew at a faster rate during exclusive breastfeeding. At 5 years, it was not the birth weight but the rate of change in body mass that modulated the attained growth. The very high linear correlation (Figure 4) between birth weight and IWG% indicates a catch-up process. We do not yet understand how the compensatory mechanism operates in breastfeeding to promote the faster move-up of centiles in the smaller babies. Considering that the breast-milk nutrients originated from the (same) maternal sources, higher rates of growth indicate more efficient transfer of nutrients (mother) or more efficient utilization (infant) or, most probably, both.

Differences in placental function (birth weight) and milk supply (postnatal growth) with regard to maternal nutrient transfer are not yet understood. Weaver (2006) has differentiated rapid weight-gain catch-up (after intrauterine restriction) and accelerated growth as a result of excessive energy intake in formula-fed infants. In our study, however, the self regulating (breastfeeding) milk supply does not seem to lead to an excessive energy intake; but the same mother, who apparently produced a smaller foetus, is capable of compensating post-natal infant growth through breast milk. After birth, the (active) infant is apparently more efficient in nutrient acquisition (sucking) and utilization than the (passive) foetus. Indeed, Karaolis-Danckert et al. (2006) recently reported that relatively smaller and lighter babies grow faster than their larger-at-birth counterparts; they appeared to diverge in growth patterns as early as 6 months. In their study only 30% of fast growers were breastfed for more than 4 months.

Rich in nutrients, hormones and complex non-nutritional factors, human milk is better suited to meet specific requirements for infant development; such a complexity of bioactive substances and neuro-stimulation factors modulates neonatal adaptation and provides protection and immunity; exclusive breastfeeding is also finely tuned to regulate infant

growth (Weaver 2006). In certain circumstances, infant growth is associated with breast-milk fat content (Fornes and Dorea 1995); however, in regard to birth weight, breast-milk's intrinsic nutritional-attributes are not sufficient to explain the inverse relationship of infant growth and birth weight.

In epidemiological studies the differences in attained growth between bottle- and breast-fed infants have been attributed to reverse causality (feeding choice influenced by child health/weight). Cole et al. (2002) discussed reverse causality in the context of feeding-mode differences: 'slow-growing infants who are 'falling off' their growth curve trajectories may be deliberately supplemented or weaned in an effort to reverse those trends'. Apparently, this is not the case in our study; our infants showed an inverse correlation (birth weight vs IWG%, Figure 4) and a direct association of extended breastfeeding (beyond 6 m) and attained *Z*-scores at 3 years (Figure 4). It is amply reported that in the developing world, absence of or insufficient breastfeeding or early weaning are all frequently associated with diarrhoea and other infections that can tax growth rate.

In poor urban environments in underdeveloped countries breastfeeding is intertwined with maternal capability to lactate and infant growth rate and health. Regardless of whether the mother lactates or not, postpartum weight and body fat changes are maternal physiological occurrences. We (Fornes and Dorea 1995) have shown that poor urban mothers (in Goiania, Brazil) can sustain a successful exclusive breastfeeding of 3 months with BMI values comparable to the present study. The socio-economically disadvantaged urban mothers of that study, taxed by milk supply demanded by the growing infant, did not show significant BMI changes (from 15 to 90 days) but showed significant differences in subcutaneous fat change (Fornes and Dorea 1995). Indeed our study did not show significant association between maternal BMI (at 36 months) and attained infant weight. Collectively, there is a distinct pattern of postpartum changes in body weight and adiposity between affluent and socially disadvantaged mothers (in developing countries): Lactating mothers in less developed countries lose subcutaneous fat when measured singly (triceps, more sensitive) or as a sum of several skin-fold measurements. Comparing different studies of different countries it was shown that these effects tend to occur mainly during the second trimester of lactation, but will occur in the first semester of lactation in circumstances of relative maternal malnutrition (Dorea 1997). This is in agreement with the changes observed in the BMI of the mothers in the present study: The profile between pre-pregnancy and at 6 months and 3 years are indistinguishable.

Fish is important in the diets and health of many poor people suffering from vitamin and mineral deficiencies, and yet we lack studies of its consumption by populations most vulnerable to nutrient deficiencies (Roos et al. 2007). The unique feature of this study is to be able to evaluate the impact of a dietary item (fish consumption) on critical periods of infant development (gestation, breastfeeding) which is sensitive to the maternal effects of nutritional factors. Although HHg – the signature of fish consumption – was not significantly associated with infant growth, the study revealed that extended breastfeeding had a statistically significant impact on profiles of attained height and weight at 3 years thus indicating that breastfeeding can be an important modifying factor of weaning.

Conclusion

Fish consumption rates can be traced through HHg; this biomarker showed that nutrient intakes *in tandem* with maternal fish consumption rate had no impact on outcomes of growth

and development of breastfed children. In the food transition scenario of Porto Velho, regardless of the animal-source food, the habitual plane of nutrition of these urban mothers was sufficient to sustain satisfactory *Z*-scores for weight and height at 5 years.

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(Anexo 7)

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Projeto: Avaliação da Exposição ao Mercúrio e Seus Compostos Sobre o Desenvolvimento Neuropsicomotor em Crianças de Porto Velho – RO.

Estou sendo orientado (a) quanto ao estudo Avaliação da Exposição ao Mercúrio e Seus Compostos Sobre o Desenvolvimento Neuropsicomotor em Crianças de Porto Velho – RO, cujos dados serão colhidos nas pacientes do Centro Obstétrico do Hospital de Base Ary Pinheiro, Maternidade Regina Pacis e Hospital Panamericano. Este estudo tem por objetivo estudar a exposição por mercúrio nos recém-nascidos da comunidade. É importante que se estude a mãe e o filho, pois a exposição é passada de mãe para filho e continuada por alimentos contaminados, entre eles o peixe. Esta exposição pode levar a problemas de saúde em crianças e adultos. O estudo será feito com perguntas de importância para o estudo da exposição e análise dos seguintes materiais: cortes de pedaços de cabelo da mãe e recém-nascido, amostra de sangue e leite materno, sangue e pedaço de cordão umbilical e placenta. Os participantes devem ser moradores da cidade de Porto Velho. A pesquisa será realizada sem riscos para quem participa, uma vez que o exame clínico não machuca, o cabelo cresce de novo e será utilizado material descartável para coleta de sangue. Os materiais acima citados serão estudados na busca da quantidade de mercúrio no corpo. Os pesquisadores comprometem-se a utilizar os materiais e dados coletados exclusivamente para os fins previstos no protocolo e a publicar os resultados, sejam eles favoráveis ou não.

Rejane Corrêa Marques
Endereço: Rua 27, casa 14, condomínio Fabiane Asfuri,
Bairro: Jardim das Mangueiras II, Porto Velho – RO.
Telefone: 225-5436/216-8550.

Estou ciente de que não sou obrigado (a) a participar ou deixar nenhum familiar participar, e que posso desistir a qualquer momento da pesquisa. Concordo com o que foi dito, permitindo que meu filho (a) e eu participemos da pesquisa. E estou recebendo cópia deste papel assinado por mim e pela pesquisadora.

Nome da mãe e/ou responsável pela criança: ._____

Porto Velho, _____de _____de 2000.

(Anexo 8)
Questionário mães e recém-nascidos

UNIVERSIDADE FEDERAL DE RONDÔNIA – UNIR

Projeto: Avaliação da Exposição ao Mercúrio e Seus Compostos Sobre o Desenvolvimento Neuropsicomotor em Crianças de Porto Velho – RO.

Área de atuação: Porto Velho (RO)

Ficha n.º :..... Data...../...../.....

MÃE

Nome:.....
Data de nascimento:...../...../..... Idade:..... Cor: branca( ) parda( ) negra( ) índia ( )
Lugar de nascimento:.....
Endereço Atual:.....FONE:.....
Pré-natal: sim( ) não( ) Início:..... Local:.....
Início dos movimentos fetais:..... Intensidade:.....
Intercorrências/Terapêutica:.....
Trabalho durante a gestação:.....
Tipo de parto: ( )normal ( )fórceps ( )cesárea: Indicação:.....
DUM: ...../...../..... IG: .....semanas DPP:..... Informante:.....

RECÉM-NASCIDO:

Sexo: ( ) feminino ( ) masculino Peso:.....g Estatura:.....cm PC:.....cm
Condições do RN ao nascer: Apgar 1º minuto.....5º minuto.....
Circular de cordão: ( )não ( )sim Reanimado: ( )não ( )sim: ( )O_2 nasal ( )ambu ( )entubação
Amniorrexe prematura: ( ) não ( )sim quanto tempo:.....horas
Uso de antibiótico pré-parto: ( )não ( )sim tipo:.....
Dose:..... Tempo de uso:.....
Eliminou mecônio antes da expulsão: ( )não ( )sim
Maturidade: ( )a termo ( )prematuro ( )pós-maduro **IG** pelo Índice de capurro:.....sem e....dias
Classificação quanto ao peso: ( )AIG ( ) PIG ( ) GIG
Medicação na sala de parto: ( )não ( )sim; especificar a medicação:.....
Internação no berçário: ( ) não ( ) sim: período: .....
Intercorrências no berçário:.....
Malformação: ( )não ( )sim qual: .....
Observações:.....
Condições de alta do RN: ( )com a mãe ( )do berçário para o ambulatório ( )TFD

ANTECEDENTES FAMILIARES MATERNOS:

Tipagem sangüínea: ( )O ( )A ( )B ( )AB Fator Rh: ( )Positivo ( )Negativo
Pais consangüíneos: ( ) sim ( ) não
Gesta:..... Para..... Abortos..... (espontâneos:..... provocados:.....)
Tipo de parto: ( ) normais ( )cesarianas ( ) complicações: .....
( )Natimortos ( )Prematuro ( )malformados tipo de malformações:.....
Antecedentes patológicos:
( )malária ( )leishmaniose ( )chagas ( )amebíase ( )esquistossomose ( )diabetes ( )sífilis ( )hepatite ( )toxoplasmose ( )rubéola ( )citomegalovírus ( )HIV ( )caxumba ( )tuberculose

Outras:.....
Educação Materna (anos): ..... Renda mensal familiar: R$.....
Quanto tempo mora no endereço atual:.....
Trabalho em garimpo: ( )não ( )sim período:.....
Quantas refeições faz por dia:..... Come peixe: ( )não ( )sim, quantidade/dia:.....
Freqüência: ..... Tipo:..... Procedência:.....
Usa medicamentos: ( )não ( )sim, qual:..... Quanto tempo:..... Dose/dia:.....
Contato com substâncias praguicidas ou semelhantes: ( )não ( )sim Quais:.....
Uso de drogas narcóticas: ( )não ( )sim, Qual:..... tempo de uso:.....
Usa xampu: ( )não ( )sim, marca:..... Tinge os cabelos: ( )não ( ) sim, periodicidade:.....
Ingere bebida alcoólica: não ( ) sim( ) Freqüência: ..... Quantidade/dia:.....
Tipo de bebida.....
Viagens ao exterior: ( )não ( )sim : país.....período:.....

ANTECEDENTES FAMILIARES PATERNOS:

Saúde: ( )normal ( )com problemas; quais:.....
Educação Paterna (anos): ..... Trabalho em garimpo: ( )não ( )sim período:.....
Quantas refeições/dia:..... Come peixe: ( )não ( )sim quantidade/dia:.....
Qual tipo:..... Procedência:.....
Usa medicamentos: ( )não ( )sim qual:..... Quanto tempo:..... dose/dia:.....
Ingere bebida alcoólica: não ( ) sim( ) Freqüência: ..... Quantidade/dia:.....
Tipo de bebida.....
Uso de drogas narcóticas: ( )não ( )sim tipo de droga:.....tempo de uso:.....

IRMÃOS DO RECÉM-NASCIDO: Número total:..... Sexo e idade em ordem decrescente:

Idade	Sexo

Saúde dos irmãos: ( )boa ( )com problemas, quais:.....

CONDIÇÕES DE MORADIA DA FAMÍLIA:
Situação da residência: ( )própria ( )alugada ( )de parentes ( )cedida pelo empregador
( )madeira ( )alvenaria ( )mista ( )enchimento ( )em terreno alagado
No de moradores:..... Entra luz em todos os cômodos: ( ) sim ( ) não
Presença de insetos e roedores: ( )não ( )sim, qual:.....
Destino das fezes: ( )fossa seca ( )enterrado ( )céu aberto ( )rio ( )fossa negra
Abastecimento de água: ( )rio ( )encanada ( )Poço ( )torneira pública ( ) outra:.....

NÍVEIS DE MERCÚRIO ENCONTRADOS:

No cabelo da mãe:.....
No cabelo do recém nascido:.....
No sangue da mãe:.....
No sangue umbilical:.....
No cordão umbilical:.....
Na placenta:.....
No leite materno: .....

Avaliador:.....

(ANEXO 9)

Questionário Para Crianças 6 meses

UNIVERSIDADE FEDERAL DE RONDÔNIA – UNIR

Projeto: Avaliação da Exposição ao Mercúrio e Seus Compostos Sobre o Desenvolvimento Neuropsicomotor em Crianças de Porto Velho – RO.

Ficha n.º _____ Data ___/___/____

IDENTIFICAÇÃO

Nome: _____

Data de nascimento: ___/___/____ Idade:_____ Sexo: ( )Masc ( )Fem Cor:_____

Endereço Atual:_____

Informante/Responsável:_____ Telefone para Contato:_____

Nome da mãe: _____ Profissão: _____

Nome do Pai: _____Profissão:_____

SAÚDE

História Vacinal

Apresentou cartão de vacinação: ( )sim ( )não

Vacinas	Datas					
BCG						
Anti-Hepatite B						
DPT						
Outras						

Antecedentes patológicos:_____

HISTÓRIA ALIMENTAR

Tempo de amamentação:_____ n.º de refeições/dia:_____

Alimentos mais freqüentes:_____

Come Peixe: ( )não ( )sim, tipo de peixe:_____

Freqüência:_____Quantidade:_____ Procedência/peixe:_____

EDUCAÇÃO:

Freqüenta creche: ( )sim ( )não Tempo de permanência na creche:_____horas

Educação Paterna (anos):_____ Educação Materna (anos):_____

Renda Familiar:_____

Irmãos (total):.....

Idade	**Sexo**

Saúde dos irmãos: ( )boa ( )com problemas, quais:.....

CONDIÇÕES DE MORADIA DA FAMÍLIA:

Situação da residência: ( )própria ( )alugada ( )de parente/amigos ( )cedida pelo empregador ( )madeira ( )alvenaria ( )enchimento ( )em terreno alagado
Presença de insetos e roedores:( )não ( )sim, qual:_____
Quantos moram na casa:_____ Entra luz em todos os cômodos: ( )sim ( )não
Destino das fezes: ( )fossa seca ( )enterrado ( )céu aberto ( )rio ( ) fossa negra
Abastecimento de água: ( )rio ( )encanada ( )Poço ( )torneira pública ( )outra:_____

EXAME FÍSICO

Mãe:

Peso:_____ Altura:_____ Paridade:_____

Criança:

Peso:_____ Altura:_____ PC:_____
Permanece sentado: ( )sim ( )não Idade que começou a sentar:_____
Engatinha: ( )sim ( )não Idade que começou a engatinhar:_____
Outros achados:_____
Impressão diagnóstica:_____

DESENVOLVIMENTO NEUROPSICOMOTOR:

Idade cronológica:_____

CONDUTA		Idade (meses)	%
MOTORA			
ADAPTATIVA			
LINGUAGEM	compreensiva		
	expressiva		
PESSOAL-SOCIAL			

Níveis de Hg no cabelo da criança:_____
Níveis de Hg no cabelo da mãe:_____

Avaliador:_____

(Anexo 10)

UNIVERSIDADE FEDERAL DE RONDÔNIA – UNIR
UNIVERSIDADE DE BRASÍLIA – UnB
UNIVERSIDADE FEDERAL DO RIO DE JANEIRO – UFRJ

Projeto: Avaliação da exposição ao mercúrio e suas repercussões sobre o desenvolvimento neuropsicomotor em crianças de Porto Velho

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Estou sendo orientada (o) quanto ao estudo “Avaliação da Exposição ao Mercúrio e suas Repercussões Sobre o Desenvolvimento Neuropsicomotor em Crianças de Porto Velho – RO”, cujos dados serão colhidos com o objetivo estudar a exposição por mercúrio nas crianças da comunidade. É importante que se estude as crianças, pois a exposição pode ser passada de mãe para filho e continuada por alimentos contaminados, entre eles o peixe. Esta exposição pode levar a problemas de saúde em crianças e adultos. O estudo será feito com exame físico completo, perguntas de importância para o estudo da exposição e amostras de cabelo da criança. A pesquisa será realizada sem riscos para os participantes, uma vez que o exame clínico não machuca e o cabelo cresce de novo. O material acima citado será estudado na busca da quantidade de mercúrio no corpo. A pesquisadora compromete-se a utilizar o material e dados coletados exclusivamente para os fins previstos no protocolo e a publicar os resultados, sejam eles favoráveis ou não.

Rejane Corrêa Marques
Endereço: Laboratório de Biogeoquímica
Br 364, km 9,5, Campus UNIR.
Telefone: (69) 2182-2122

Estou ciente de que não sou obrigada (o) a participar ou deixar nenhum familiar participar, e que posso desistir a qualquer momento da pesquisa, tendo sido informada (o) dos endereços para contato com a pesquisadora. Concordo com o que foi dito, permitindo que meu filho (a) e eu participemos da pesquisa, e estou recebendo cópia deste papel assinado por mim e pela pesquisadora.

Nome da mãe e/ou responsável pela criança_____

Porto Velho,_____ de _____ de 200__.

(ANEXO 11)

Questionário Para Crianças de 3 a 5 anos

UNIVERSIDADE FEDERAL DE RONDÔNIA – UNIR
UNIVERSIDADE DE BRASÍLIA – UnB
UNIVERSIDADE FEDERAL DO RIO DE JANEIRO – UFRJ

Projeto: Avaliação da exposição ao mercúrio e suas repercussões sobre o desenvolvimento neuropsicomotor em crianças de Porto Velho

Ficha n.º _____ Data ___/___/____

IDENTIFICAÇÃO

Nome: _____
Data de nascimento: ___/___/____ Idade:_____ Sexo: ( )Masc ( )Fem
Lugar de nascimento: _____
Endereço Atual:_____
Nome da mãe: _____ Profissão: _____
Nome do Pai: _____ Profissão:_____
FONE:_____Informante:_____

SAÚDE

História Vacinal: Apresentou carteira: ( )sim ( )não

Vacinas	Datas					
BCG						
Anti-Hepatite B						
DPT + Hib						
Anti-polio						
Anti-Sarampo						
Anti-Amarílica						
Triplice viral s						
Outras						

Teve alguma reação não esperada após receber alguma vacina? ( ) sim ( )não
Se sim, qual (s)_____
Usou algum tipo de remédio: ( )sim ( )não) Qual:_____

DOENÇAS:

( )parasitoses ( )catapora ( )dengue ( )hepatite ( ) pneumonia ( ) malária
outras:_____

HISTÓRIA ALIMENTAR

Tempo de amamentação (meses):_____N.º de refeições/dia:_____
Alimentos mais freqüentes:_____
Come Peixe: ( )não ( )sim, tipo de peixe:_____
Freqüência do consumo de peixe:_____ Quantidade/dia:_____

Consome enlatado: ( )sim ( )não Quais:_____
Come ovos de aves domésticas: ( )sim ( ) não tipo de ave:_____

EDUCAÇÃO

Educação Paterna (anos):_____ Educação Materna (anos):_____
Renda Familiar:_____
Freqüenta creche/escola: ( )sim ( )não Tempo de permanência no local:_____horas
Qual o meio de informação que a criança dispõe:_____

Irmãos: número total:..... Sexo e idade em ordem decrescente:

Idade	Sexo

Saúde dos irmãos: ( )boa ( )com problemas, Quais:_____

CONDIÇÕES DE MORADIA DA FAMÍLIA:

Situação da residência: ( )própria ( )alugada ( )de parente/amigos ( )cedida pelo empregador ( )madeira ( )alvenaria ( )enchimento ( )em terreno alagado
Presença de insetos e roedores:( )não ( )sim, qual:_____
Quantos moram na casa:_____ Entra luz em todos os cômodos: ( )sim ( )não
Destino das fezes: ( )fossa seca ( )enterrado ( )céu aberto ( )rio ( ) fossa negra
Abastecimento de água: ( )rio ( )encanada ( )Poço ( )torneira pública ( ) outra:_____

EXAME FÍSICO:

Peso:_____ Altura:_____ PC:_____
Idade que começou a andar:_____ Idade que começou a falar:_____
Outros achados:_____
_____
_____

DESENVOLVIMENTO NEUROPSICOMOTOR:

Idade Cronológica: _____

CONDUTA		Idade (meses)	%
MOTORA			
ADAPTATIVA			
LINGUAGEM	compreensiva		
	expressiva		
PESSOAL-SOCIAL			

Quociente de Desenvolvimento (QD) = _____
Impressão diagnóstica:_____

Avaliador:_____

(ANEXO 12)

Etapas do desenvolvimento infantil segundo Gesell

Idade	Adaptativo	Motor Grosseiro	Motor Fino	Linguagem	Pesssoal-Social
4 semanas	Seguimento restrito do olhar	Reflexo tônico-cervical	Punhos fechados	¾	Olha para a face do examinador
16 semanas	Olha para chocalho na mão	Postura simétrica	Mãos abertas; arranha e agarra	Sorri; vocaliza socialmente	Brinca com as mãos; reconhece a mamadeira
28 semanas	Transfere o cubo de mão	Senta; projeta-se para frente apoiando nas mãos; sustenta peso com MMII	Agarra o cubo com as mãos; tenta pegar objetos pequenos	Vocaliza para brinquedos; emite sons consonantais	Brinca com os pés; Toca na imagem no espelho
40 semanas	Segura mamadeira; segura objetos pequenos	Senta-se sem ajuda; engatinha; fica de pé	Solta objetos; aponta com o dedo em direção a objeto	Palavra-frase; imita sons da fala	Brinca de jogos simples de berçário; come biscoito com as mãos
52 semanas	Coloca cubo dentro do copo; tenta torre com 2 cubos	Anda com ajuda; dá alguns passos sem apoio	Preensão em pinça	Fala 2-3 palavras; reconhece objeto pelo nome	Coopera no vestir-se; brinca com a bola
18 meses	Retira pequeno objeto do copo; rabisca espontaneamente	Anda com equilíbrio; agacha-se	Torre de 3 cubos; folheia 2-3 páginas de livro de uma só vez	Jargões; reconhece figuras	Usa a colher derramando; puxa o brinquedo andando
24 meses	Constrói torre com 6 cubos; imita círculo com lápis	Corre com equilíbrio; chuta bola	Torre com 6 cubos; folheia páginas 1 por vez	Usa frases; compreende ordens simples diretas	Coloca partes simples da vestimenta; brinca com bonecos
36 meses	Imita ponte com 3 cubos; copia círculo	Fica num pé só; pula	Torre com 10 cubos; segura o lápis de forma adequada	Fala formando sentenças; responde a questões simples	Usa a colher adequadamente; coloca sapatos; aguarda a vez
48 meses	Imita portão com 5 cubos; copia linhas cruzadas	Pula num pé só; pulo amplo	Traços entre linhas	Usa conjunções; compreende preposições	Lava e enxuga a face; brinca de forma interativa
60 meses	Conta 10 objetos; copia triângulo	Pula no pé só alternadamente	¾	Fala sem dislalias; pergunta “por que?”	Veste-se sem auxílio; pergunta o significado das palavras

APÊNDICE

Calendário Básico de Vacinação da Criança 2007

IDADE	VACINAS	DOSES	DOENÇAS EVITADAS
Ao nascer	BCG - ID	dose única	Formas graves de tuberculose
	Vacina contra hepatite B ***(1)***	1ª dose	Hepatite B
1 mês	Vacina contra hepatite B	2ª dose	Hepatite B
2 meses	Vacina tetravalente (DTP + Hib) ***(2)***	1ª dose	Difteria, tétano, coqueluche, meningite e outras infecções causadas pelo *Haemophilus influenzae* tipo b
	VOP (vacina oral contra pólio)	1ª dose	Poliomielite (paralisia infantil)
	VORH (Vacina Oral de Rotavírus Humano) (3)	1ª dose	Diarréia por Rotavírus
	Vacina tetravalente (DTP + Hib)	2ª dose	Difteria, tétano, coqueluche, meningite e outras infecções causadas pelo *Haemophilus influenzae* tipo b
4 meses	VOP (vacina oral contra pólio)	2ª dose	Poliomielite (paralisia infantil)
	VORH (Vacina Oral de Rotavírus Humano) *(4)*	2ª dose	Diarréia por Rotavírus
6 meses	Vacina tetravalente (DTP + Hib)	3ª dose	Difteria, tétano, coqueluche, meningite e outras infecções causadas pelo *Haemophilus influenzae* tipo b
	VOP (vacina oral contra pólio)	3ª dose	Poliomielite (paralisia infantil)
	Vacina contra hepatite B	3ª dose	Hepatite B
9 meses	Vacina contra febre amarela ***(5)***	dose inicial	Febre amarela
12 meses	SRC (tríplice viral)	dose única	Sarampo, rubéola e caxumba
15 meses	VOP (vacina oral contra pólio)	reforço	Poliomielite (paralisia infantil)
	DTP (tríplice bacteriana)	1º reforço	Difteria, tétano e coqueluche
4 - 6 anos	DTP (tríplice bacteriana	2º reforço	Difteria, tétano e coqueluche
	SRC (tríplice viral)	reforço	Sarampo, rubéola e caxumba
10 anos	Vacina contra febre amarela	reforço	Febre amarela

(1) A primeira dose da vacina contra a hepatite B deve ser administrada na maternidade, nas primeiras 12 horas de vida do recém-nascido. O esquema básico se constitui de 03 (três) doses, com intervalos de 30 dias da primeira para a segunda dose e 180 dias da primeira para a terceira dose.

(2) O esquema de vacinação atual é feito aos 2, 4 e 6 meses de idade com a vacina Tetravalente e dois reforços com a Tríplice Bacteriana (DTP). O primeiro reforço aos 15 meses e o segundo entre 4 e 6 anos.

(3) É possível administar a primeira dose da Vacina Oral de Rotavírus Humano a partir de 1 mês e 15 dias a 3 meses e 7 dias de idade (6 a 14 semanas de vida).

(4) É possível administrar a segunda dose da Vacina Oral de Rotavírus Humano a partir de 3 meses e 7 dias a 5 meses e 15 dias de idade (14 a 24 semanas de vida). O intervalo mínimo preconizado entre a primeira e a segunda dose é de 4 semanas.

(5) A vacina contra febre amarela está indicada para crianças a partir dos 09 meses de idade, que residam ou que irão viajar para área endêmica (estados: AP, TO, MA MT, MS, RO, AC, RR, AM, PA, GO e DF), área de transição (alguns municípios dos estados: PI, BA, MG, SP, PR, SC e RS) e área de risco potencial (alguns municípios dos estados BA, ES e MG). Se viajar para áreas de risco, vacinar contra Febre Amarela 10 (dez) dias antes da viagem.