UNIVERSITÉ DU QUÉBEC À MONTRÉAL

LA CONSOMMATION DE POISSONS ET DE FRUITS EN AMAZONIE : UNE APPROCHE ÉCOSYSTÉMIQUE À L'ÉTUDE DU RÔLE DE L'ALIMENTATION TRADITIONNELLE SUR LA DYNAMIQUE D'EXPOSITION AU MERCURE

THÈSE

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L'enfer ne sera jamais suffisamment mauvais

si l'on n'arrive pas à connaître le ciel

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AVANT-PROPOS

La présente thèse analyse le rôle de l'alimentation traditionnelle amazonienne sur la dynamique d'exposition humaine au mercure. Elle est constituée de 4 chapitres, lesquels sont issus d'une étude épidémiologique à large échelle dans la région de la rivière Tapajós en Amazonie brésilienne. Parmi ces quatre chapitres, un premier est déjà publié dans la revue *The Science of the Total Environment*, un deuxième est sous presse dans *Environmental Research*, un troisième a été accepté pour publication dans *Journal of Exposure Science and Environmental Epidemiology,* et un quatrième est actuellement en révision dans *Environmental Health.*

Dans le premier chapitre on retrouve une revue exhaustive de la littérature scientifique concernant l'exposition humaine au mercure en Amazonie et les effets sur la santé. Il est en forme d'article et a été soumis à une revue scientifique. L'auteur de la thèse a planifié le projet de révision et mené lui-même la recherche bibliographique; il a évalué les documents retrouvés, monté la structure du rapport et effectué la rédaction de ce dernier.

Le deuxième chapitre présente des analyses confirmant régionalement l'influence de la fréquence de consommation de fruits sur la relation entre la consommation de poissons et les teneurs en mercure, non seulement dans les cheveux mais aussi dans le sang; le devis de l'étude a été établi par l'auteur de cette thèse et ce dernier a également coordonné la collecte de données sur le terrain ainsi que la saisie de celles-ci avec l'assistance de deux collègues du Projet CARUSO. L'auteur a aussi effectué les dosages du mercure dans les échantillons de cheveux au laboratoire de la division de recherche en santé environnementale de Santé Canada. Il a fait les analyses statistiques, pour enfin écrire le manuscrit.

Dans le troisième chapitre, on calcule l'apport quotidien de mercure à partir des données sur la concentration du métal dans les poissons et montre l'influence de la consommation de fruits sur la relation entre l'apport quotidien en Hg et les teneurs du métal dans le sang et dans les cheveux. L'auteur de la thèse a planifié l'étude et a effectué la collecte de toutes les données alimentaires et sociodémographiques utilisées dans les analyses; à partir des données générées par d'autres collègues (le mercure dans les poissons, les données anthropométriques et les quantités de poisson consommé par repas), l'auteur a pu calculer l'apport quotidien et mener toutes les analyses statistiques ainsi que la rédaction du manuscrit.

Le quatrième chapitre comporte des données montrant que ces populations riveraines consommatrices de poissons sont exposées non seulement au méthylmercure, mais aussi à la forme inorganique du contaminant, ce qui a des répercussions propres au point vue toxicologique. L'auteur a planifié et mené l'étude sur le terrain avec l'aide d'une étudiante de maîtrise, a effectué les analyses statistiques et a rédigé l'article.

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RÉSUMÉ

En Amazonie, les enjeux entourant la pollution environnementale par le mercure et l'exposition humaine par la consommation de poissons ont fait l'objet d'un très grand nombre de recherches au cours des deux dernières décennies. Malgré cela, les niveaux d'exposition au mercure par voie alimentaire sont toujours très élevés, et de nombreux effets sur la santé ont été rapportés dans la littérature scientifique chez des adultes de même que chez des enfants. Le projet CARUSO dans lequel s'insère la présente recherche, a adopté une approche écosystémique à la pollution du mercure dans le bassin de la rivière Tapajós, un affluent important du fleuve Amazone. C'est dans ce cadre que compte tenu de l'action toxique de ce métal lourd chez les communautés consommatrices de poissons vivant au bord de la rivière, notre groupe de recherche a déployé des efforts pour étudier et proposer des mesures viables permettant la réduction de l'exposition au mercure tout en maintenant la consommation de poissons. Ces derniers constituent non seulement une importante source de protéine animale et d'autres nutriments très importants à la nutrition humaine, mais très souvent ils constituent la seule source de ces nutriments pour ces populations relativement isolées dans la forêt.

Suite à une étude exploratoire antérieure suggérant que la consommation de fruits régionaux pourrait réduire l'absorption du mercure chez une communauté riveraine en Amazonie, nous avons mené une large étude épidémiologique et régionale visant à mieux comprendre le rôle des fruits sur la dynamique d'exposition au mercure dans toute la région riveraine du moyen Tapajós. En raison des variations saisonnières dans la disponibilité des fruits et des poissons, cette étude transversale a été réalisée en 2 périodes : pendant les périodes de descente et de montée des eaux. Cette thèse présente les résultats d'un grand nombre d'analyses issues de cette étude.

Dans un premier temps, une étude d'observation transversale basée sur une enquête alimentaire pour la consommation de poissons et de fruits a été réalisée auprès de 13 communautés riveraines durant la période de descente des eaux. Des échantillons de cheveux de 449 personnes et des échantillons de sang d'un sous-groupe de 225 personnes ont été collectés afin de déterminer les teneurs en mercure total et inorganique, et ce par la méthode d'Absorption Atomique. En moyenne, les participants ont consommé 6.6 repas de poissons par semaine, et ils ont mangé 11 fruits/semaine. La teneur moyenne en mercure sanguin était 57.1 ± 36.3 µg/L (médiane: 55.1 µg/L), et la teneur moyenne en mercure dans les cheveux était 16.8 ± 10.3 µg/g (médiane: 15.7 µg/g). La consommation de poissons ainsi que la consommation de fruits ont été significativement reliées aux bioindicateurs d'exposition au mercure dans les modèles d'analyse multivariées : pour le sang (poissons: $\beta = 5.6$, $p < 0.0001$; fruits: $\beta = -0.5$, $p = 0.0011$; R2 ajusté = 36.0%) et pour les cheveux (poissons: $\beta = 1.2$, $p < 0.0001$; fruits: $\beta = -0.2$, $p = 0.0002$; R2 ajusté = 21.0%). Des modèles ANCOVA ont montré que pour le même nombre de repas de poissons, les personnes consommant des fruits plus fréquemment présentaient des teneurs en mercure significativement inférieures pour les deux bioindicateurs utilisés. Pour les faibles consommateurs de fruits, chaque repas de poisson augmentait le mercure sanguin de 9.8 µg/L comparé à seulement 3.3 µg/L pour les grands consommateurs de fruits.

L'intégration des données de mercure dans les poissons avec les informations issues des questionnaires alimentaires nous a permis de calculer l'apport quotidien en mercure dans cette population, pour ensuite vérifier le possible effet protecteur des fruits contre cet apport quotidien toxique. Cette étude, réalisée à la descente des eaux (Juin), a ciblé les 256 personnes des 6 villages pour lesquels nous avions des données sur les concentrations de mercure dans les poissons de leur écosystème aquatique local. Les quantités de poissons par repas ont été évaluées. De plus, nous avons eu accès aux données sur les concentrations de mercure dans 1,123 spécimens de poissons locaux. L'ingestion quotidienne de mercure (µg/kg/jour) a été estimée pour les hommes et pour les femmes de chaque village à partir des moyennes de concentrations de mercure dans les poissons capturés dans leurs zones de pêche, la quantité moyenne de poissons par repas, la fréquence de consommation des différentes espèces de poissons et le poids corporel des participants. La concentration moyenne de mercure dans les poissons était 0.33µg/g ± 0.33, et l'ingestion quotidienne de mercure variait entre 0 et 11.8 µg/kg/jour (moyenne : 0.92µg/kg/jour ± 0.89), et a fluctué selon le genre et le village. Les concentrations moyennes de mercure sanguin et dans les cheveux étaient 60.1 ± 38.4µg/L et 17.9 ± 11.5µg/g, respectivement. On observe une forte relation positive entre le mercure sanguin et l'ingestion quotidienne de mercure, avec une relation inverse par rapport à la consommation de fruits et la scolarité des individus. Des variations significatives ont été observées selon le statut d'immigration régionale et entre les différents villages. Le mercure dans les cheveux a été directement associé à l'ingestion quotidienne de mercure et inversement relié à la scolarité ainsi qu'à la consommation de fruits. La consommation de fruits a été la seule variable qui modifiait la relation entre l'ingestion quotidienne de mercure et les teneurs sanguines du métal : pour une même ingestion quotidienne de mercure, les personnes mangeant plus de fruits ont des concentrations inférieures de mercure sanguin. Ces résultats révèlent des niveaux élevés d'ingestion quotidienne de mercure dépassant les valeurs de référence établies par l'Organisation mondiale de la santé ainsi que d'autres normes d'exposition établies par d'autres agences.

Des analyses ultérieures ont cherché à identifier de possibles pistes de recherche sur les voies physiologiques de l'action des fruits. Nous avons donc examiné la relation entre la consommation de poissons et les teneurs en mercure total, inorganique et organique dans le sang et dans l'urine. Une étude transversale a été menée auprès de 171 personnes de sept villages riverains de la région du Tapajós lors de la période de montée des eaux en 2004. Ces personnes n'avaient pas d'histoire d'exposition aux vapeurs de mercure en milieu de travail, et elles ne possédaient pas des amalgames dentaires. Une enquête alimentaire basée sur la fréquence de consommation de poissons et de fruits a été administrée, et des renseignements sociodémographiques ont été cueillis. Des échantillons de sang et d'urine ont été collectés, et les teneurs en mercure total, organique, et inorganique tout comme le mercure urinaire ont été déterminés par Spectrométrie d'Absorption Atomique. En moyenne, les participants ont consommé 7.4 repas de poissons par semaine, et 8.8 fruits par semaine. La teneur moyenne en mercure total dans le sang a été 38.6 ± 21.7 µg/L, et le pourcentage moyen de mercure inorganique sanguin était 13.8%. La valeur moyenne de mercure organique (méthylmercure) a été 33.6 ± 19.4 µg/L, alors que celle de mercure inorganique était 5.0 ± 2.6 µg/L. La teneur moyenne en mercure urinaire était 7.5 ± 6.9 µg/L, avec 19.9% des participants ayant des niveaux de mercure urinaire au-dessus de 10 µg/L. Le mercure inorganique sanguin a été hautement et significativement relié au nombre de repas de poissons carnivores, mais aucune

relation n'a été observée avec les poissons non-carnivores. Le mercure inorganique sanguin était aussi négativement relié à la fréquence de consommation de fruits et positivement associé à l'âge. De plus, il variait selon les communautés et était plus élevé parmi les personnes nées dans la région du Tapajós. Le mercure urinaire a également été associé à l'ingestion de poissons carnivores, et il a montré une tendance vers une relation négative avec la consommation de fruits; il était plus élevé chez les hommes que chez les femmes et supérieur parmi les participants natifs de la région. Le mercure urinaire était fortement relié au mercure inorganique et organique et total dans le sang. Le rapport de cotes (OR) pour des teneurs en mercure urinaire supérieures à 10µg/L chez les participants consommant plus de 4 repas de poissons carnivores par semaine est 4.00 [1.83 – 9.20]. Malgré la relation négative avec le mercure inorganique, la consommation des fruits n'influençait pas la relation entre les divers bioindicateurs de mercure. Les résultats de cette étude appuient une relation directe entre la consommation de poissons et les teneurs en mercure inorganique sanguin et urinaire.

La revue exhaustive de la littérature scientifique concernant la pollution environnementale et l'exposition humaine au mercure, tout comme ses effets à la santé des populations traditionnelles amazoniennes au cours de presque deux dernières décennies, montre de manière assez claire que malgré les niveaux d'exposition élevés, la grande majorité des recherches n'ont pas proposé des mesures concrètes et réalistes pour l'atténuation de la charge corporelle du métal chez ces populations. Ce cadre plutôt décevant renforce davantage la pertinence du thème qui a été abordé dans la présente recherche puisque les résultats obtenus se montrent très encourageants et prometteurs du point de vue de leur potentiel en tant que possible outil d'intervention alimentaire. Toutefois, des études ultérieures sont nécessaires pour mieux comprendre les conséquences toxicocinétiques de la consommation des fruits sur le mercure. Un des plus grands défis pour les scientifiques et les gestionnaires en santé publique dans cette région en voie de développement assez accéléré est la réduction de la charge mercurielle et le maintien de la consommation élevée de poissons. Les études cherchant des outils permettant l'atténuation de l'exposition humaine devraient être poursuivies pendant que d'autres mesures s'attaquent aux sources primaires de pollution.

Mots clés : Poissons, fruits, Amazonie, approche écosystémique, exposition, mercure

INTRODUCTION GÉNÉRALE

Au cours des dernières années des faits nouveaux ont permis de mieux comprendre la problématique de la présence du mercure (Hg) dans l'environnement amazonien ainsi que l'exposition humaine à ce métal. Les sols amazoniens disposent de réserves du métal qui dépassent largement celles qui sont attribuées à l'activité d'orpaillage et qui peuvent être à l'origine de l'exposition au méthylmercure, la forme la plus neurotoxique et bioaccumulable du contaminant (Roulet et al., 1998, 1999; Guimarães et al., 2000; Farella et al., 2001). Plusieurs activités dans cette région favorisent la mobilisation du mercure (déboisement, feux, mines) (Roulet et al., 1999; Farella et al., 2006;Veiga, 1994; Spiegel et al., 2005) vers des milieux aquatiques propices à la méthylation du mercure et à son accumulation au sein de la chaîne alimentaire (Guimarães, 2001). De plus, la santé des populations riveraines consommatrices de poissons contaminés au méthylmercure est altérée à partir de seuils de contamination bien plus bas que ceux qui déclenchent les signes cliniques spécifiques à l'intoxication mercurielle (Lebel et al., 1996, 1998; Grandjean et al., 1999; Amorim et al., 2000; Dolbec et al., 2000; Harada et al., 2001; Cordier et al., 2002; Yokoo et al., 2003; Silva et al., 2004; Fillion et al., 2006).

Au delà de dix ans de recherches participatives menées par des chercheurs brésiliens et canadiens en collaboration avec des communautés riveraines du Tapajós en Amazonie brésilienne ont grandement contribué à définir les bases de cette problématique et à initier des actions permettant de réduire l'exposition (CARUSO, 2007). Ces études ont permis de mieux comprendre les sources de contamination par le mercure, les caractéristiques de sa circulation au sein des écosystèmes aquatiques, les relations entre la consommation de poissons et l'exposition, ainsi que les effets sur la santé humaine.

Dans une optique de recherche de solutions à court et long terme pour réduire l'exposition au mercure tout en maintenant la consommation de poissons, ces études ont permis de mettre sur pied, dans un village de la région du Tapajós au Brésil, un projet pilote basé sur les habitudes alimentaires et les pratiques de pêche, en vue de réduire l'exposition au mercure à court terme, mais aussi de jeter les bases pour l'élaboration de solutions à long terme qui s'appuient sur le développement de systèmes agro-forestiers, et ce afin de diminuer les sources de contamination et d'offrir des options de développement durable pour les communautés du Tapajós. Quant aux solutions à court terme, une intervention participative pilote a permis de réduire considérablement les niveaux de mercure chez les habitants du village, tout en maintenant la consommation de cet aliment riche en nutriments, le poisson. La comparaison des pratiques de consommation de poissons et de l'exposition au mercure chez les mêmes personnes, en 1995 et 2000, a montré qu'elles continuaient à manger les mêmes quantités de poissons, mais que la proportion des poissons non carnivores consommés avait augmenté, avec pour conséquence une diminution de près de 40% des niveaux de mercure dans les cheveux. En plus de ce travail éducatif au niveau des pratiques de consommation de poissons, un suivi quotidien et annuel du régime alimentaire général a permis d'identifier la capacité de l'ingestion de fruits régionaux à moduler la forte relation entre la consommation de poissons et les teneurs en mercure dans les cheveux.

À partir de ces expériences pilotes encourageantes, l'objectif principal de la troisième phase du projet CARUSO était d'étendre les recherches participatives, du niveau local au niveau régional à l'échelle du bassin du Bas-Tapajós, pour définir des interventions adaptées à la diversité des écosystèmes et des communautés, en vue de diminuer la contamination par le mercure et d'améliorer la santé et le bien-être des populations au sein d'une approche écosystémique.

Toutes les phases du développement de la présente thèse, à savoir les enquêtes alimentaires ainsi que d'autres échantillonnages au Brésil, l'analyse des concentrations de mercure dans les cheveux et dans le sang, les analyses statistiques et la rédaction du rapport ont été réalisées dans le cadre de la troisième phase du projet CARUSO.

1. Problématique

En Amazonie, la problématique de pollution environnementale par le mercure (Hg) et l'exposition humaine à ce toxique font l'objet de nombreuses recherches scientifiques depuis la fin des années 1980 (Couto et al. 1988; Malm et al., 1990; Veiga, 1994; Lebel et al., 1997; Cordier et al., 1998; Roulet et al., 1998, 1999, Farella et al., 2001; Dolbec et al., 2001; Bastos et al., 2006). Malgré cela, les niveaux d'exposition humaine sont toujours très élevés et répandus (Santos et al., 2003; Bastos et al., 2006; Pinheiro et al., 2007), et plusieurs effets néfastes à la santé sont rapportés chez des travailleurs des sites d'orpaillage tout comme chez des populations traditionnelles consommatrices de poissons (Branches et al., 1993; Counter et al., 2006; Lebel et al., 1996, 1998; Grandjean et al., 1999; Amorim et al., 2000; Dolbec et al., 2000; Harada et al., 2001; Cordier et al., 2002; Yokoo et al., 2003; Silva et al., 2004; Fillion et al., 2006). Compte tenu de la toxicité du mercure chez l'humain, il est devenu important de trouver des moyens permettant la réduction de la charge corporelle de ce métal sans pour autant priver les individus de la consommation de poissons, un aliment de haute importance pour les populations traditionnelles amazoniennes, et très souvent la seule source de protéine animale ainsi que d'autres substances de grande valeur nutritive pour ces communautés (Mertens et al., 2005).

Un grand défi donc pour les scientifiques et pour les autorités sanitaires est de garantir le maintien de la consommation de cette ressource alimentaire, tout en évitant à la fois l'intoxication au mercure. Ce défi est d'autant plus important lorsqu'on observe que dans le cadre de cette problématique, l'expérience montre que la simple interdiction de la consommation de poissons n'est pas suffisante pour résoudre le problème, puisqu'une telle mesure peut entraîner d'autres problèmes de santé à cause d'une qualité inférieure de l'alimentation (Chan & Receveur, 2000; Receveur et al., 1997). Usher et al. (1995) ont suggéré, à partir de leurs études chez des populations autochtones au Canada, que des changements majeurs dans l'alimentation traditionnelle peuvent entraîner une perte de la culture et de l'identité au sein de ces populations, car toute leur structure socioéconomique et culturelle est altérée suite aux changements alimentaires.

Plus récemment, ce dilemme a fait l'objet d'un certain nombre de publications analysant le cas amazonien, où compte tenu de l'absence d'épidémies cliniques majeures causées par l'exposition au mercure, l'on arrive même à proposer que les populations riveraines et indigènes continuent à manger des poissons sans aucune crainte, malgré les niveaux élevés de contamination mercurielle chez ces derniers (Dórea, 2003, 2004; Dórea et al., 2005). Il va sans dire que de tels auteurs ne tiennent pas compte des risques toxiques liés à l'exposition à faibles doses et à long terme.

Une approche alternative susceptible d'atténuer les niveaux d'exposition tout en maintenant la consommation de poissons, serait d'identifier des éléments du régime alimentaire traditionnel pouvant influencer le métabolisme du mercure tout comme sa toxicité. Il existe un certain nombre d'études expérimentales qui appuient cette hypothèse, mais avant de passer en revue une telle littérature, un bref rappel du métabolisme du méthylmercure s'impose.

2. Le métabolisme du méthylmercure chez l'humain

Le méthylmercure (MeHg) est la forme organique du métal incorporée pas les ressources halieutiques des lacs et rivières, suite à des processus de méthylation bactérienne (Guimarães et al., 2000 ; Guimarães, 2001). Cette forme chimique est absorbée quasi totalement à travers la paroi gastro-intestinale et au-delà de 90% de ce qui est ingéré atteint le courant sanguin (WHO, 1990). Une fois parvenu à la circulation sanguine, le MeHg établit des liaisons avec les molécules qui contiennent des groupements thiolés, notamment les groupes sulfhydriques des protéines plasmatiques, les acides aminés et les peptides (Aschner & Clarkson, 1988), et il est par la suite distribué aux organes dans un délai moyen de 4 jours (Clarkson, 1987). Selon cet auteur, le cerveau présente une forte affinité pour le MeHg; dans cet organe, l'on retrouve des concentrations trois à six fois supérieures à celles des autres organes. Bien que l'affinité de l'organe soit importante, la distribution et la rétention du MeHg dans les divers systèmes sont aussi influencées par des facteurs indépendants de l'exposition, notamment l'âge, le sexe, l'alimentation, la consommation d'alcool, le tabagisme, ainsi que l'activité métabolique de la microflore intestinale (Clarkson, 1992; WHO, 1990).

Dans les tissus biologiques, une partie du MeHg est convertie en une forme inorganique (Dunn & Clarkson, 1980), et ce notamment au niveau des intestins (Clarkson & Magos, 2006). L'hypothèse plus soulevée pour expliquer cette conversion accorde un rôle particulièrement important à l'activité métabolique de la flore intestinale et aux macrophages tissulaires comme des agents promoteurs de la déméthylation du MeHg, ce qui est suivi par l'excrétion des formes inorganiques via les selles (NRC, 2000 ; WHO, 1990). Cependant, les mécanismes exacts et les microrganismes impliqués dans ces processus ne sont toujours pas connus (Clarkson & Magos, 2006). Une cinétique de premier ordre caractérise l'élimination du méthylmercure, lequel présente une demi-vie de 70 jours chez l'humain et est éliminé à 90% par voie fécale (Inskip & Piotrowski, 1985; WHO, 1990).

3. Les bioindicateurs d'exposition

Le sang et les cheveux constituent les deux choix disponibles pour l'évaluation de l'exposition alimentaire au méthylmercure. Le sang représente l'exposition récente alors que les cheveux peuvent être utilisés pour faire une évaluation rétrospective de l'exposition, puisque ceux-ci croissent à raison d'environ 1 cm par mois (NRC, 2000). Quant aux cheveux, en plus d'être spécifique au MeHg et de permettre l'évaluation rétrospective, leur utilisation est privilégiée puisque leur collecte est non-invasive et peut être faite sans supervision médicale (NRC, 2000). S'ajoute à cela le fait que les échantillons peuvent être préservés longtemps avant d'être analysés sans que ceux-ci ne subissent de détérioration.

Classiquement, le sang peut aussi être utilisé pour déterminer l'exposition au mercure inorganique de même que l'urine chez des individus inhalant des vapeurs de mercure en milieu de travail (Clarkson & Magos, 2006). Par contre, des études récentes menées dans différentes régions du globe montrent que des individus consommant des quantités considérables de poissons peuvent présenter d'importantes teneurs de mercure inorganique (Apostoli et al., 2002; Johnsson et al., 2005; Berglund et al., 2005; Levy et al., 2004).

4. La toxicité du mercure inorganique et du méthylmercure

Les expositions aux formes inorganiques du mercure surviennent notamment en milieu de travail et incluent l'inhalation de vapeurs de mercure métallique ainsi que des expositions mixtes aux aerosols mercuriques (Kazantzis, 2002). Dans le cas de l'Amazonie, les premiers registres d'activités d'orpaillage remontent au siècle XVIII, avec une ruée majeure vers l'or pendant les années 1970, laquelle a suivi les grands projets d'occupation et de développement mis en place par le gouvernement militaire (Santos et al., 2003). Selon ces auteurs, l'utilisation massive de mercure dans les processus artisanaux d'exploitation aurifère, et en particulier par les personnes chargées du raffinage et du travail de l'or (bijoutiers) au Brésil, entraîne des exposistions très élevées au mercure par voie respiratoire, les concentrations urinaires du métal pouvant atteindre 663 μg/L.

Des études récentes en Guyane Française montrent que les activités d'orpaillage sont à l'origine d'une double pollution mercurielle liée aux rejets de la forme élémentaire du métal (Hg^0) utilisé en tant qu'agent d'amalgamation et à l'érosion des sols très anciens du bassin amazonien, naturellement riches en mercure inorganique (Hg^{++}) (Boudou et al., 2006). Ces auteurs indiquent qu'une exposition forte aux vapeurs de mercure peut entraîner des signes respiratoires et fièvre semblables à ceux d'une grippe, pouvant être mortels en l'absence de traitement, accompagnés éventuellement de manifestations gastrointestinales, de douleurs musculaires et d'une atteinte rénale. De plus, l'intoxication chronique par le mercure métallique se manifeste par des atteintes du système nerveux central (tremblements, troubles de caractère, pertes de mémoire), du système nerveux périphérique (troubles sensitifs dans les mains et les pieds; constriction du champ visuel), par des lésions rénales glomérulaires et tubulaires, parfois accompagnées de gingivite ou stomatite (Lauwerys, 2000; cité dans Boudou et al., 2006).

Par ailleurs, à des niveaux bien plus inférieurs, des amalgames dentaires libèrent des vapeurs de mercure qui peuvent exercer une toxicité comme les vapeurs inhalées de sources externes telles les expositions en milieu de travail (Kazantzis, 2002). Cependant, à part quelques cas d'allergies par contact, aucune évidence convaincante n'est disponible à l'effet que des amalgames dentaires peuvent causer des effets néfastes à la santé (Clarkson & Magos, 2006).

Des formes inorganiques de mercure peuvent donc endommager les reins et causer dans quelques cas un syndrome néphrotique. Des vapeurs de mercure inhalées peuvent aussi causer des dommages au système nerveux central dû à leur capacité de traverser la barrière hémato-encéphalique. On croit que les formes ioniques de ce mercure sont les agents causals de la maladie infantile acrodynie ou la « maladie rose ». Des auteurs suggèrent que le cation Hg^{++} est l'agent toxique approximatif pour toutes ces formes inorganiques de mercure (Clarkson & Magos, 2006). Toutes les espèces de mercure inorganique ont la capacité de causer des réactions idiosyncratiques, mais sa prévalence et sa sévérité ne semble pas être reliée à la dose. Le syndrome néphrotique et la « maladie rose » en constituent deux exemples (pour une révision approfondie voir Kazantizis, 2002; Clarkson & Magos (2006) Magos & Clarkson (2006)).

La forme organique du mercure (méthylmercure) est particulièrement connue pour son potentiel de neurotoxicité. Il s'agit d'une toxine puissante qui est biomagnifiée au long des chaines trophiques, tout en mettant à risque des populations côtières et/ou riveraines consommatrices de poissons. Même si des indicateurs d'effets tératogènes constituent depuis quelques années la base pour l'évaluation des risques et la formulation de politiques de protection à la santé publique, le méthylmercure peut aussi causer des effets cardiovasculaires et reproductifs (pour une révision approfondie voir Boudou et al., 2006; Virtanen et al., 2007; Mergler et al., 2007).

5. La nutrition en lien avec l'exposition et la toxicité des xenobiotiques

Depuis longtemps les liens entre la nutrition et la toxicologie attirent l'attention des professionnels œuvrant dans le domaine de la santé. En combinant les connaissances produites par ces deux disciplines, on peut proposer des moyens de soulager un certain nombre d'atteintes à la santé humaine causées par l'exposition aux substances toxiques (Levander and Cheng, 1980). Par exemple, il a été suggéré que des facteurs nutritionnels peuvent jouer un rôle important au niveau de l'absorption de quelques métaux, ainsi prévenant ou réduisant ainsi l'exposition à ces contaminants (Fox, 1979; More, 1979; Goyer, 1995). Les teneurs dans les tissues biologiques en plomb, en cadmium et en mercure, des métaux toxiques et xenobiotiques, peuvent être réduites ou alors augmentées par des interactions ou déficiences d'éléments essentiels à la nutrition (Goyer, 1997). Malgré cela, très peu d'études ont vérifié directement les effets d'une déficience ou d'une compensation nutritionnelle sur la toxicité causée par un métal, et on doit donc déduire l'effet des altérations nutritionnelles à partir des résultats d'études expérimentales de mécanismes physiologiques généraux (Peraza et al, 1998).

Des recherches suggèrent que quelques nutriments peuvent influencer l'absorption et/ou la toxicité du mercure. Parmi ceux-ci, le sélénium (Se) est le plus étudié (Chapman & Chan, 2000). À partir d'études animales, Parizek & Ostadalova (1967) ont été les premiers à suggérer que le sélénium pourrait protéger contre la toxicité du méthylmercure. Le sélénium jouerait un rôle protecteur grâce à sa capacité de ralentir l'expression de la toxicité du mercure (Ganther et al., 1973 ; Ganther & Sunde, 1974). Ces études pionnières ont ouvert un tout nouveau champ d'investigation sur les relations antagonistiques entre ces deux éléments. Quelques années plus tard un certain nombre d'hypothèses ont été proposées en vue d'expliquer les mécanismes favorisant cette action protectrice, à savoir : i) redistribution du mercure dans l'organisme; ii) compétition pour des sites de liaison cellulaire; iii) formation d'un complexe mercure-sélénium; iv) conversion des formes toxiques du mercure en d'autres formes ; et v) prévention du stress d'oxydation (Cuvin-Aralar & Furness, 1991). Chez les humains, les relations entre le Se et le Hg sont moins claires. Certains auteurs ont observé une corrélation entre des bioindicateurs de sélénium et de mercure (Campos et al, 2002; Dewailly, 1997; Grandjean et al, 1992), alors que d'autres n'ont pas observé une telle corrélation (Hansen et al., 1984; Alexander et al., 1983). Par ailleurs, aucune étude épidémiologique n'a jamais démontré un véritable effet protecteur contre la toxicité du MeHg (Chapman & Chan, 2000), et même dans les études expérimentales des résultats assez contradictoires ont été observés (Magos, 1991). Malgré cela, plusieurs auteurs mettent de l'avant l'utilisation du Se dans le traitement de l'empoisonnement humain par le mercure, même si le Se est aussi toxique si ingéré à de fortes doses (Chowdhury & Chandra, 1987 ; Plessi et al., 2001). Le rôle de ce nutriment reste toujours à confirmer.

D'autres nutriments qui ont été mis en relation avec les niveaux ou la toxicité du méthylmercure, sont les vitamines E et C (Calabrese, 1980 ; Levander & Cheng, 1980; Solomons et al., 1982), ainsi que quelques minéraux essentiels, comme le zinc (Zn) et le fer (Fe) (Chowdhury & Chandra, 1987). Quant à la vitamine E, son effet protecteur a été rapporté pour la première fois en 1974, quand on a observé que des niveaux élevés de cette vitamine diminuaient la mortalité chez des animaux de laboratoire nourris avec 30 µg/g de méthylmercure (Welsh et al., 1976). Plusieurs hypothèses ont été mises de l'avant pour expliquer ce phénomène : la neutralisation des radicaux libres qui commenceraient la dégradation du méthylmercure ; la capacité de la vitamine E de réagir avec les radicaux méthyles qui peuvent être formés lors de la dégradation, ainsi qu'à sa capacité de stabiliser les membranes cellulaires à travers des interactions avec les chaînes d'acides gras non saturées (Peraza et al., 1998). L'effet protecteur de la vitamine E a été étudié dans plusieurs systèmes et les résultats des études indiquent que cette vitamine peut maintenir l'état d'agrégation des cellules nerveuses en présence de mercure (Kleinschuster et al., 1983), réduire le taux de péroxidation des lipides dans le foie (Andersen & Andersen, 1993), s'associer à d'autres antioxydants pour diminuer la neurotoxicité du méthylmercure (Gassó et al, 2001), et prévenir les changements des paramètres biochimiques dans le processus de toxicité du mercure sur le système reproducteur (Rao & Sharma, 2001).

Pour la vitamine C, des études animales suggèrent l'existence d'une influence de celle-ci sur le métabolisme de quelques métaux à travers d'interactions rendant ces métaux moins disponibles pour l'organisme (Hill, 1980). Grâce à ses propriétés réductrices, on croit que cette vitamine peut diminuer l'état d'intoxication causée par divers éléments, et il a été démontré qu'elle peut aussi interférer dans le processus de biotransformation et modification de la toxicité du mercure, tout en diminuant les teneurs en méthylmercure (Sharma et al., 1982). Par contre, comme pour le sélénium, la vitamine C fait l'objet de quelques controverses concernant sa capacité de protection. Fujimoto et al. (1985) suggèrent qu'en présence de la vitamine C, le méthylmercure a un effet stimulateur puissant sur la péroxidation de lipides dans les mitochondries, et trois ans plus tard le même auteur ajoute que la vitamine C augmente légèrement l'accumulation de méthylmercure dans les reins (Fujimoto et al., 1988). Dans une autre étude, l'ingestion de suppléments de vitamine C n'a pas affecté de façon significative les teneurs en mercure, en plomb et en cadmium dans les cheveux et dans le sang d'une population humaine adulte (Calabrese et al., 1987). De plus, l'addition de vitamine C dans le régime alimentaire a entraîné une augmentation des teneurs en méthylmercure dans les tissus nerveux et musculaires, surtout en association avec d'autres vitamines, comme la vitamine B_{12} (Zorn & Smith, 1990). Dirks et al. (1994) ont étudié l'effet d'injection intraveineuse de la vitamine C sur le taux d'excrétion du mercure et leurs résultats n'indiquaient aucun effet. Même si la plupart des études n'indiquent pas un effet protecteur de la vitamine C sur les niveaux et/ou la toxicité du mercure, une étude récente suggère que l'addition de vitamine C au régime alimentaire peut éviter les effets genotoxiques du mercure sur des cultures cellulaires (Rao et al., 2001). Évidemment, l'analyse des résultats des recherches doit tenir compte des différentes approches méthodologiques utilisées, ce qui suggère que le rôle de cette vitamine reste aussi à confirmer.

Un certain nombre d'études ont examiné l'influence de quelques minéraux essentiels ainsi que des fibres alimentaires et des niveaux protéiniques sur l'action néfaste des métaux. Dans le but d'estimer si la teneur en fer (Fe) dans le lait pouvait être la raison d'une absorption élevée des métaux, Kostial et al. (1980) ont réalisé une étude dont les résultats indiquent que la forte absorption des métaux n'est probablement pas due au faible teneur en Fe dans le lait et aussi que le Fe n'interagit pas avec le cadmium, le mercure et le manganèse dans le processus d'absorption. Une année plus tard, le même auteur a présenté des données additionnelles sur l'effet du régime alimentaire sur l'absorption du mercure, tout en suggérant que des animaux de laboratoire nourris au lait retiennent plus de mercure que ceux nourris sans lait (Kostial et al., 1981). Par ailleurs, les résultats d'une autre étude indiquent que le prétraitement d'animaux de laboratoire avec le zinc (Zn) avant d'être exposés au mercure, peut non seulement induire la production de métallothioneine, mais aussi augmenter l'activité d'enzymes protectrices, ce qui favoriserait la diminution de la toxicité du mercure (Furkino et al., 1984). Rowland et al. (1986) suggèrent que la consommation de fibres alimentaires peut réduire les teneurs en mercure dans le cerveau suite à une exposition au méthylmercure, pouvant ainsi réduire les effets neurotoxiques du métal. Finalement, l'influence des niveaux protéiniques dans l'organisme sur la cinétique du méthylmercure a aussi été examinée et les résultats indiquent qu'un régime alimentaire pauvre en protéines pourrait diminuer l'excrétion urinaire du mercure à cause d'une augmentation de la rétention des métabolites de MeHg dans les cellules rénales. Les auteurs concluent que le statut nutritionnel en protéines jouerait un rôle important dans la détermination de la cinétique du MeHg (Adachi et al, 1992).

La figure 1 montre de façon schématique la recirculation entero-hépatique du méthylmercure ainsi que ses mouvements vers le cerveau, les reins, les cheveux et les tissus des foetus (Cernichiari et al., 2007). Le méthylmercure est efficacement absorbé de l'alimentation et ensuite il entre dans la circulation portière. Lors de son passage par le foie une fraction de la dose absorbée est secrétée dans la bile et une autre partie est reabsorbée par le compartiment sanguin. Une partie de ce qui reste dans le tract gastrointestinal est convertie en mercure inorganique par la microflore intestinale et excrétée par voie fécale. Un certain nombre d'études ont suggéré que le processus de déméthylation dans les intestins pourrait constituer un site d'interaction entre des nutriments et l'accumulation du MeHg dans le corps (Chapman & Chan, 2000), comme pour le cas des fibres alimentaires qui augmenteraient l'excrétion intestinale du MeHg (Rowland et al., 1986). Ainsi, pour réduire l'absorption du mercure, un nutriment pourrait réduire l'absorption ou accélérer l'excrétion par une ou plusieurs voies impliquées dans la distribution du mercure.

6. Les études précédant la présente thèse

À la recherche de moyens permettant de maintenir la consommation de poissons tout en minimisant le risque toxique résultant de l'exposition au mercure, l'auteur de la présente thèse et ses collaborateurs ont réalisé une étude exploratoire portant sur les possibles influences du régime alimentaire traditionnel riverain sur les niveaux de mercure chez la population d'un petit village en Amazonie brésilienne (Passos et al., 2001, 2003, 2004). Ces études préliminaires se sont inscrites dans une démarche transdisciplinaire basée sur une approche écosystémique à la santé (Lebel, 2003), en s'appuyant sur la participation communautaire et sur l'analyse du genre. On cherchait à identifier et à mettre en pratique des solutions viables au problème d'exposition humaine au mercure en Amazonie brésilienne (CARUSO, 2007). En effet, parce que la santé ne peut pas être considérée de manière isolée et puisqu'elle est étroitement liée à la qualité de l'environnement dans lequel les gens évoluent, Lebel (2003) propose un cadre de recherche qui réconcilie la santé des écosystèmes avec celle de leurs habitants, tout en accueillant à la fois l'apport des scientifiques, des membres des communautés et des représentants des autorités et des groupes d'intérêts. Selon ce dernier auteur, ce n'est qu'à partir de démarches intégrées que l'on arrivera à construire des solutions viables et surtout durables aux problèmes de santé humaine liés à la qualité et à l'intégrité des écosystèmes.

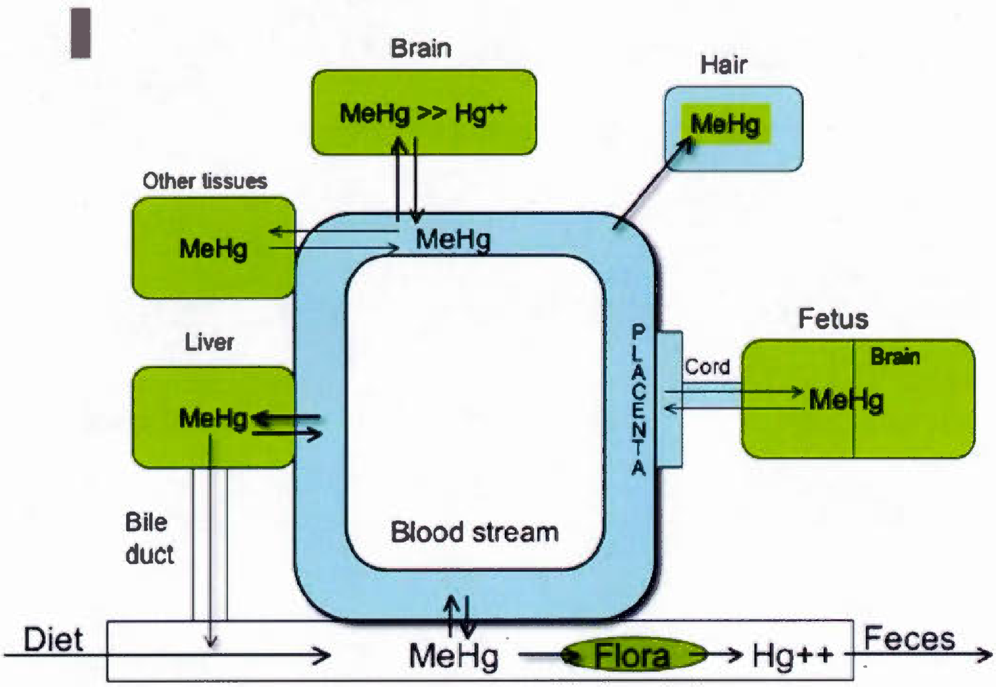

Figure 1 : Schéma montrant la toxicocinétique du mercure chez l'humain (Tirée de Cernichiari et al., 2007).

Les résultats de l'étude exploratoire mentionnée ci-haut et menée avec 26 femmes d'un village du Tapajós, ont d'une part permis de voir que le régime alimentaire chez les riverains est assez varié, et d'autre part que la consommation de fruits pouvait influencer la relation entre la consommation de poissons et l'exposition au mercure. Ainsi, pour une même consommation de poissons, des femmes consommant plus fréquemment des fruits présentaient des teneurs inférieures en mercure dans les cheveux. Toutefois, la mise en lumière de cet effet des fruits n'a constitué qu'une toute première étape de nos efforts de compréhension du rôle de l'alimentation traditionnelle amazonienne sur la dynamique d'exposition humaine au mercure, puisque l'étude a été réalisée sur un nombre très restreint de personnes du sexe féminin seulement, et les méthodologies avaient été choisies en vue d'examiner l'influence de l'ensemble du régime alimentaire (tous les aliments) sur l'exposition. Cette étude exploratoire nous a permis de mettre de l'avant les hypothèses ci-dessous.

7. Hypothèses

Trois hypothèses ont été testées dans le cadre de la thèse :

- La consommation de fruits régionaux peut influencer la relation entre la consommation de poissons et le mercure sanguin ;
- Les fruits modifieraient la relation entre l'apport en Hg provenant des poissons et les bioindicateurs d'exposition ;
- La consommation de fruits agit sur la déméthylation du mercure, tout en augmentant son excrétion urinaire.

8. Objectifs

L'objectif général de la présente thèse est d'analyser le rôle de la consommation des fruits sur la dynamique d'exposition humaine au mercure provenant des poissons contaminés. Les objectifs spécifiques sont les suivants :

- À l'aide d'une étude épidémiologique, déterminer la fréquence de consommation de fruits et de poissons des riverains sur deux saisons ;
- Chez cette population, déterminer les concentrations de mercure dans le sang, les cheveux et l'urine

- Chez cette population, déterminer l'apport en mercure à l'aide des concentrations connues de mercure dans les poissons locaux ;
- Examiner les relations entre la consommation de fruits, de poissons et l'apport de mercure sur les bioindicateurs d'exposition.

9. Méthodes

Les méthodes détaillées sont présentées dans les chapitres. Ici nous présentons les grandes lignes méthodologiques.

Tout d'abord pour ce qui des stratégies d'échantillonage de la population, une analyse de puissance statistique ainsi que des calculs pour déterminer la taille de l'échantillon ont été réalisés selon la méthode de comparaison de deux corrélations (transformation fisher r-to-z), en utilisant UnifyPow, une macro pour le système SAS Inc. (O'Brien, 1998). À partir des données de l'étude exploratoire réalisée auprès du groupe de 26 femmes (Passos et al, 2003), et en considérant un scénario où la fréquence de consommation de fruits diminuerait de 1,5 fois la valeur du coefficient de corrélation entre la fréquence de consommation de poissons et les niveaux de Hg, on a obtenu une taille minimale d'échantillon équivalente à 300 personnes pour une puissance statistique de 0,865 à un niveau de signification statistique $\alpha = 0{,}05$.

Les démarches pour le choix des communautés participant à l'étude ont été faites conjointement avec les autres chercheurs du projet plus large dans lequel la thèse a été réalisée. Suite à un voyage exploratoire dans la région du Tapajós réalisé au mois de Janvier 2003, nous avons mis en commun les besoins de chaque volet de recherche en lien avec les caractéristiques des villages et ses environs. De cette façon, 6 communautés ont été initialement sélectionnées (Tableau 1). Ce tableau montre les besoins de chaque volet ainsi que les communautés qui ont été choisies selon ces besoins.

Dans le cas de la présente thèse, il convient de remarquer qu'en plus des communautés montrées au Tableau 1 (Groupe I), d'autres communautés ont été inclues dans le plan d'échantillonnage, compte tenu des besoins particuliers en termes de la taille de l'échantillon qui permettrait l'obtention d'une puissance statistique satisfaisante ainsi que la nécessité de comparer des sites présentant d'autres variations en termes de la disponibilité des ressources alimentaires. En ayant à l'esprit l'importance d'une bonne représentativité de la diversité régionale, le choix des communautés additionnelles considérées dans la zone d'étude a été fait en tenant compte de trois blocs : une région autour de la ville d'Aveiro (bloc 1), une région autour de la communauté de Brasília Legal (bloc 2) et une région proche de la ville d'Itaituba (bloc 3) (Voir carte de la région d'étude, Figure 1 du chapitre II). Dans chacun de ces blocs, nous avons choisi deux communautés tout en tenant compte de leurs localisations (au bord de la rivière *vs.* dans la forêt, ruisseaux et lacs) et de leurs tailles (plus petites *vs.* plus grandes). Déjà lors du premier terrain d'échantillonnage, une troisième communauté avait été inclue dans le bloc 1, et ce à la demande des riverains du village (*Campo Alegre*) qui voulaient participer à l'étude. La figure 2 présente le nombre total de communautés participant à l'enquête alimentaire.

Tableau 1 : Les communautés sélectionnées pour l'étude (Groupe I), selon les besoins des différents volets du projet.

	Volets				
Communautés	**Milieu biophysique et pratiques agricoles**	**Réseaux Sociaux**	**Poissons & Pratiques de pêche**	**Alimentation & Santé**	**Occupation du territoire**
Capituã	Présence de berges intactes et de forêts d'igapós âgées	Présence de pêcheurs occasionnels	Milieu témoin	-	Intérêt quant à la structure foncière
Mussum/Vista Alegre	Présence de *terra preta do indio* et de végétaux variés	Communauté grande et organisée	Présence de petits cours d'eau (*ruisseaux d'eaux claires*).	Grande consommation de poissons et de fruits	Diversité des activités commerciales
São Luís do Tapajós	Terres pauvres; présence d'arbres fruitiers sur les terrains des villageois; culture de manioc et de riz	Communauté organisée	Grand nombre de pêcheurs; présence de *riverains natifs* et *nordestins*.	Consommation de poissons et de fruits; absence d'étude neurotoxique chez les humains	Colonisation ancienne par les riverains natifs de la région

Açaituba	Communauté très pauvre; protestante; exploitation de bois; cultures de subsistance	Communauté très pauvre; protestante; personnes engagées	Manque d'organisation communautaire; pêche lointaine	Pauvreté; santé précaire	Colonisation récente (<10 ans); contact avec Fordlândia
Nova Canaã	Village entouré par des fermes	Communauté très organisée autour de la religion; conflit de pêche	Pêcheurs originaires de la région	Pas d'arbres fruitiers sur les lots des habitants	Présence d'un conflit entre les pêcheurs et les fermiers; communauté bien organisée
Santo Antônio	Mosaïque d'usage du territoire	Communauté catholique	Activité de pêche réalisée sur l'igarapé pour la consommation et sur le Tapajós pour la vente	Consommation de poissons et de fruits; localisation à l'intérieur des terres	Cultures de manioc pour le marché d'Itaituba et présence d'élevage d'animaux

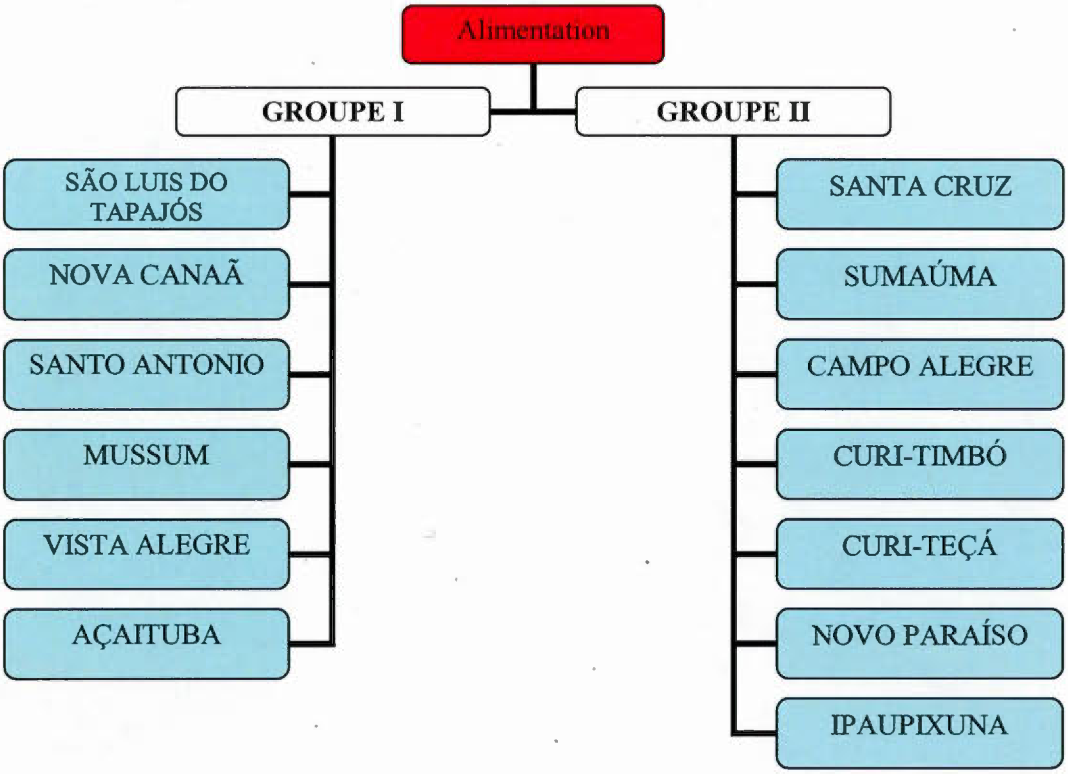

Figure 2 : Liste des communautés ayant participé à l'étude sur l'alimentation et la santé.

À cause des différences saisonnières dans la biodisponibilité des fruits et des poissons, nous avons mené une étude épidémiologique transversale en deux temps : à la saison des eaux descendantes (en juin 2003) et à la saison des eaux montantes (en janvier 2004). Lors de la saison des eaux descendantes, nous avons rejoint 449 personnes dans 13 villages. Chaque personne a répondu aux questionnaires sur la fréquence de consommation de fruits, et des poissons et sur les informations socio-démographiques (age, sexe, communauté d'appartenance, consommation d'alcool, tabagisme, scolarité, etc.). Des échantillons de sang et de cheveux ont été collectés. À la saison des eaux montantes, nous avons rejoint 171 personnes vivant dans 7 villages. Les protocoles et les outils méthodologiques ont été les mêmes que dans la saison antérieure, mais pour cette saison en plus du sang et des cheveux, nous avons aussi récolté des échantillons d'urine pour examiner les bioindicateurs du mercure inorganique dans la population.

10. Présentation des chapitres

Le premier chapitre présente une analyse critique sur la littérature scientifique concernant les niveaux d'exposition humaine au mercure en Amazonie, ainsi que leurs effets néfastes sur la santé des populations. Les chapitres suivants présentent les méthodes et les analyses réalisées dans le cadre de cette étude épidémiologique. Le deuxième chapitre présente l'étude qui examine l'influence de la consommation de fruits régionaux sur la relation entre la consommation de poissons et les teneurs en mercure, tant dans le sang que dans les cheveux des villageois. Dans le troisième chapitre on calcule les taux d'ingestion quotidienne de mercure et on examine les associations des taux d'ingestion quotidienne avec des bioindicateurs d'exposition. Le quatrième chapitre porte sur les relations entre la consommation de poissons et de fruits et les bioindicateurs de mercure inorganique. La thèse se termine avec les conclusions générales concernant l'ensemble des études qui ont été menées, et propose de nouvelles études nécessaires à l'approfondissement des connaissances.

11. Références bibliographiques

Adachi, T., Yasutake, A., Hirayama, K. 1992. Influence of dietary protein levels on the fate of methylmercury and glutathione metabolism in mice. Toxicology 72:17-26.

Alexander, J., Thomassen, Y. 1983. Increased urinary excretion of selenium among workers exposed to elemental mercury vapor. J Appl Toxicol 3:143-145.

Amorim, M.I.M., Mergler, D., Bahia, M.O., Dubeau, H., Miranda, D., Lebel, J., Burbano, R.R., Lucotte, M. 2000. Cytogenetic damage related to low levels of methyl mercury contamination in the Brazilian Amazon. An. Acad. Bras. Ci. 72 (4):497-507.

Andersen, H.R., Andersen, O. 1993. Effects of dietary α-Tocopherol and β-Carotene on lipid peroxidation induced by methyl mercuric chloride in mice. Pharmacology and Toxicology 73:192-201.

Apostoli, P., Cortesi, I., Mangili, A., Elia, G., Drago, I., Gagliardi, T., Soleo, L., Valente, T., Sciarra, GF., Aprea, C., Ronchi, A., Minoia, C., 2002. Assessment of reference values for mercury in urine : the results of an Italian polycentric study. Sci Total Environ 289-13-24.

Aschner, M., Clarkson, TW., 1988. Distribution of mercury 203 in pregnant rats and their fetuses following systemic infusions with thiol-containing amino acids and glutathione during late gestation. Teratology 38(2): 145-155.

Bastos, WR., Gomes, JPO., Oliveira, RC., Almeida, R., Nascimento, EL., Bernardi, JVE., Lacerda, LD., Silveira, EG., Pfeiffer, WC., 2006. Mercury in the environment and riverside population in the Madeira River Basin, Amazon, Brazil. Sci Total Environ 368: 344-351.

Berglund, M., Lind, B., Bjornberg, A., Palm, B., Einarsson, O., Vahter, M., 2005. Inter-individual variations of human mercury exposure biomarkers: a cross-sectional assessment. Environ Health 4:20.

Boudou, A., Dominique, Y., Cordier, S., Fréry, N., 2006. Les chercheurs d'or et la pollution par le mercure en Guyane française : conséquences environnementales et sanitaires. Environnement, Risques & Santé 5(3) : 167-179.

Branches, F.J.P., Erickson, T.B., Aks, S.E. et Hryhorczuk, D.O. 1993. The price of gold: mercury exposure in the Amazonian rain forest. Journal of Toxicology and Clinical Toxicology 31:295-306.

Calabrese, E.J. 1980. Nutrition and Environmental Health: the influence of nutritional status on pollutant toxicity and carcinogenicity, volume 1, the vitamins. John Wiley & Sons, New York.

Calabrese, E.J., Stoddard, A., Leonard, D.A., Salvatore, R.D. 1987. The effects of vitamin C supplementation on blood and hair levels of cadmium, lead, and mercury. Ann N Y Acad Sci 498:347-353.

Campos, M.S., Sarkis, E.S., Müller, R.C.S., Brabo, E.S., Santos, E.O. 2002. Correlation between mercury and selenium concentrations in Indian hair from Rondônia State, Amazon region, Brazil. The Science of the Total Environment 287: 155-161.

CARUSO. Mercury exposure and ecosystem health in the Amazon. 2007. http://www.unites.uqam.ca/gmf/caruso/caruso.htm

Cernichiari, E., Myers, GJ., Ballatori, N., Zareba, G., Vyas, J., Clarkson, T., 2007. The biological monitoring of prenatal exposure to methyl mercury. Neurotoxicology, doi: 10.1016/j.neuro.2007.02.009

Chan, H.M. & Receveur, O. 2000. Mercury in the traditional diet of indigenous peoples in Canada. Environmental Pollution 110 : 1-2.

Chowdhury, B.A., Chandra, R.K. 1987. Biological and health implications of toxic heavy metals and essential trace element interactions. Progress in Food and Nutrition Science 11: 55-113.

Chapman, L. & Chan, H. M. 2000. The influence of Nutrition on Methylmercury Intoxication. Environ Health Perspect 108 (suppl 1): 29-56.

Clarkson, TW., 1987. Metal toxicity in the central nervous system. Environ Health Perspect 75: 59-64.

Clarkson, T.W. 1992. Mercury: Major issues in environmental health. Environmental Perspectives 100: 31-38.

Clarkson, TW., Magos, L., 2006. The toxicology of mercury and its chemical compounds. Crit Rev Toxicol 36: 609-662.

Cordier, S., Grasmick, C., Paquier-Passelaigue, M., Mandereau, L., Weber, J-P., Jouan, M., 1998. Mercury exposure in French Guiana: levels and determinants. Arch Environ Health 53(4): 299-305.

Cordier, S., Garel, M., Mandereau, L., Morcel, H., Doineau, P., Gosme-Seguret, S., Josse, D., White, R., Amiel-Tison, C., 2002. Neurodevelopmental investigations among methyl mercury-exposed cildren in French Guiana. Environ Res 89: 1-11.

Counter, SA., Buchanan, LH., Ortega, F., 2006. Neurocognitive screening of mercury-exposed children of Andean gold miners. Int J Occup Environ Health 12: 209-214.

Couto, RCS., Câmara, VM., Sabroza, PC., 1988. Intoxicação mercurial: resultados preliminares em duas áreas garimpeiras – PA. Cad Saúde Pública 4(3): 301-315.

Cuvin-Aralar, M.L.A., Furness, R.W. 1991. Mercury and selenium interaction: a review. Ecotoxicology and Environmental Safety 21: 348-364.

Dewailly, E. 1997. Evaluation of prenatal exposure to organochlorines and heavy metals in Nunavik newborns 1993-1996. In: Environmental Studies, 74: Synopsis of research conducted under the 1995-1997 Northern Contaminants Program (Jensen J, ed). Ottawa, Canada: Indian and Northern Affairs Canada, p. 293.

Dirks, M.J., Davis, D.R., Cheraskin, E., Jackson, J.A. 1994. Mercury excretion and intravenous ascorbic acid. Archives of Environmental Health 49(1): 49-52.

Dolbec, J., Mergler, D., Sousa Passos, C.J., Morais, S.S., Lebel, J. 2000. Methylmercury exposure affects motor performance of a riverine population of the Tapajos River, Brazilian Amazon. Int Arch Occup Environ Health 73: 195-203.

Dolbec, J., Mergler, D., Larribe, F., Roulet, M., Lebel, J., Lucotte, M. 2001. Sequential analysis of hair mercury levels in relation to fish diet of an Amazonian population, Brazil. The Science of the Total Environment 271: 87-97.

Dórea, JG., 2003. Fish are central in the diet of Amazonian riparians: should we worry about their mercury concentrations? Environ Res 92: 232-244.

Dórea, JG., 2004. Cassava cyanogens and fish mercury are high but safely consumed in the diet of native Amazonians. Ecotoxicol Environ Safety 57: 248-256.

Dórea, JG., Souza, JR., Rodrigues, P., Ferrai, I., Barbosa AC., 2005. Hair mercury (signature of fish consumption) and cardiovascular risk in Mundurucu and Kayabi Indians of Amazonia. Environ Res 97:209-219.

Dufour, D. & Teufel, N. 1995. Minimum Data Sets for the Description and Measurement of Food Intake and Nutritional Status. In: Moran. E.F. (ed), The comparative analysis of human societies: toward common standards for data collection and reporting, Boulder, Lynne Rienner Publishers.

Dunn, JD., Clarkson, TW., 1980. Does mercury exhalation signal demethylation of methylmercury? Health Phys. 38(3): 411-414.

Farella, N., Lucotte, M., Louchouarn, P., Roulet, M. 2001. Deforestation modifying terrestrial organic transport in the Rio Tapajós, Brazilian Amazon. Organic Geochemistry 32: 1443-1458.

Fillion, M., Mergler, D., Passos, CJS., Larribe, F., Lemire, M., Guimarães, JRD., 2006. A preliminary study of mercury exposure and blood pressure in the Brazilian Amazon. Environ Health 5:29.

Fox, M.R.S. 1979. Nutritional influences on metal toxicity: cadmium as a model toxic element. Environmental Health Perspectives 29: 95.

Fujimoto, Y., Yoshida, A., Morisawa, K., Ueno, T., Fujita, T. 1985. Enhancement of methylmercury-induced lipid peroxidation by the addition of ascorbic acid. Research Communications in Chemical Pathology and Pharmacology 49(2): 267-275.

Fujimoto, Y., Sakamoto, N., Kitaura, H., Ikeda, E., Fujita, T. 1988. Stimulatory action of ascorbic acid on methylmercury-induced lipid peroxidation in rabbit kidney cortex slices. Research Communications in Chemical Pathology and Pharmacology 60(1): 1988.

Furkino, H., Hirai, M., Hsueh, Y.M., Yamane, Y. 1984. Effect of zinc pretreatment on mercuric chloride-induced lipid peroxidation in the rat kidney. Toxicology and Applied Pharmacology 73: 395-401.

Furst, A., 2002. Can nutrition affect chemical toxicity? International Journal of Toxicology 21: 419 – 424.

Ganther H.E. et al. 1973. Protective effects of selenium against heavy metal toxicities. In: Trace Substances in Environmental Health, VI.D.D. Hemphill, Ed., University of Missouri Columbia Mo., p. 247

Ganther, H.E. et Sunde, M.L. 1974. Effect of tuna fish and selenium on the toxicity of methylmercury: a progress report. Journal of Food Science 39: 1-5.

Gassó, S., Cristòfol, M., Selema, G., Rosa, R., Rodrígues-Farré, E., Sanfeliu, C. 2001. Antioxidants compounds and Ca2+ pathways blockers differentially protect against methylmercury and mercuric chloride neurotoxicity. Journal of Neuroscience Research 66: 135-145.

Goyer, R.A. 1995. Nutrition and metal toxicity. American Journal of Clinical Nutrition 61 (suppl): 646-650.

Goyer, R.A. 1997. Toxic and essential metal interactions. Ann Rev Nutr 17: 35-50.

Grandjean, P., Weihe, P., Jorgensen, P.J., Clarkson, T., Cernichiari, E., Videro, T. 1992. Impact of maternal seafood diet on fetal exposure to mercury, selenium, and lead. Arch Environ Health 47: 185-195.

Grandjean, P., White, R.F., Nielsen, A., Cleary, D., De Oliveira Santos, E.C. 1999. Methylmercury neurotoxicity in Amazonian children downstream from gold mining. Environmental Health Perspectives 107(7): 587-591.

Guimarães, JRD., Meili, M., Hylander, LD., Silva, EC., Roulet, M., Mauro, JBN., Lemos, RA., 2000. Mercury net methylation in five tropical flood plain regions of Brazil: high in the rood zone of floating macrophyte mats but low in surface sediments and flooded soils. Sci Total Environ 261: 99-107.

Guimarães, JRD., 2001. Les processus de méthylation du mercure en milieu amazonien. *In*: Carmouze JP, Lucotte M and Boudou A (coord.), Le mercure en Amazonie: Rôle de l'homme et de l'environnement, risques sanitaires. IRD Éditions, Paris.

Hansen, J.C., Kromann, N., Wulf, H.C., Alboge, K. 1984. Selenium and its interrelation with mercury in wholeblood and hair in an East Greenlandic population. The Science of the Total Environment 38: 33-40.

Harada, M., Nakanishi, J., Yasoda, E., Pinheiro, MCN., Oikawa, T., Guimarães, GA., Cardoso, BS., Kizaki, T., Ohno, H., 2001. Mercury pollution in the Tapajós River Basin, Amazon: mercury level of haid hair and health effects. Environ Int 27: 285-290.

Hill, C.H. 1980. Interactions of vitamin C with lead and mercury. Ann N Y Acad Sci, 355: 262-266.

Huss-Ashmore, R. & Curry, J. 1991. Diet, Nutrition, and Agricultural Development in Swaziland. 2. Patterns of Food Consumption. Ecology of Food and Nutrition 26: 167-185.

Inskip, MJ., Piotrowski, JK., 1985. Review of the health effects of methyl mercury. J Appl Toxicol 5(3): 113-133.

Iyengar, G.V., Nair, P.P. 2000. Global outlook on nutrition and environment: meeting the challenges of the next millennium. The Science of the Total Environment 249: 331-346.

Johnsson, C., Schutz, A., Sallsten, G., 2005. Impact of consumption of freshwater fish on mercury levels in hair, blood, urine, and alveolar air. J Toxicol Environ Health Part A; 68: 129-140.

Kazantzis, G., 2002. Mercury exposure and early health effects: an overview. Med Lav 93(3): 139-147.

Kleinschuster, S.J., Yoneyama, M., Sharma, R.P. 1983. A cell aggregation model for the protective effect of selenium and vitamin E on methylmercury toxicity. Toxicology 26: 1-9.

Kostial, K., Rabar, I., Blanusa, J., Simonovic, I. 1980. The effect of iron additive to milk on cadmium, mercury, and manganse absorption in rats. Environmental Research 22: 40-45.

Kostial, K., Blanusa, M., Rabar, I., Simonovic, I. 1981. More data on mercury absorption in relation to dietary treatment in rats. Toxicology Letters 7: 201-205.

Lebel, J., Mergler, D., Lucotte, M., Amorim, M., Dolbec, J., Miranda, D., Arantes, G., Rheaulth, I., Pichet, P. 1996. Evidence of early nervous system dysfunctions in Amazonian populations exposed to low-level of methylmercury. Neurotoxicology 17(1) : 157-168.

Lebel, J., Roulet, M., Mergler, D., Lucotte, M. et Larribe, F. 1997. Fish diet and mercury exposure in a riparian Amazonian population. Water Air Soil Pollut 97:31-44.

Lebel, J., Mergler, D., Branches, F., Lucotte, M., Amorim, M., Larribe, F., Dolbec, J. 1998. Neurotoxic effects of low-level methylmercury contamination in the Amazonian Basin. Environmental Research (Section A) 79 :20-32.

Lebel, J., 2003. La santé : une approche écosystémique. Centre de recherches pour le développement international, Ottawa, 84 p.

Levander, O.A. 1977. Nutritional factors in relation to heavy metal toxicants. Federation Proceedings 36(5): 1683-1687.

Levander, O.A., Cheng, L. 1980. Micronutrient interactions, vitamins, minerals, and hazardous elements. Ann N Y Acad Sci 355: 1-372.

Levy, M., Schwartz, S., Dijak, M., Weber, J-P., Tardif, R., Rouah, F., 2004. Childhood urine mercury excretion: dental amalgam and fish consumption as exposure factors. Environ Res 94:283-290.

Magos, L. 1991. Overview on the protection given by selenium against mercurials. In: Advances in mercury toxicology (Suzuki T, Imura N, Clarkson TW, eds). New York: Plenum Press, p. 289-297.

Magos, L., Clarkson, TW., 2006. Overview of the clinical toxicity of mercury. Ann Clin Biochem. 43(4): 257-268.

Malm, O., Pfeiffer, W.C., Souza, C.M.M. et Reuther, R. 1990. Mercury pollution due to gold mining in the Madeira river basin, Brazil. Ambio 19: 11-15.

Mergler, D., Anderson, HA., Chan, LHM., Mahaffey, KR., Murray, M., Sakamoto, M., Stern, AH., 2007. Methyl mercury exposure and health effets in humans: a worldwide concern. Ambio 36(1): 3-11.

Mertens, F., Saint-Charles, J., Mergler, D., Passos, CJ., Lucotte, M., 2005. A network approach for analysing and promoting equity in participatory ecohealth research, EcoHealth 2: 113-126.

More, M.R. 1979. Diet and lead toxicity. Proc. Nutr. Soc 38: 243.

National Research Council, 2000. Toxicological effects of methyl mercury. National Academy Press, Washington DC, 344 pp.

O'Brien, RG., 1998. A Tour of UnifyPow: A SAS Module/Macro for Sample-Size Analysis. Proceedings of the 23rd SAS Users Group International Conference, Cary NC: SAS Institute, 1346-1355.

Pace, V., Iannucci, E. 1994. The importance of vitamins in relation to the presence of heavy metals in food. Panminerva Med 36 : 80-82.

Passos, CJ., Mergler, D., Gaspar, E., Morais, S., Lucotte, M., Larribe, F., Grosbois, S., 2001. Caracterização do consumo alimentar de uma população ribeirinha na Amazônia Brasileira. Rev Saúde & Ambiente 4(1/2): 72-84.

Passos, C.J., Mergler, D., Gaspar, E., Morais, S., Lucotte, M., Larribe, F., Davidson, R., Grosbois, S., 2003. Eating tropical fruit reduces mercury exposure from fish consumption in the Brazilian Amazon. Environ Res 93: 123-130.

Passos, CJS., Mergler, D., Larribe, F., 2004. Response to "Fruits, fish and mercury: further considerations". Environ Res 96: 102-108.

Parizek, J., Ostadalova, I. 1967. The protective effect of small amounts of selenium in sublimate intoxication. Experientia 23: 142.

Parizek, J., et al. 1971. Effect of mercuric compounds on the maternal transmission of selenium in the pregnant and lactating rat. J. Reprod. Fertil 25: 157.

Peraza, M.A., Ayala-Fierro, F., Barber, D.S., Casarez, E., Rael, L.T. 1998. Effects of micronutrients on metal toxicity. Environmental Health Perspectives 106 (suppl 1): 203-216.

Pinheiro, MCN., Crespo-López, ME., Vieira, JLF., Oikawa, T., Guimarães, GA., Araújo, CC., Amoras, WW., Ribeiro, DR., Herculano, AM., Nascimento, JLM., Silveira, LCL., 2007. Mercury pollution and childhood in Amazon riverside villages. Environ Int 33: 56-61.

Plessi, M., Bertelli, D., Monzani, A. 2001. Mercury and selenium content in selected seafood. Journal of Food Composition and Analysis 14: 461-467.

Rao, M.V., Chinoy, N.J., Suthar, M.B., Rajvanshi, M.I. 2001. Role of ascorbic acid on mercuric chloride-induced genotoxicity in human blood cultures. Toxicology in Vitro 15: 649-654.

Rao, M.V., Sharma, P.S.N. 2001. Protective effect of vitamin E against mercuric chloride reproductive toxicity in male mice. Reproductive Toxicology 15: 705-712.

Receveur, O., Boulay, M., Kuhnlein, HV., 1997. Decreasing traditional food use affects diet quality for adult Dene/Métis in 16 communities of the Canadian Northwest Territories. J. Nutr. 127: 2179 – 2186.

Roulet, M., Lucotte, M., Canuel, R., Rheaulth, I., Tran, S., De Freitas Goch, Y., Farella, N., Souza do Vale, R., Sousa Passos, C.J., De Jesus da Silva, E., Mergler, D. et Amorim, M. 1998. Dissolved and particulate mercury in the Tapajos River Basin, Brazilian Amazon. The Science of the Total Environment 213 : 203-211.

Roulet, M., Lucotte, M., Farella, N., Serique, G., Coelho, H., Sousa Passos, C.J., De Jesus da Silva, E., Scavone de Andrade, P., Megler, D., Guimarães, J.R.D. et Amorim, M. 1999. Effects of human colonization on the presence of mercury in Amazonian ecosystems. Water Air and Soil Pollution 12: 297-313.

Rowland, I.R., Mallett, A.K., Flynn, J., Hargreaves, R.J. 1986. The effect of various dietary fibers on tissue concentration and chemical form of mercury after methylmercury exposure in mice. Arch Toxicol 59: 94-98.

Sandstead, H.H. 1980. Interactions of toxic elements with essential elements: introduction. Ann N Y Acad Sci 355: 282-284.

Santos, ECO., de Jesus, IM., Brabo, ES., Fayal, KF., Filho, GCS., Lima, MO., Miranda, AMM., Mascarenhas, AS., Sá, LLÇ., da Silva, AP., Câmara, VM., 2003. Exposição ao mercúrio e ao arsênio em Estados da Amazônia: síntese dos estudos do Instituto Evandro Chagas/FUNASA. Rev Bras Epidemiol 6(2): 171-185.

Sharma, D.C., Davis, P.S., Sharma, P.K. 1982. Effect of ascorbic acid on biotransformation and modification of the toxicity of mercurials in goldfish (Carassius auratus). Experientia 38: 565-567.

Shrimpton, R., Giugliano, R. 1979. Consumo de alimentos e alguns nutrientes em Manaus, Amazonas. 1973-4. Acta Amazônica 9(1) : 117-141.

Silva, IA., Nyland, JF., Gorman, A., Perisse, A., Ventura, AM., Santos, ECO., Souza, JM., Burek, CL., Rose, NR., Silbergeld, EK., 2004. Mercury exposure, malaria, and serum antinuclear/antinucleolar antibodies in Amazon populations in Brazil: a cross-sectional study. Environ Health 3:11.

Solomons, N.W., Viteri, E.E. 1982. Biological interaction of ascorbic acid and mineral elements (iron, selenium, copper, nickel, manganese, zinc, cobalt, cadmium, mercury, vitamin C). Adv Chem Ser 200: 551-569.

Spiegel, SJ., Yassi, A., Spiegel, JM., Veiga, MM., 2005. Reducing mercury and responding to the global gold rush. The Lancet 366: 2070-2072.

Usher, P.J., Baiki., M., Demmer, M., Nakashima, D., Stevenson, M.G., Stiles, M. 1995. Communicating about contaminants in country food: the experience in aboriginal communities. Inuit Tapirisat of Canada. Ottawa.

Veiga, M.M., Mech, J.A., Onate, N. 1994. Nature 368: 816.

Virtanen, JK., Rissanen, TH., Voutilainen, S., Tuomainen, T-P., 2007. Mercury as a risk factor for cardiovascular diseases. Journal of Nutritional Biochemistry 18:75-85.

Welsh, S.O. & Soares, J.H. 1976. The protective effect of vitamin E and selenium against methylmercury toxicity in the Japanese quail. Nutr Rep Int 13: 43.

WHO. 1990. International Programme on Chemical Safety. Environmental Health Criteria 101: Methylmercury. Geneva, 144 p.

Yokoo, EM., Valente, JG., Grattan, L., Schimidt, SL., Platt, I., Silbergeld, EK., 2003. Low level methyl mercury exposure affects neuropsychological functions in adults. Environ Health 2: 8.

Zorn, N.E., Smith, J.T. 1990. A relationship between vitamin B_{12}, folic acid, ascorbic acid, and mercury uptake and methylation. Life Sciences 47: 167-173.

CHAPITRE I

MERCURY EXPOSURE AND ADVERSE HEALTH EFFECTS IN THE AMAZON: A REVIEW

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Cet article est en révision dans

Environmental Health

RÉSUMÉ

Au cours des deux dernières décennies il y a eu beaucoup de recherches sur la pollution mercurielle et l'exposition humaine en Amazonie. Cet article a pour finalité d'analyser la littérature scientifique concernant les niveaux d'exposition humaine au mercure en Amazonie ainsi que leurs effets sur la santé des populations. Des niveaux d'exposition très élevés ont été mesurés chez plusieurs populations minières et traditionnelles, et on a rapporté une série d'effets adverses tant chez des adultes que chez des enfants. Les effets neurotoxiques ont été les plus étudiés, et on a observé des atteintes non seulement précoces mais aussi cliniques. Des altérations cytogénétiques, immunologiques et cardiovasculaires ont été également rapportées, mais l'évidence est plus controversée. Malgré des appels pour des interventions communautaires visant à réduire l'exposition en milieu de travail et dans l'environnement, très peu d'initiatives sont documentées. La seule intervention documentée en milieu riverain porte sur l'utilisation de la grande biodiversité des poissons et des fruits amazoniens pour réduire l'exposition humaine tout en permettant le maintien de la consommation de poissons. Il y a un besoin urgent de joindre les efforts des scientifiques et des décideurs afin de mener des interventions communautaires basées sur l'action concrète et permettant de maximiser l'apport nutritionnel de l'alimentation traditionnelle amazonienne, tout en minimisant le plus possible les risques toxiques de l'exposition au mercure.

Mots clés : Mercure, exposition, effets, santé, Amazonie

ABSTRACT

There has been much research on mercury pollution and human exposure in the vast Amazonian region. Deforestation and soil erosion, as well as gold-mining operations, are major sources of mercury release into this environment. Elevated exposure levels were initially reported in mining communities exposed to mercury vapors and later in traditional populations in several Amazonian countries, where fish is a dietary mainstay. This paper reviews the scientific literature on mercury exposure through fish consumption, adverse health effects and their repercussions for intervention and environmental health. A considerable range of adverse health effects have been reported both for adults and children. Neurotoxic outcomes have received the most attention, including early deterioration of several specific domains and, in some instances, clinical insults. There is some evidence of cytogenetic damage, immunologic changes and cardiovascular toxicity. Dietary studies suggest that shift in fish-eating practices to less contaminated fish can successfully reduce exposure on a short-term basis, and fruit consumption is associated with lower blood and hair mercury concentrations at similar levels of fish consumption. Despite early calls for intervention to reduce occupational and environmental exposures, few actions have been initiated and debate still rages on the appropriateness of intervention in this region with prevalent tropical diseases. Environmental conditions and ecosystem degradation are at the source of mercury exposure as well as many tropical diseases. There is an urgent need to join efforts and conduct action-based transdisciplinary research that will provide this area of the world with the means to preserve its rich biodiversity, reduce the source of mercury exposure and improve human health.

Key words: Mercury exposure, health effects, Amazon

Introduction

Mercury (Hg) contamination of the large Amazonian Basin has been the subject of much concern over the last two decades. In 1979, a major 'gold rush' began in the Brazilian Amazon, which, over time, brought several hundred thousand persons (*garimpeiros*) to the region searching for riches [1]. A health study carried out between 1986 and 1991, revealed elevated levels of urinary and blood Hg concentrations in miners with signs and symptoms of Hg intoxication [2]. World attention focused on the plight of miners and those exposed to the vapors of gold-refining [1-3]. Biogeochemical studies showed that these uncontrolled gold-mining activities released thousands of tons of Hg into the environment and elevated levels of this metal were reported in water, sediments and fish [3-4]. Gold-mining became synonymous with Hg pollution [5]. In the Amazon, the bioaccumulation and biomagnification of methyl-Hg in fish as well as the link between fish consumption and increased hair Hg levels were made in the early nineties [4]. In comparison to the northern regions of the world, the Amazonian environment can be much more propitious for the formation of methyl-Hg. Flooded forest soils are generally considered highly favorable for the production and bioaccumulation of methyl-Hg [6], and the processes of bioaccumulation of Hg in the aquatic biota coupled to biomagnification through the aquatic food chain explain the presence of high quantities of Hg in predatory fish of this region [7]. As data accumulated on elevated levels of Hg in human and fish populations, even among those living far away from gold-mining activities, other primary Hg sources were proposed. Veiga et al. [8] estimated that forest fires and biomass burning could make an important contribution to the environmental Hg burden. Subsequent studies on the distribution and partition of total Hg in waters of the Tapajós River in Brazil showed that Hg content in the water column was influenced by the amount of particulate matter, independently from upstream gold-mining activities [9]. Pioneering studies, conducted in the Tapajós region, further showed that Amazonian soils constitute major natural Hg reservoirs, which release substantial amounts of the metal into the aquatic ecosystems through soil erosion and lixiviation resulting from deforestation practices, such as 'slash and burn' agriculture and/or cattle raising [10-13]. Since then, a growing number of publications has corroborated the role of deforestation and soil erosion as a major source of Hg release into the waterways, and hence to halieutic resources and human fish-eating communities [14-18]. Deforestation and soil erosion resulting from gold mining activities as well as Hg releases from abandoned mining sites constitute additional sources of Hg to the environment [19]. Because of the multiple sources and continuing environmental degradation leading to increased Hg in the food web, Hg exposure through fish consumption is very pervasive throughout the large Amazonian region.

Much of our knowledge of environmental methyl-Hg clinical toxicity is strongly linked to the disasters that occurred over 40 years ago in Minamata and Niigata, Japan, as well as in Iraq [20-21]. These high level exposures, which caused death and neurologic damage to thousands who were directly exposed or exposed *in utero*, have recently been reviewed elsewhere [22, 23]. While current international public health concerns on the toxicological risks of methyl-Hg have focused almost exclusively on developmental neurotoxicity associated with chronic prenatal exposures [24, 25], comparatively little attention has been paid to the protection of traditional populations in the Amazon who, despite many years of research and biomonitoring, are currently still exposed to elevated levels of this toxicant through their fish diet [26-27].

The purpose of this paper is to critically review the scientific literature on Hg exposure through fish consumption and adverse health effects in the Amazon, with a view to understanding the implications for intervention, risk management and environmental health in this developing region.

Methods

Computerized database searching (MEDLINE, LILACS, SCIELO, SCOPUS), secondary reference searching and hand-searching were used to identify relevant primary studies and reviews dealing with biogeochemical and ecotoxicological issues, human exposure, health risk assessment and communication, human health effects as well as dietary/nutritional studies and interventions to reduce exposure and toxicity.

We systematically searched for cohort, case-control and cross-sectional studies preferably based on multivariate analytical methods. In addition to peer-reviewed documents, we also reviewed published reports by international organizations dealing with policy and research approaches for programs to reduce the adverse ecological and human health impacts of Hg pollution. The bibliographic research covered articles and reports written in English, Portuguese, Spanish, and French.

In all, we examined 24 primary studies covering the anthropogenic and natural sources of Hg into the environment and its related ecotoxicological issues. Fifty-four (54) studies addressed questions relating to human exposure in mining, riparian, indigenous and urban populations, while only 8 investigations assessed health risk and communication. Human toxicological outcomes were examined in 34 studies, and a limited amount of work was retrieved on community-based interventions and dietary, nutritional as well as genetic factors that could influence Hg exposures in the Amazon. For the sake of comparisons, many primary studies and review papers as well as authoritative sources analyzing Hg issues around the globe are included in the present report, which make a total of 42 documents.

Results and discussion

Mercury exposure through fish consumption in the Amazonian Basin

Hair Hg is generally considered to be a good indicator for dietary exposures through fish consumption [22, 25, 28], and many studies in the Amazon have examined hair Hg concentrations in occupational settings at gold-mining and refining sites and for environmental exposures in urban and remote riparian and indigenous settlements. Table 1 provides a chronological summary by country for studies conducted in several Amazonian watersheds and cities (Figure 1). The first published assessment of hair Hg levels was made in gold miners and dates back to 1988 in the Brazilian literature [60], while the first evaluation in remote riparian villagers was reported by Malm et al. [4] for communities along the Madeira River. At around the same time, Boischio and Barbosa [29] reported that out of 311 hair samples analyzed in the Madeira River area, 51% had Hg levels over 10μg/g, with wide inter-individual variations and concentrations as high as 303μg/g. To our knowledge, Malm et al. [4] authored the first report that recognized fish consumption as the route of exposure for communities not living in the vicinity of gold-mining sites. Since those initial studies, consistently elevated levels of hair Hg have been reported in different watersheds of the Brazilian Amazonian Basin. In 1995, Olivero et al. [57] reported elevated hair Hg levels in a mixed population living in a mining area of the Colombian Amazon, with fishermen showing higher levels than miners, and in 1998 a study in French Guiana, another Amazonian country, likewise revealed increased Hg levels in fish consumers of different ethnic groups [54]. Dietary exposures through fish consumption have since been reported for the Amazonian areas of Bolivia, Ecuador, and Suriname [56, 58-59, 61].

Some studies have provided information on the percentage of methyl-Hg in hair samples (Table 1). In general methyl-Hg is above 70%, but a few studies show wider variations.

Table 1: Hair mercury levels reported for fish-eating groups in the Amazon Basin

Location	Population	Age (years)	n	Total hair Hg (μg/g)		%MeHg (Range)	References
				Mean ± SD	Range		
Brazil							
Madeira River	Riparian	-	713	15.2 ± 9.6	6 - 150	-	[26]
Tapajós River	Riparian	< 15 - 65	69	$\cong 15 \pm$ n/a	-	-	[27]
Madeira River	Riparian	-	241	$17.2 \pm$ n/a	N.A – 303^{d}	e	[29]
Xingu Park	Indigenous	-	27	18.5 ± 5.9	6.9 - 34	-	[30]
Cuniã Lake Reserve	Riparian	-	75	$8.7 \pm$ n/a	N.A – 31.9	70 -80	[31]
Fresco River	Indigenous	-	419	$8.0 \pm$ n/a	-	-	[31]
Tapajós River	Riparian	-	101	$21 \pm$ n/a	4.7 - 151	85 - 91	[32]
Santarém City	Urban	-	10	$2.7 \pm$ n/a	-	-	[32]
Gurupi goldfield	Riparian	-	10	-	0.2 - 15	-	[33]
Negro River	Riparian	-	101	38.6 ± 14.4	-	-	[34]
Teles Pires, Juruena Rivers	Indigenous	-	55	$34.2 \pm$ n/a	10 - > 50	87.2^{f}	[35]
Yanomami Indians	Indigenous	-	14	3.3 ± 1.2^{d}	1.5- 6.0^{d}	58 – 97	[36]
Tapajós & Madeira Rivers	Riparian	-	82	16.7 ± 7.3^{d}	1.0 – 59.4^{d}	8.7 - 100	[36]
Tapajós River	Riparian	≥ 15	96	12.9^{b}	0 – 145	73 - 94	[37]
Fresco River	Indigenous	< 2 – 45	28	8.1 ± 3.2	0.8 – 13.7	-	[38]

Madeira River	Riparian	< 2 – 45	98	14.1 ± 10.7	2.6 – 94.7	-	[39]
Madeira River	Riparian	0.1 - 32	28	-	4.0 – 84.4	84 - 90	[38]
Pracuúba Lake	Riparian	-	15	16.7 ± n/a	-	-	[41]
Alta Floresta City	Urban	14 - 45	75	1.1 ± 1.17	0.1 – 8.2	-	[42]
Tapajós River	Riparian	-	-	17 ± 8.5	2.9 – 71.5	-	[43]
Xingu Park	Indigenous	-	-	13.6 ± 4.7	4.3 – 32.8[d]	-	[44]
Negro River	Riparian	< 15 - 40	76	21.4 ± 12.7	1.7 - 59	34 – 100	[45]
Tapajós River	Riparian	12 - 68	36	12.5[b]	2.9 - 27	-	[46]
Rio Branco City	Urban	0 - > 60	2318	2.4 ± 3.9	0.8 – 72.7	-	[47]
Tapajós River	Riparian	< 1	-	12 ± n/a	9.6 – 14.6	-	[48]
Santarém City	Urban	17 - 25	44	2.0 ± 1.8	0.08 – 15.2	-	[49]
Pakaanóva Indians	Indigenous	0.5 - 90	910	8.4 ± 6.4	0.5 – 83.9	-	[50]
Tapajós River	Indigenous	< 10	203	8.1 ± 5.2	-	-	[51]
Negro River	Urban	-	399	11.4 ± n/a	0.1 – 83.1	-	[52]
Tapajós, Tocantins Rivers	Riparian	0.1 - 12	168	5.1[c]	0.4 – 53.8	-	[53]
French Guiana							
Maroni & Oyapock Rivers	Indigenous	<15 - 80	156	5.4[c]	0.2 – 530	-	[54]
Maroni River	Indigenous	23[f]	235	11.4 ± 4.2	1.9 – 27.2	-	[55]
Bolivia							

Beni River	Indigenous	-	80	9.8 ± n/a	4.3 – 19.5	-	[56]
Colombia							
San Jorge River	Riparian	15 - 65	94	4.9 ± 0.55	0.5 - 40	-	[57]
Ecuador							
Napo River Valley	Urban	-	46	1.9 ± n/a	0.03 – 10	-	[58]
Napo River Valley	Indigenous	-	54	7.0 ± n/a	1.5 – 13.6	-	[58]
Nambija mining area	Indigenous	0 - 14	80	6.0 ± 17.5	1.0 - 135	-	[59]

[a] Not available [b] Median [c] Geometric mean [d] Hair MeHg [e]: authors indicate that 70% of a sub-sample had >80% MeHg [f] Arithmetic mean

Figure 1: Amazonian watersheds and important cities and towns where Hg studies were carried out.

In 3 pregnant women from French Guiana with exceptionally elevated levels of hair Hg (88μg/g, 420μg/g, and 530μg/g), speciation of Hg showed that it was mainly inorganic Hg; the authors suggested that this probably resulted from direct exposures to metallic Hg in the households [54]. Indeed, pregnant women constitute a group deserving particular attention since Hg is actively transferred through the placenta to the fetus [62-64].

Among fish-eating populations, riparian communities present the highest degree of exposure, with community hair Hg means as high as 38.6 ± 14.4μg/g [34] and 65 ± 58μg/g

[65]. Overall, urban fish-consumers are the least exposed, presenting hair Hg means of 2.4µg/g [47], 2µg/g [49] and 1.9µg/g [58]. However, a recent pilot study carried out in two municipalities of the Negro River in the Brazilian Amazon (São Gabriel da Cachoeira and Barcelos) revealed average hair Hg concentrations of 13µg/g and 9.7µg/g, with upper values as high as 81.3µg/g [52]. While these latter authors indicate the existence of gold mining in the Upper Negro River (Colombian Amazon) as the probable source, recent biogeochemical studies have shown the important impact of naturally occurring Hg in this basin [14, 66-67], concluding that Hg leaching from soils constitutes the largest regional reservoir of this metal [14]. These findings are supported by studies analyzing environmental determinants of Hg levels in predatory fish as well as dietary human exposure, all of which indicate that fish and human Hg levels in the Negro River Basin are strongly influenced by naturally high background levels of Hg [34, 68-69, 173].

The widespread evidence of elevated Hg levels in Amazonian communities can be seen as a reflection of the multiplicity of Hg sources into the environment and halieutic resources. While in some areas of Colombia, Suriname , and Venezuela, the use of Hg in gold mining predominates over deforestation and/or hydroelectric damming [57, 61, 70], in other regions, like in Peru, it is difficult to ascertain whether fish-Hg contamination is attributable to mining or other causes [71], and in countries like Brazil, Ecuador, and Bolivia there seems to be an interplay of these various sources [17, 18, 26, 56 72, 73], which contribute to environmental and aquatic biota contamination in a synergistic manner, as recently proposed in the French Guiana Amazon [19].

Fish eating practices are the most important predictor of Hg exposure, explaining a substantial proportion of the variation of total and methyl-Hg concentrations in both blood and hair. Based on these biomarkers, as well as Hg measurements in fish tissue, many studies in the Amazon have associated Hg exposure with fish consumption [4, 26-27, 31, 32-37, 40, 42-43, 46-47, 50, 54, 56, 74], especially for members of fishing families [41, 55, 75]. These studies support the universal observation that fish consumption constitutes the most important source of Hg exposure worldwide [22, 28]. What distinguishes communities in the Amazon from many others around the world is that for an important proportion of this population, fish is the dietary mainstay and many eat fish at least once a day.

Studies on Hg in fish have noted a very sizeable variation in concentrations among a large number of species, ranging from 0.01 up to 3.77µg/g, depending on the feeding habits and location [26, 32, 37, 40, 56]. There are however few studies of Hg speciation in fish from the Amazonian area, and notable exceptions are Kehrig et al. [40] and Maurice-Bourgoin et al. [56] who respectively reported methyl-Hg fractions above 95% and varying from 73% to 98% of the total Hg in fish from the Balbina Reservoir and the Madeira River areas. Recent studies of total fish-Hg concentrations in the Amazon have stressed the particular influence of annual flooding [69, 76] and the role of fish species' food regimes [77, 78] on the bioaccumulation patterns in different ecosystems. Other factors controlling Hg levels in predatory fish species are pH and dissolved organic carbon [68]. Flooded forests and macrophyte mats are specific features of many of the waterways of the Amazonian Basin, and are important links between Hg inputs from natural or anthropogenic sources and Hg exposure of local populations through fish intake [7].

Investigators who examined the strength of the associations between fish consumption frequency and hair Hg concentrations [65, 37, 46, 49, 57], have shown correlation coefficients varying between 0.2 and 0.89, depending on the frequency of fish intake as well as the fish species consumed. In addition, significant seasonal differences in hair Hg levels have been reported for villages on the Tapajós River [37, 46], where water levels can vary by around six meters between the rainy and dry seasons, affecting fish habitats and availability. In contrast, no seasonal variations were observed for communities in the Andean Amazon [58] although the local habits of fish consumption as well as the different fish-eating patterns among communities were important determinants of exposure.

To date, hair concentrations in most studies from the Amazon surpass those reported in many areas of the world. In the children's cohort study in the Seychelles Islands, maternal hair level of total Hg during pregnancy ranged from 0.5 to 26.7µg/g [62], while Grandjean et al. [63] reported a geometric hair Hg average concentration of 4.27µg/g in Faroese mothers at parturition. A report from China indicated mean hair Hg of 4.0µg/g for male fish consumers [79], and hair Hg levels ranging from 0.01 to 15.2µg/g were recently correlated to monthly fish meals in U.S. [80]. Based on follow-up data of childbearing age women living in the Arctic Canada, Van Oostdan et al. [81] indicated hair Hg levels ranging from 0.5 to 41.4µg/g for the period of 1976-1979 as compared to concentrations varying between 0 – 11.5µg/g for the period between 1998 and 1999. Indeed in some countries where studies have focused on Hg, there has been a reduction in exposure over time [82-85]. In the Amazon, despite a pilot and localized participatory intervention experience showing reductions in Hg exposure over time [123, 169], overall levels remain elevated.

Urinary Hg is the most commonly used biomarker to assess exposure from inhalation of elemental Hg vapours [22]. In the Amazonian mining regions, gold burning activities have contributed to substantial Hg exposure, with urinary Hg values ranging from 0.2 to 663µg/L in adults [2, 31, 33, 86, 87-88], while in children concentrations between 26 and 159µg/L [59, 89] have been reported. However, consistent with recent findings in other areas of the world [90-94], in non-occupationally exposed populations in the Brazilian Amazon, fish-eating has been associated with relatively elevated levels of urinary Hg. Passos and colleagues [95] reported urinary Hg levels above 10µg/L for almost 20% of a population with no evidence of exposure from Hg vapors. In this study, urinary Hg was related to consumption of carnivorous fish, suggesting that inorganic Hg may be absorbed from certain fish or possible demethylation following absorption of a bolus dose of methyl-Hg.

Given the elevated levels of exposure, some researchers have attempted to estimate the rates of Hg intake for purposes of health risk assessment. Table 2 lists studies which measured daily fish and Hg intake rates in different settings. Large amounts of fish are consumed throughout the Amazon; average daily intake in one area was around 340 g/day and reached up to more than 600 g/day in periods of abundance [55]. For many in the Amazonian watershed, fish constitutes the dietary mainstay, with intake depending on cultural practices and seasonal availability of this food. Different investigators have estimated the health risks of Hg exposure using predictive models. Kehrig et al. [40] estimated that the population living near the Barbina Reservoir ingested 35.2µg of methyl-Hg during the whole year, while Fréry et al. [55], who performed extensive calculations using data from fish-Hg concentrations as well as amounts and frequency of fish consumed by each household, estimated Hg intake varying from 40 to 60µg/day. Others have presented their estimates in µg/kg/day; these Hg intake rates, presented in Table 2, range from 0 to more than 4µg/kg/day, largely exceeding regulatory guidelines for Hg intake [24]. Boischio and Henshel [96] and Hacon et al. [75] likewise estimated potential health risks using hazard quotients (HQ), based on the ratio of the estimated dose rate to the selected reference dose. If

HQ is less than one, there is no anticipated risk for adverse health effects, while there is a possibility for such risks in sensitive groups if it is equal or greater than one [75]. Based on a reference dose of 0.3μg/kg/day, these studies showed that the highest hazard quotients were observed for the riparian populations of the Madeira River.

Table 2: Summary of risk assessment studies in Amazonian settings

Location	Setting	Mean fish intake (g/day)	Hg intake			Mean Hazard Quotient	References
			Total population	Women	Children		
Madeira River	Riparian	200	2.2 μg/kg/day	1.2[a]	6.4[b]	0.02[w]; 21[c]	[96]
Tartarugalzinho River	Riparian	200	1.6 μg/kg/day	-	-	-	[97]
Alta Floresta City	Urban	8[gp]; 5[c], 110[f]	2.2 μg/kg/day[f]	-	3.5[f]	1.4[gp]; 8.6[f]	[75]
Barbina Reservoir village	Riparian	110	35.2 μg/y	-	-	-	[40]
Madeira River	Riparian	243	2.6 μg/kg/day	-	-	-	[98]
Upper Maroni River	Indigenous	163.1	40 – 60 μg/day	41.4[e]	17.5[e]	-	[55]
Northern Mato Grosso State Municipalities	Urban	-	0.01 – 3.9 μg/kg/day	-	-	-	[99]
Tapajós River	Riparian	141[m]	0 – 4.3 μg/kg/day	-	-	-	[100]

[a] 15 - 48 years old [b] < 5 years old [w] Women [c] Children [gp] General population [e] μg/day [f] Fisher families [m] g/meal

Human Health Studies

Over the last decade a number of human health studies have been carried out in different Amazonian communities. We identified 34 studies, of which the large majority was conducted in the Brazilian Amazon (82%). Sixty-three percent (63%) focused on adult populations, while 24% examined children and 12% surveyed both adults and children. All of the studies used cross-sectional designs. Hair Hg was the most used biomarker of exposure (76%), followed by blood (21%) and urinary Hg concentrations (12%). Neurotoxic outcomes were the most extensively examined: 36% sought to identify clinical neurological signs of Hg intoxication, whereas 42% tested the hypothesis of long-term low-dose exposure as a risk factor for neurobehavioral deficits at the population level. The remaining 12% included cytogenetic damage and more recent emerging evidence on immunologic and cardiovascular effects.

Neurotoxic Effects

Table 3 summarizes the results of studies which examined associations between exposure and neurofunctional deficits in fish-eating communities. Significant dose-effect associations were reported for motor, visual and/or cognitive functions in adults [101-105]. Two child studies identified deficits in attention and visuo-spatial performance as well as poorer coordination of the legs [106-107], whereas two other studies did not detect effects [108-109]. These latter negative studies examined riparian and urban children respectively, with substantially lower levels of exposure compared to other Amazonian communities; both used different neurobehavioral and statistical approaches from previous investigations, which makes it difficult to compare their findings with the others. On the other hand, the positive findings in the Amazon child studies are consistent with other investigations conducted around the world, such as the Faeroe Islands and New Zealand prospective cohort studies [110-112] and cross-sectional investigations in the Canadian Arctic and Japan [113-114].

In the Amazonian region, Hg exposure has been negatively associated with hearing thresholds as well as auditory brainstem evoked responses in both children and adults living in the Nambija gold mining area of Ecuador [115-116]. Although the studied population lived in a mining area, the authors suggest that these persons may be at neurological risk not only from elemental Hg vapors inhaled during amalgam burning, but also from Hg exposure through consumption of contaminated foods. In another study conducted in the Brazilian

Amazon, auditory deficits were not observed for children and adolescents exposed through fish consumption [117]. It is difficult to ascertain whether or not the differences in exposure scenario (inorganic Hg vapors vs. dietary methyl-Hg) might be the underlying reason for the inconsistencies.

Other investigators used clinical methods including neurological examinations to identify symptoms typical of methyl-Hg poisoning (e.g. glove- and stocking-type sensory disturbance, constriction of visual field, tremor, and cognitive disturbances such as difficulty in concentrating and recent memory losses). We identified 7 clinical cross-sectional studies of fish-eating communities, of which two reported neurological deficits related to Hg exposure, including sensory and balance disturbances, tremor, hyperreflexia, dysarthria, among others [118-119]. In addition, the study of Lebel et al. [102] included a neurological examination which showed that although participants had normal results on most items, those with higher hair Hg levels presented a significantly high prevalence of disorganized movements on the Branches Alternate Movement Task (BAMT) as well as restricted visual fields. The BAMT, which requires the subject to perform coordinated hand-knee movements, continually passing one arm over the other, as rapidly as possible [102], was developed by Dr. Fernando Branches, a physician and pioneer in Hg neurotoxicity in the Brazilian Amazon [102, 32, 2]. Reduced performance on the BAMT has also been observed in connection with low level Hg exposures through fish consumption as well as with occupational exposures in Italian cohorts [120-121]. The results of the clinical investigations of Amazonian communities provide supplementary evidence of Hg neurotoxicity in fish-eating riparian villagers, despite the non-specific nature of a number of signs.

Non-neurologic outcomes

Only one study has reported cytogenetic damage (decreased mitotic index and increased frequency of polyploid cells) related to hair Hg levels in a fish-eating community on the Tapajós River [122]. In a follow-up of this population, five years later, after a 40% decrease in hair Hg levels, the same cytogenetic endpoints no longer displayed significant associations with hair Hg. The authors suggested that this would be consistent with a reduced biological impact resulting from the reduction in Hg exposure [123]. There is, however, a lack of evidence in the literature concerning the association of both organic and inorganic Hg with respect to cytogenetic outcomes, and more studies are needed to better comprehend these early biomarkers of cytogenetic insult.

Table 3: Summary of neurobehavioral outcomes examined in relation to hair Hg levels in Amazonian fish-eating communities.

References	**101**	**102**	**103**	**104**	**105**	**106**	**107**	**108**	**109**
	Adult populations					**Child populations**			
Hair Hg (μg/g)	14^{a}	$\cong 13^{b}$	11 ± 6	4 ± 2	$0.6 - 72^{c}$	11^{a}	10.2^{a}	5 ± 3	1.81^{b}
Color vision	↓*	↓	-	-	↓*	-	-	-	-
Near visual contrast sensitivity	↓	↓*	-	-	↓*	-	-	-	-
Grip strength	↓	↓	-	-	-	-	-	-	-
Muscular fatigue	↑	↑	-	-	-	-	-	-	-
Visual field	↓	↓*	-	-	-		-	-	-
Manual dexterity	↓*	↓*	↓*	-	-	↓*	-	-	-
Fine motricity	-	-	↓*	↓*	-	↓*	↓	-	-
Logical memory	-	-	-	↓*	-	-	-	-	-
Digit span	-	-	-	↓*	-	↓*	↓*	-	-
Easy learning	-	-	-	↓*	-	-	-	-	-
Concentration	-	-	-	↓*	-	-	-	-	-
Visuospatial functions	-	-	-	-	-	↓*	↓*	-	-
Leg coordination	-	-	-	-	-		↓*	-	-
Static balance	-	-	-	-	-	-	-	n/s	-
Dynamic balance	-	-	-	-	-	-	-	n/s	-
Appendicular coordination	-	-	-	-	-	-	-	n/s	-
Motor	-	-	-	-	-	-	-	n/s	-

persistence									
Trunk-limb coordination	-	-	-	-	-	-	-	n/s	-
Sensory perception	-	-	-	-	-	-	-	n/s	-
Gesell developmental schedules	-	-	-	-	-	-	-	-	n/s

[a] Geometric mean [b] Median [c] Range [n/s] Non-significant

* Statistically significant

Some investigations have recently focused on possible immunologic outcomes associated with Hg exposure. One epidemiological study reported that even if no relation was observed between biomarkers of Hg exposure and prevalent malaria, the odds ratio for reporting past malaria infection was four times higher for those also reporting a history of working with inorganic Hg [124]. Further testing of the relation between Hg exposure and prevalent malaria in the Amazon has produced consistent results in terms of Hg-induced effects on biomarkers of autoimmunity. One study reports on immunologic changes indicative of autoimmune dysfunction in environmentally exposed villagers [125], and another investigation shows that positive serum antinuclear antibodies are more frequently observed in riparian fish-eaters than in low fish consumers from an urban center [126]. The experimental and human evidence of possible inorganic Hg-induced immunologic changes [127] are particularly important in the Amazon, given considerable genetic variability among riparian, indigenous and other traditional populations, as well as concomitant exposures to vector-born diseases, such as malaria and Chagas infection.

Only two studies have analyzed cardiovascular outcomes in relation to Hg in the Amazon; one examined four indigenous tribes [128] and the other surveyed six riparian communities on the Tapajós [129]. Dórea et al. [128] reported that while no significant differences in body mass index were observed between tribes, there was a trend of lower increase in blood pressure with age among the higher fish consumers. On the other hand, Fillion et al. [129] showed blood pressure directly and significantly associated with total hair

Hg levels, and no association between fish consumption and blood pressure. Given the higher levels of exposure in many communities of the Amazon as compared to European and North American cohorts [130-131], and because of possible toxicokinetic interactions promoted by traditional diet [132-134], future epidemiological studies should attempt to include a larger range of cardiovascular endpoints, and further examine the possible influence of dietary and genetic factors.

The role of dietary and nutritional factors

Because of protective effects observed in a certain number of animal studies, selenium (Se) has received the most attention as a potential protector against Hg toxicity in populations consuming seafood [135-136], and more recently there has been a search for other candidate nutrients that could protect against Hg absorption and/or toxicity [137]. A few studies have analyzed blood and/or hair Se concentrations among fish-eating communities in areas of the Brazilian Amazon [44, 138-140]. While most are in the normal range, Lemire et al. (2006) [140] reported very elevated blood Se concentrations for a group of persons in one village, suggesting that there may be wide variations throughout the region. There are some reports [44, 138, 140] of positive relations between biomarkers of Se and Hg, suggesting that fish may constitute a source of dietary Se. Although the authors of these studies point out that Se may play a role with respect to Hg toxicity, no investigation has yet been carried out.

Recent studies by Passos and collaborators [100, 132-134] show that the strong relationship between fish consumption and Hg exposure is significantly modified by fruit consumption, which may indicate possible toxicokinetic interactions promoted by fruit intake in Amazonian traditional communities. Despite general recognition of the antioxidant and nutraceutical properties of many nutrients contained in fruits [141], including Amazonian fruits [142-145], this food has received little attention as a dietary factor with a potential to counteract Hg exposure and/or toxicity. And yet, fruit gathering and consumption are repeatedly reported by many studies dealing with Hg issues in traditional communities of the Amazon [26, 34, 56, 98, 146-147]. Fruit is a particularly important component of riparian and indigenous diets, contrary to the diet of communities occupationally exposed in the mining areas [148]. Notwithstanding the encouraging and promising results concerning the role of fruits on Hg exposure, the studies conducted so far have not examined the possible physiologic events that may be involved in the interactions between fruit nutrients and Hg absorption, and the potential protective effect of fruit intake with respect to toxicological outcomes also remains to be demonstrated.

Interventions

There has been some debate in the Amazon as to what should be done or whether anything should be done about Hg exposures through fish consumption. Some researchers have suggested that "normal" safe values, higher than those proposed by WHO, could be established in the Amazon [147], while others have alleged that notwithstanding the high concentrations of Hg, daily consumption of large amounts of fish does not necessarily pose a health hazard [149-150]. A contrasting opinion was expressed even in the early nineties, when some authors urged for community-based interventions for both the occupational settings [2] and the environmental context [34]. Considering the potential for Hg-induced deleterious health effects, these latter authors indicated that environmental biomonitoring would be pointless if not directed at active action, and suggested that any approach seeking solutions for the high exposure problem should include a major educational component.

Most mitigation actions have been undertaken to reduce the use of Hg in gold-mining activities. In recent years, international organizations and researchers have deployed considerable efforts to introduce cleaner artisanal gold mining and extraction technologies in many developing countries [151-152]. These initiatives center on capacity- and awareness-building about the links of small-scale gold mining practices with human and ecosystem health [153]. Initial and encouraging results from these initiatives in terms of reduction of Hg use and emissions through the adoption of homemade solutions have recently been reported in Africa [154]. In the Amazon, during the early nineties, major projects proposed alternative techniques to reduce Hg use and exposure in gold mining activities [152, 155-156], but the results of the efficiency of these interventions are not available. There are ongoing and valuable educational and capacity-building efforts in Brazil, Venezuela, Ecuador and French Guyana [157, 19]. These initiatives have encountered several barriers to change, the most important and challenging one being poverty; lack of resources and political support are also cited [154, 158]. Hilson et al. [159-160] have pointed out that despite extensive research conducted over the past 15-20 years in Latin America and in Africa, most of the investigations have failed to identify appropriate mitigation measures, and education strategies have at best been marginally effective due to socioeconomic and poor living conditions in mining areas.

To date, there has been little effort linked to the mitigation of Hg contamination through reduction in soil erosion, although there are current attempts to reforest areas on the Upper Tapajós region (Brazil), where gold mining was extensive and many studies have been carried out [161]. In addition, the multiple pressures for "development" of the Amazon through extensive exploitation of several natural resources and, more particularly, the recent push of the agricultural frontier, constitute a further barrier for reduction of deforestation and soil erosion [162-163]

A few efforts have addressed the question of fish-eating practices [19, 98, 169], although in general there has been little focus on this issue possibly due to lack of political interest and the difficult trade-offs in public health regarding the reduction of toxic exposures through fish consumption. Indeed, these trade-offs have been the subject of much international debate over the last years because of the nutritional benefit from fish consumption [164-167]. Modifying fish-eating habits through the use of fish advisories has constituted the most popular approach to reduce exposures and health risks in North America, Europe and Japan, even if such approach is considered by many policy makers an unfortunate and interim public health need [168]. In the Amazon, debate has been raging on the appropriateness of intervention not only because fish is a very important dietary mainstay, but according to some authors, priority should rather be given to the high prevalence of other general health problems such as tropical diseases and intestinal parasites [149-150, 156]. Passos et al. [100] discuss the suitability of using fish advisories as a policy intervention in the Amazonian context, and suggest that a risk management strategy that focuses solely on limiting the number of fish meals might deprive these populations of this important food source. In the short-term, and due to the high biodiversity of fish and fruit in the Amazon, dietary Hg exposures in the environment may be reduced through changes in fish-eating practices without necessarily limiting fish consumption frequency [100,169].

One such intervention was carried out in a village on the Tapajós River in Brazil. The investigators used a participatory strategy based on capacity building, which aimed at maintaining fish consumption but shifting towards less contaminated fish species [169]. Through community involvement and education based on posters showing the status of Hg contamination in relation to fish species, villagers continued to eat the same quantity of fish, but decreased the proportion of carnivores, which resulted in a reduction of close to 40% of hair Hg levels. An interesting aspect explored in this process was the role of community participation to achieve exposure reduction [169]. Using a social communication network analysis [170], these authors observed that participation was associated with awareness of the critical information that led to changing dietary habits towards the preferential consumption of the less contaminated fish species.

Conclusions

The studies performed in the Amazon have added important information to our knowledge on Hg sources, exposure and effects. Despite the many gaps, a picture of a complex situation, reflecting the biodiversity of the region, is emerging. Hg pollution is coming from multiple anthropogenic and natural sources and the degree of contamination of the halieutic resources is further influenced by the dynamics of the regional and local ecosystems. For many Amazonian communities, fish is a dietary mainstay, resulting in elevated Hg exposures throughout the vast region. There is strong evidence for dose-related neurofunctional deficits in both adults and children, consistent with studies throughout the world [28], while other health outcomes (cardiovascular, cytogenetic, immunologic) require more investigation. These effects are observed at exposure levels below the Benchmark dose (BMD) for blood Hg (58μg/L) that was used to calculate the U.S. EPA reference dose [25], as well as the BMD for hair Hg (14μg/g) used to determine the recently revised World Health Organization reference dose [24]. At this point in time, we do not know whether the effects observed in children and adults of the Amazon are due to *in utero* exposure or to cumulative exposure throughout life.

Recent interest has focused on particularities of the Amazonian ecosystem and how this may affect Hg absorption and toxicity. The influence of the consumption of tropical fruits on the relation between fish consumption, Hg intake and bioindicators of Hg exposure needs to be better understood. In studies that have examined Se status in the Brazilian Amazon, levels appear to be relatively high, and more work needs to be done on the sources of Se as well as its possible effect and interaction with Hg.

The historical data presented here clearly and sadly shows that despite many years of research, Hg exposure remains high throughout the region. In this population that is already challenged by poor living and sanitary conditions, a high prevalence of tropical diseases and limited access to health care, further assault to physiological functions through exposure to Hg increases their vulnerability and diminishes their capacity for development. Mitigation efforts in mining activities have hopefully reduced direct exposure of many miners and their families, but for the moment, do not appear to be reflected in a reduction of Hg concentrations in fish or humans. Deforestation is extensive, among other reasons because of the advancement of the agricultural frontier. The literature likewise reflects a reticence on the part of public health authorities in some Amazonian countries to provide guidelines on fish consumption, in part due to the importance of the nutritional value of fish and the consideration that the health effects of Hg are less severe than those of other health problems.

Could and should Hg reduction become a priority in the Amazon region? If one makes a long list of priorities, because of the multitude of environmental, social and health problems, it might not be very near the top. But if one adopts an ecosystem approach that examines how it fits into current concerns for deforestation, environmental degradation from mining, social and economic development of the region and improvement of human health, Hg reduction could be included as an outcome of research and intervention programs on many levels. To achieve this, we need to identify the social, political and economic factors underlying deforestation and small-scale gold-mining operations [171, 162-163], as well as the socio-cultural factors which influence regional agricultural and forestry practices and diet [18, 98, 172]. We need to increase our understanding of the biogeochemical issues that will allow for control of the sources that contribute to the release of Hg into the aquatic ecosystems, and increase our knowledge of the factors that influence Hg absorption and toxicity.

Because of the ongoing severity of the problem, on a short term basis we could take advantage of the high biodiversity of fish and fruits readily available in these ecosystems, by coupling traditional knowledge and cultural practices to scientific information on Hg transmission from fish to humans and the factors that influence this relation. The use of this information within a public health program could provide the means for modifying dietary habits and reduce Hg exposures, while maintaining the health benefits of a diet rich in fish intake.

Competing interests

The authors declare they have no competing interests.

Authors' contributions

CJSP is a doctoral fellow in environmental sciences. He designed the review project, carried out the bibliographic research and wrote the report. DM is thesis supervisor. She participated in the design and planning of the manuscript, literature appraisal and co-wrote the manuscript. Both authors read and approved the final text.

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References

1. Cleary, D., 1990. Anatomy of the Amazon Gold Rush. Iowa City: University of Iowa Press, 245 p.
2. Branches, FJ., Erickson, TB., Aks, SE., Hryhorczuk, DO., 1993. The price of gold: mercury exposure in the Amazonian rain forest. J Toxicol Clin Toxicol 31(2):295-306.

3. Pfeiffer WC., Lacerda, LD., Malm, O., Souza, CM., Silveira, EG., Bastos, WR., 1989. Mercury concentrations in inland water of gold-mining areas in Rondonia, Brazil. Sci Total Environ 87-88:233-240.

4. Malm, O., Pfeiffer, WC., Souza, CMM., Reuther, R., 1990. Mercury pollution due to gold mining in the Madeira River Basin, Brazil. Ambio 19(1): 11-15.

5. Malm, O., 1998. Gold mining as a source of mercury exposure in the Brazilian Amazon. Environmental Research Section A 77: 73-78.

6. Roulet, M., Maury-Brachet, R., 2001. Le mercure dans les organismes aquatiques amazoniens. *In:* Carmouze JP, Lucotte M and Boudou A (coord.), Le mercure en Amazonie: Rôle de l'homme et de l'environnement, risques sanitaires. IRD Éditions, Paris.

7. Guimarães, JRD., 2001. Les processus de méthylation du mercure en milieu amazonien. In: Carmouze JP, Lucotte M and Boudou A (coord.), Le mercure en Amazonie: Rôle de l'homme et de l'environnement, risques sanitaires. IRD Éditions, Paris.

8. Veiga, MM., Meech, JA., Onate, N., 1994. Mercury pollution from deforestation. Nature 368:815-816.

9. Roulet, M., Lucotte, M., Canuel, R., Rheault, I., Tran, S., Goch, YGF., Farella, N., Vale, RS., Passos, CJS., Silva, EJ., Mergler, D., Amorim, M., 1998. Distribution and partition of total mercury in waters of the Tapajós River Basin, Brazilian Amazon. Sci Total Environ 213:203-211.

10. Roulet M., Lucotte, M., Saint-Aubin, A., Tran, S., Rheault, I., Farella, N., Silva, EJ., Dezincourt, J., Passos, CJS., Soares, GS., Guimarães, JRD., Mergler, D., Amorim., 1998. The geochemistry of Hg in Central Amazonian soils developed on the Alter-do-Chão formation of the lower Tapajós river valley, Pará state, Brazil. Sci Total Environ 223: 1-24.

11. Roulet, M., Lucotte, M., Farella, N., Serique, G., Coelho, E., Passos, CJS., Silva, EJ., Andrade, PS., Mergler, D., Guimarães, JRD., Amorim, M., 1999. Effects of recent human colonization on the presence of mercury in Amazonian ecosystems. Water Air Soil Poll 112: 297-313.

12. Roulet, M., et al., 2000. Increase in mercury contamination recorded in lacustrine sediments following deforestation in the central Amazon. Chem Geol 165: 243-266.

13. Farella N, Lucotte M, Louchouarn P and Roulet M., 2001. Deforestation modifying terrestrial organic transport in the Rio Tapajós, Brazilian Amazon. *Org Geochem* 32: 1443-1458.

14. Fadini, PS., Jardim, WF., 2001. Is the Negro River Basin (Amazon) impacted by naturally occurring mercury? Sci Total Environ 275: 71-82.

15. Lacerda, LD., Souza, M., Ribeiro, MG., 2004. The effects of land use change on mercury distributions in soils of Alta Floresta, Southern Amazon. Environ Poll 129: 247-255.

16. Almeida, MD., Lacerda, LD., Bastos, WR., Herrmann., 2005. Mercury loss from soils following conversion from forest to pasture in Rondônia, Western Amazon, Brazil. Environ Poll 137: 179-186.

17. Mainville, N., et al., 2006. Decrease of soil fertility and release of mercury following deforestation in the Andean Amazon, Napo River Valley, Ecuador. Sci Total Environ 368: 88-98.

18. Farella, N., Lucotte, M., Davidson, R., Daigle, S., 2006. Mercury release from deforested soils triggered by base cation enrichment. Sci Total Environ 368: 19-29.

19. Boudou, A., Dominique, Y., Cordier, S., Frery, N., 2006. Les chercheurs d'or et la pollution par le mercure en Guyane française : conséquences environnementales et sanitaires. Environnement, Risques & Santé 5(3) : 167-179.

20. Igata, A., 1993. Epidemiological and clinical features of Minamata disease. Environ Res 63: 157-169.

21. Clarkson, TW., 1992. Mercury: major issues in environmental health. Environ Health Persp 100: 31-38.

22. Clarkson, TW., Magos, L., 2006. The toxicology of mercury and its chemical compounds. Crit Rev Toxicol 36: 609-662.

23. Hachiya, N., 2006. The history and the present of Minamata disease – entering the second half a century. Jap Med Assoc J 49(3): 112-118.

24. WHO., 2003. World Health Organization, Joint FAO\WHO Expert Committee on Food Additives JECFA/61/SC.

25. NRC, 2000. Toxicological effects of methyl mercury. National Academy Press, Washington DC, 344 pp.

26. Bastos, WR., Gomes, JPO., Oliveira, RC., Almeida, R., Nascimento, EL., Bernardi, JVE., Lacerda, LD., Silveira, EG., Pfeiffer, WC., 2006. Mercury in the environment and riverside population in the Madeira River Basin, Amazon, Brazil. Sci Total Environ 368: 344-351.

27. Pinheiro, MCN., Oikawa, T., Vieira, JLF., Gomes, MSV., Guimarães, GA., Crespo-López, ME., Muller, RCS., Amoras, WW., Ribeiro, DRG., Rodrigues, AR., Côrtes, MIT., Silveira, LCL., 2006. Comparative study of human exposure to mercury in riverside communities in the Amazon region. Braz J Med Biol Res 39: 411-414.

28. Mergler, D., Anderson, HA., Chan, LHM., Mahaffey, KR., Murray, M., Sakamoto, M., Stern, AH., 2007. Methyl mercury exposure and health effects in humans: a worldwide concern. Ambio 36(1): 3-11.

29. Boischio, AAP., Barbosa, A., 1993. Exposição ao mercúrio inorgânico em populações ribeirinhas do Alto Madeira, Rondônia, 1991: resultados preliminares. Cad Saúde Públ 9(2): 155-160.

30. Vasconcelos, MBA., Saiki, M., Paletti, G., Pinheiro, RMM., Baruzzi, RG., Spindel, R., 1994. Determination of mercury in head hair of Brazilian population groups by neutron activation analysis. J Radio Anal Nuclei Chem 179(2): 369-376.

31. Barbosa, AC., Boischio, AA., East, GA., Ferrari, I., Gonçalves A., Silva, PRM., Cruz, TME., 1995. Mercury contamination in the Brazilian Amazon: environmental and occupational aspects. Water Air Soil Pollut 80: 109-121.

32. Malm, O., Branches, FJP., Akagi, H., Castro, MB., Pfeiffer, WC., Harada, M., Bastos, WR., Kato, H., 1995. Mercury and methyl mercury in fish and human hair from the Tapajós river basin, Brazil. Sci Total Environ 175: 141-150.

33. Palheta, D., Taylor, A., 1995. Mercury in environmental and biological samples from a gold mining area in the Amazon region of Brazil. Sci Total Environ 168: 63-69.

34. Eve, E., Oliveira, EF., Eve, C., 1996. The mercury problem and diets in the Brazilian Amazon: planning a solution. Environ Cons 23(2): 133-139.

35. Barbosa, AC., Garcia, AM., Souza, JR., 1997. Mercury contamination in hair of riverine populations of Apiacás reserve in the Brazilian Amazon. Water Air Soil Poll 97: 1-8.

36. Kehrig, HA., Malm, O., Akagi, H., 1997. Methyl mercury in hair samples from different riverine groups, Amazon, Brazil. Water Air Soil Poll 97:17-29.

37. Lebel, J., Roulet, M., Mergler, D., Lucotte, M., Larribe, F., 1997. Fish diet and mercury exposure in a riparian Amazonian population. Water Air Soil Poll 97: 31-44.

38. Barbosa, AC., Silva, SRL., Dórea, JG., 1998. Concentrations of mercury in hair of indigenous mothers and infants from the Amazon Basin. Arch Environ Contam Toxicol 34: 100-105.

39. Boischio AAP., Cernichiari, E., 1998. Longitudinal hair mercury concentrations in riverside mothers along the upper Madeira River (Brazil). Environ Res Section A 77: 79-83.

40. Kehrig, HA., Malm., O., Akagi, H., Guimarães, JRD., Torres, JPM., 1998. Methyl mercury in fish and hair samples from the Balbina Reservoir, Brazilian Amazon. Environ Res 77: 84-90.

41. Guimarães, JRD., Fostier, AH., Forti, MC., Melfi, JA., Kehrig, H., Mauro, JBN., Malm, O., Krug, JF., 1999. Mercury in human and environmental samples from two lakes in Amapá, Brazilian Amazon. Ambio 28(4): 296-301.

42. Hacon, S., Yokoo, E., Valente, J., Campos, RC., Silva, VA., Menezes, ACC., Moraes, LP., Ignotti, E., 2000. Exposure to mercury in pregnant women from Alta Floresta – Amazon Basin, Brazil. Environ Res Section A 84: 204-210.

43. Pinheiro, MCN., Guimarães, GA., Nakanishi, J., Oikawa, T., Vieira, JL., Quaresma, M., Cardoso, B., Amoras, W., 2000. Avaliação da contaminação mercurial mediante análise do teor de Hg total em amostras de cabelo em comunidades ribeirinhas do Tapajós, Pará, Brasil. Rev Soc Bras Med Trop 33(2): 181-184.

44. Vasconcelos, MBA., Bode, P., Paletti, G., Catharino, MGM., Ammerlaan, K., Saiki, M., Fávaro, DIT., Byrne, AR., Baruzzi, R., Rodrigues, DA., 2000. Determination of mercury and selenium in hair samples of Brazilian Indian populations living in the Amazonic region by NAA. J Radioanaly Nuc Chem 244(1): 81-85.

45. Barbosa, AC., Jardim, W., Dórea, JG., Forsberg, B., Souza, J., 2001. Hair mercury speciation as a function of gender, age, and body mass index in inhabitants of the Negro River basin, Amazon, Brazil. Arch Environ Contam Toxicol 40: 439-444.

46. Dolbec, J., Mergler, D., Larribe, F., Roulet, M., Lebel, J., Lucotte, M., 2001. Sequential analysis of hair mercury levels in relation to fish diet of an Amazonian population, Brazil. Sci Total Environ 271: 87-97.

47. Santos, ECO., Jesus, IM., Brabo, ES., Câmara, VM., Loureiro, ECB., Macarenhas, AF., Faial, KF., Silva, AP., Silva, RU., Luiz, RR., Higuchi, H., 2002. Exposure to mercury in the urban population of Rio Branco City, State of Acre, Brazil. Bull Environ Contam Toxicol 69: 314-319.

48. Boischio, AAP., Mergler, D., Passos, CJ., Gaspar, E., Morais., 2003. Segmental hair mercury evaluation among mothers, their babies and breast milk along the Tapajós River, Amazon, Brazil. Environ Sci MY Tokyo 10(2): 107-120.

49. Passos, CJ., Lucotte, M., Queiroz, A., Mergler, D., Peleja, R., Goch, Y., Morais, S., 2003. Condições socio-econômicas e exposição ao mercúrio (Hg) através do consumo de peixe: um estudo de caso em Santarém, Pará, Brasil. Rev Saúde & Ambiente 6(1/2): 3-11.

50. Santos, ECO., Câmara, VM., Brabo, ES., Loureiro, ECB., Jesus, IM., Fayal, K., Sagica, F., 2003. Avaliação dos níveis de exposição ao mercúrio entre indios Pakaanóva, Amazônia, Brasil. Cad Saúde Pública 19(1): 199-206.

51. Dórea, JG., Barbosa, AC., Ferrari, I., Souza, JR., 2005. Fish consumption (hair mercury) and nutritional status of Amazonian Amerindian children. Am J Hum Biol 17:507-514.

52. Santos, ECO., Sá, GC., Jesus, IM., Brabo, ES., Câmara, VM., Lima, MO., Faial, KF., Mendes, RA., Mascarenhas, AFS., 2005. Mercúrio no Rio Negro, Amazonas, Brasil – estudo preliminar de indicadores de exposição no pescado e em populações humanas. Cad Saúde Coletiva (RJ) 13(1): 225-236.

53. Pinheiro, MCN., Crespo-López, ME., Vieira, JLF., Oikawa, T., Guimarães, GA., Araújo, CC., Amoras, WW., Ribeiro, DR., Herculano, AM., Nascimento, JLM., Silveira, LCL., 2007. Mercury pollution and childhood in Amazon riverside villages. Environ Int 33: 56-61.

54. Cordier, S., Grasmick, C., Paquier-Passelaigue, M., Mandereau, L., Weber, J-P., Jouan, M., 1998. Mercury exposure in French Guiana: levels and determinants. Arch Environ Health 53(4): 299-305.

55. Fréry, N., Maury-Brachet, R., Maillot, E., Deheeger, M., Mérona, B., Boudou, A., 2001. Gold mining activities and mercury contamination of native Amerindian communities in French Guiana: key role of fish in dietary uptake. Environ Health Perpect 109: 449-456.

56. Maurice-Bourgoin, L., Quiroga, I., Chincheros, J., Courau, P., 2000. Mercury distribution in waters and fishes of the upper Madeira Rivers and mercury exposure in riparian Amazonian populations. Sci Total Environ 260 : 73-86.

57. Olivero, J., Mendonza, C., Mestre, J., 1995. Mercurio en cabello de diferentes grupos ocupacionales en una zona de minería aurifera en el Norte de Colombia. Cad Saúde Pública 29(5), 376-379.

58. Webb, J., Mainville, N., Mergler, D., Lucotte, M., Betancourt, O., Davidson, R., Cueva, E., Quizhpe, E., 2004. Mercury in fish-eating communities of the Andean Amazon, Napo River Valley, Ecuador. EcoHealth 1(suppl 2): 59-71.

59. Counter, SA., Buchanan, LH., Ortega, F., 2005. Mercury levels in urine and hair of children in an Andean gold-mining settlement. Int J Occup Environ Health 11: 132-137.

60. Couto, RCS., Câmara, VM., Sabroza, PC., 1988. Intoxicação mercurial: resultados preliminares em duas áreas garimpeiras – PA. Cad Saúde Pública 4(3): 301-315.

61. Mohan, S., Tiller, M., van der Voet, G., Kanhai, H., 2005. Mercury exposure of mothers and newborns in Surinam: a pilot study. Clin Toxicol 43: 101-104.

62. Davidson, PW., Myers, GJ., Cox, C., Axtell, C., Shamlaye, C., Sloane-Reeves, J., Cernichiari, E., Needham, L., Choi, A., Wang, Y., Berlin, M., Clarkson, TW., 1998. Effects of prenatal and postnatal methyl mercury exposure from fish consumption on neurodevelopment: outcomes at 66 months of age in the Seychelles Child Development Study. JAMA 280: 701-707.

63. Grandjean, P., Weihe, P., White, RF., Debes, F., Araki, S., Yokoyama, K., Murata, K., Sorensen, N., Dahl, R., Jorgensen, PJ., 1997. Cognitive deficit in 7-year-old children with prenatal exposure to methyl mercury. Neurotoxicol Teratol. 19(6): 417-428.

64. Stern, AH., Smith, AE., An assessment of the cord blood: maternal blood methyl mercury ratio: implications for risk assessment. Environ Health Perspect 111:1465-1470.

65. Leino, T., Lodenius, M., 1995. Human hair mercury levels in Tucurui area, State of Pará, Brazil. Sci Total Environ 175: 119-125.

66. do Valle, CM., Santana, GP., Augusti, R., Filho, FBE., Windmoller, CC., 2005. Speciation and quantification of mercury in oxisol, ultisol, and spodosol from Amazon (Manaus, Brazil). Chemosphere 58:779-792.

67. do Valle, Santana, GP., Windmoller, CC., 2006. Mercury conversion processes in Amazon soils evaluated by thermodesorption analysis. Chemosphere 65:1966-1975.

68. Belger, L., Forsberg, BR., 2006. Factors controlling Hg levels in two predatory fish species in the Negro river basin, Brazilian Amazon. Sci total Environ 367:451-459.

69. Dórea, JG., Barbosa, AC., Silva, GS., 2006. Fish mercury bioaccumulation as a function of feeding behavior and hydrological cycles of the Rio Negro, Amazon. Comparative Biochemistry and Physiology, Part C 142:275-283.

70. Veiga, MM., Bermudes, D., Pacheco-Ferreira, H., Pedroso, LRM., Gunson, AJ., Berrios, G., Vos, L., Huidobro, P., Roeser, M., 2005. Mercury pollution from artisanal gold mining in Block B, El Callao, Bolívar State, Venezuela. *In:* Dynamics of mercury pollution on regional and global scales: atmospheric processes, human exposure around the world, p. 421-450. N. Pirrone & K. Mahaffey (Eds). Springer Publisher, Norwell, MA, USA.

71. Gammons, CH., Slotton, DG., Gerbrandt, B., Weight, W., Young, CA., McNearny, RL., Cámac, E., Calderón, R., Tapia, H., 2006. Mercury concentrations of fish, river water, and sediment in the Río Ramis-Lake Titicaca watershed, Peru. Sci Total Environ 368:637-648.

72. Hylander, LD., Grohn, J., Tropp, M., Vikstrom, A., Wolpher, H., Silva, EC., Meili, M., Oliveira, LJ., 2006. Fish mercury increase in Lago Manso, a new hydroelectric reservoir in tropical Brazil. J Environ Manag 81:155-166.

73. Betancourt, O., Narváez, A., Roulet, M., 2005. Small-scale gold mining in the Puyango River Basin, Southern Ecuador: a study of environmental impacts and human exposures. EcoHealth 2, 323-332.

74. Sing, KA., Hryhorczuk, DO., Saffirio, G., Sinks, T., Paschal, DC., Chen, EH., 1996. Environmental exposure to organic mercury among Makuxi in the Amazon basin. Int J Occup Environ Health 2(3): 165-171.

75. Hacon, S., Rochedo, ER., Campos, R., Rosales, G., Lacerda, LD., 1997. Risk assessment of mercury in Alta Floresta. Amazon Basin – Brazil. Water Air Soil Poll 97: 91-105.

76. Bastos, WR., de Almeida, R., Dórea, JG., Barbosa, AC., 2007. Annual flooding and fish-mercury bioaccumulation in the environmentally impacted Rio Madeira (Amazon). Ecotoxicology 16:341-346.

77. Régine, M-B., Durrieu, G., Dominique, Y., Boudou, A., 2006. Mercury distribution in fish organs and food regimes: significant relationships from twelve species collected in French Guiana (Amazonin basin). Sci Total Environ 368: 262-270.

78. Silva, DS., Lucotte, M., Roulet, M., Poirier, H., Mergler, D., Crossa, M., 2006. Mercury in fish of the Tapajós River in the Brazilian Amazon. INTERFACEHS 1(1), art 6.

79. Dickman, MD., Leung, CKM., Leong, MKH., 1998. Hong Kong sub-fertility links to mercury in human hair and fish. Sci Total Environ 214: 165-174.

80. Knobeloch, L., Gliori, G., Anderson, H., 2007. Assessment of methyl mercury exposure in Wisconsin. Environ Res. 103: 205-210.

81. Van Oostdam, J., Donaldson, SG., Feeley, M., Arnold, D., Ayotte, P., Bondy, G., Chan, L., Dewaily, E., Furgal, CM., Kuhnlein, H., Loring, E., Muckle, G., Myles, E., Receveur, O., Tracy, B., Gill, U., Kalhok, S., 2005. Human health implications of environmental contaminants in Arctic Canada: A review. Sci Total Environ 351-352: 165-246.

82. Deutch, B., Dyerberg, J., Pedersen, HS., Asmund, G., Moller, P., Hansen, JC., 2006. Dietary composition and contaminants in north Greenland, in the 1970s and 2004. Sci Total Environ 370: 372-381.

83. Schulz, C., Conrad, A., Becker, K., Kolossa-Gehring,M., Seiwert, M., Seifert, B., 2007. Twenty years of the German Environmental Survey (GerES): Human biomonitoring – Temporal and spatial (West Germany / East Germany) differences in populations exposure. Int. J. Hyg. Environ.-Health, in press.

84. Harada, M., Nakanishi, J., Konuma, S., Ohno, K., Kimura, T., Yamaguchi, H., Tsuruta, K., Kizaki, T., Ookawara, T., Ohno, H., 1998. The present mercury contents of scalp hair and clinical symptoms in inhabitants of the Minamata area. Environ Res Section A 77: 160-164.

85. Dumont, C., Girard, M., Bellavance, F., Noel, F., 1998. Mercury levels in the Cree population of James Bay, Quebec, from 1988 to 1993/94. Can Med Assoc Journal 158(11): 1439-1445.

86. Hurtado, J., Gonzales, GF., Steenland, K., 2006. Mercury exposures in informal gold miners and relatives in southern Peru. Int J Occup Environ Health 12: 340-345.

87. Rosa, RMSS., Muller, RCS., Alves, CN., Sarkis, JES., Bentes, MHS., Brabo, E., Oliveira, ES., Determination of total mercury in workers' urine in gold shops of Itaituba, Pará State, Brazil. Sci Total Environ 261: 169-176.

88. Jesus, IM., Santos, ECO., Brabo, ES., Loureiro, ECB., Câmara, VM., Mascarenhas, AF., Silva, DFL., Cleary, D., 2001. Exposure to elemental mercury in urban workers and gold miners from the Tapajós region, Pará, Brazil. Bull Environ Contam Toxicol 67: 317-323.

89. Harari, R., Forastiere, F., Axelson, O., 1997. Unacceptable "occupational" exposure to toxic agents among children in Ecuador. Am J Ind Med 32: 185-189.

90. Apostoli, P., Cortesi, I., Mangili, A., Elia, G., Drago, I., Gagliardi, T., Soleo, L., Valente, T., Sciarra, GF., Aprea, C., Ronchi, A., Minoia, C., 2002. Assessment of reference values for mercury in urine: the results of an Italian polycentric study. Sci Total Environ 289-13-24.

91. Berglund, M., Lind, B., Bjornberg, A., Palm, B., Einarsson, O., Vahter, M., 2005. Inter-individual variations of human mercury exposure biomarkers: a cross-sectional assessment. Environ Health 4:20.

92. Johnsson, C., Schutz, A., Sallsten, G., 2005. Impact of consumption of freshwater fish on mercury levels in hair, blood, urine, and alveolar air. J Toxicol Environ Health Part A; 68: 129-140.

93. Levy, M., Schwartz, S., Dijak, M., Weber, J-P., Tardif, R., Rouah, F., 2004. Childhood urine mercury excretion: dental amalgam and fish consumption as exposure factors. Environ Res 94:283-290.

94. Ohno, T., Sakamoto, M., Kurosawa, T., Dakeishi, M., Iwata, T., Murata, K., 2007. Total mercury levels in hair, toenail, and urine among women free from occupational exposure and their relations to renal tubular function. Environ Res 103: 191-197.

95. Passos, CJS., Mergler, D., Lemire, M., Fillion, M., Guimarães, JRD., 2007. Fish consumption and bioindicators of inorganic mercury exposure. Sci Total Environ 373: 68-76.

96. Boischio, AAP., Henshel, DS., 1996. Risk assessment of mercury exposure through fish consumption by the riverside people in the Madeira Basin, Amazon, 1991. Neurotoxicol 17(1): 169-176.

97. Bidone, ED., Castilhos, ZC., Santos, TJS., Souza, TMC., Lacerda, LD., 1997. Fish contamination and human exposure to mercury in Tartarugalzinho River, Amapá State, Northern Amazon, Brazil. A screening approach. Water Air Soil Poll 97: 9-15.

98. Boischio, AAP., Henshel, D., 2000. Fish consumption, fish lore, and mercury pollution – risk communication for the Madeira River people. Environ Res Section A 84: 108-126.

99. Hacon, S., Farias, RA., Barrocas, P., Campos, RC., Wasserman, J., Argento, R., 2005. Avaliação da exposição humana ao mercúrio na região norte de Mato Grosso – Amazônia Legal. Cad Saúde Coletiva (RJ) 13(4): 837-854.

100. Passos, CJS., da Silva, DS., Lemire, M., Fillion, M., Guimarães, JRD., Lucotte, M., Mergler, D., 2007. Daily mercury intake in fish-eating populations in the Brazilian Amazon. J Exp Sci Environ Epidemiol, accepted.

101. Lebel, J., Mergler, D., Lucotte, M., Amorim, M., Dolbec, J., Miranda, D., Arantes, G., Rheault, I., Pichet, P., 1996. Evidence of early nervous system dysfunctions in Amazonian populations exposed to low-level of methyl mercury. Neurotoxicol 17(1): 157-168.

102. Lebel, J., Mergler, D., Branches, F., Lucotte, M., Amorim, M., Larribe, F., Dolbec, J., 1998. Neurotoxic effects of low-level methyl mercury contamination in the Amazonian Basin. Environ Res 79: 20-32.

103. Dolbec, J., Mergler, D., Passos, C.J.S., Morais, S.S., Lebel, J., 2000. Methyl mercury exposure affects motor performance of a riverine population of the Tapajós River, Brazilian Amazon. Int Arch Occup Environ Health 73: 195-203.

104. Yokoo, EM., Valente, JG., Grattan, L., Schimidt, SL., Platt, I., Silbergeld, EK., 2003. Low level methyl mercury exposure affects neuropsychological functions in adults. Environ Health 2: 8.

105. Silveira, LCL., Ventura, DF., Pinheiro, MCS., 2004. Toxicidade mercurial – avaliação do sistema visual em indivíduos expostos a níveis tóxicos de mercúrio. Cienc Cult 56(1): 36-38.

106. Grandjean, P., White, RF., Nielsen, A., Cleary, D., Santos, ECO., 1999. Methyl mercury neurotoxicity in Amazonian children downstream from gold mining. Environ Health Perspect 107(7): 587-591.

107. Cordier, S., Garel, M., Mandereau, L., Morcel, H., Doineau, P., Gosme-Seguret, S., Josse, D., White, R., Amiel-Tison, C., 2002. Neurodevelopmental investigations among methyl mercury-exposed children in French Guiana. Environ Res 89: 1-11.

108. Tavares, LMB., Câmara, VM., Malm, O., Santos, ECO., 2005. Performance on neurological development tests by riverine children with moderate mercury exposure in Amazonia, Brazil. Cad Saúde Pública 21(4): 1160-1167.

109. Marques, RC., Dórea, JG., Bastos, WR., Rebelo, MF., Fonseca, MF., Malm, O., 2007. Maternal mercury exposure and neuro-motor development in breastfed infants from Porto Velho (Amazon), Brazil. Int J Hyg Environ Health 210: 51-60.

110. Debes, F., Budtz-Jorgensen, E., Weihe, P., White, RF., Grandjean, P., 2006. Impact of prenatal methyl mercury exposure on neurobehavioral function at age 14 years. Neurotoxicol Teratol 28: 363-375.

111. Kjellstrom, T, Kennedy, P, Wallis, S, and, Mantell, C., 1986. Physical and Mental Development of Children with Prenatal Exposure to Mercury From Fish, Stage I:

Preliminary Tests at Age 4, National Swedish Environmental Protection Board, Solna, Sweden. Report, 3080.

112. Kjellstrom, T, Kennedy, P, and, Wallis, S, *et al.*, 1989. Physical and Mental Development of Children with Prenatal Exposure to Mercury From Fish, Stage II: Interview and Psychological Tests at Age 6, National Swedish Environmental Protection Board, Solna, Sweden. Report, 3642.

113. Saint-Amour, D., Roy, M-S., Bastien, C., Ayotte, P., Dewailly, É., Després, C., Gingras, S., Muckle, G., 2006. Alterations of visual evoked potentials in pre-school children exposed to methyl mercury and polychlorinated biphenyls from a marine diet. Neurotoxicol 27: 567-578.

114. Murata, K., Sakamoto, M., Nakai, K., Dakeishi, M., Iwata, T., Liu, X-J., Satoh, H., 2006. Subclinical effects of prenatal methyl mercury exposure on cardiac autonomic function in Japanese children. Int Arch Occup Environ Health 79: 379-386.

115. Counter, SA., Buchanan, LH., Laurell, G., Ortega, F., 1998. Blood mercury and auditory neuro-sensory responses in children and adults in the Nambija gold-mining area of Ecuador. Neurotoxicol 19(2): 185-196.

116. Counter, SA., 2002. Elevated blood mercury and neuro-otological observations in children of the Ecuadorian gold-mines. J Toxicol Environ Health Part A 65:149-163.

117. Arruda, AGO., Câmara, V., Lima, MAMT., 2002. Avaliação otoneurológica em uma população exposta ao metilmercúrio. Arquivos Internacionais de Otorrinolaringologia 6(1): 1.

118. Harada, M., Nakanishi, J., Yasoda, E., Pinheiro, MCN., Oikawa, T., Guimarães, GA., Cardoso, BS., Kizaki, T., Ohno, H., 2001. Mercury pollution in the Tapajós River Basin, Amazon: mercury level of haid hair and health effects. Environ Int 27: 285-290.

119. Pacheco-Ferreira, H., 2001. Mercúrio na Amazônia: efeitos sobre a saúde das populações ribeirinhas. Doctoral Thesis. NAEA, Federal University of Pará, Belém, Pará, Brazil, 176 p.

120. Carta, P., Flore, C., Alinovi, R., Ibba, A., Tocco, MG., Aru, G., Carta, R., Girei, E., Mutti, A., Lucchini, R., Randaccio, FS., 2003. Sub-clinical neurobehavioral abnormalities

associated with low level of mercury exposure through fish consumption. Neurotoxicol 24: 617-623.

121. Lucchini, R., Calza, S., Camerino, D., Carta, P., Decarli, A., Parrinello, G., Soleo, L., Zefferino, R., Alessio, L., 2003. Application of a latent variable model for a multicenter study on early effects due to mercury exposure. Neurotoxicol 24: 605-616.

122. Amorim, M.I.M., Mergler, D., Bahia, MO., Dubeau, H., Miranda, D., Lebel, J., Burbano, RR., Lucotte, M., 2000. Cytogenetic damage related to low levels of methyl mercury contamination in the Brazilian Amazon. An Acad Bras Cienc 72(4): 497-507.

123. Bahia, MO., Corvelo, TC., Mergler, D., Burbano, RR., Lima, PDL., Cardoso, PCS., Lucotte, M., Amorim, MIM., 2004. Environmental biomonitoring using cytogenetic endpoints in a population exposed to mercury in the Brazilian Amazon. Environ Mol Mutagen 44: 346-349.

124. Crompton, P., Ventura, AM., Souza, JM., Santos, E., Strickland, GT., Silbergeld, E., 2002. Assessment of mercury exposure and malaria in a Brazilian Amazon riverine community. Environ Res Section A 90: 69-75.

125. Silva, IA., Nyland, JF., Gorman, A., Perisse, A., Ventura, AM., Santos, ECO., Souza, JM., Burek, CL., Rose, NR., Silbergeld, EK., 2004. Mercury exposure, malaria, and serum antinuclear/antinucleolar antibodies in Amazon populations in Brazil: a cross-sectional study. Environ Health 3:11.

126. Alves, MFA., Fraiji, NA., Barbosa, AC., Lima, DSN., Souza, JR., Dórea, JG., Cordeiro, GWO., 2006. Fish consumption, mercury exposure and serum antinuclear antibody in Amazonians. Int J Environ Health Res 16(4): 255-262.

127. Silbergeld, EK., Silva, IA., Nyland, JF., 2005. Mercury and autoimmunity: implications for occupational and environmental health. Toxicol Appl Phamacol 207: S282-S292.

128. Dórea, JG., Souza, JR., Rodrigues, P., Ferrai, I., Barbosa AC., 2005. Hair mercury (signature of fish consumption) and cardiovascular risk in Munducucu and Kayabi Indians of Amazonia. Environ Res 97:209-219.

129. Fillion, M., Mergler, D., Passos, CJS., Larribe, F., Lemire, M., Guimarães, JRD., 2006. A preliminary study of mercury exposure and blood pressure in the Brazilian Amazon. Environ Health 5:29.

130. Virtanen, JK., Rissanen, TH., Voutilainen, S., Tuomainen, T-P., 2007. Mercury as a risk factor for cardiovascular diseases. J Nut Biochem 18: 75-85.

131. Stern, AH., 2005. A review of the studies of the cardiovascular health effects of methyl mercury with consideration of their suitability for risk assessment. Environ Res 98: 133-142.

132. Passos, C.J., Mergler, D., Gaspar, E., Morais, S., Lucotte, M., Larribe, F., Davidson, R., Grosbois, S., 2003. Eating tropical fruit reduces mercury exposure from fish consumption in the Brazilian Amazon. Environ Res 93: 123-130.

133. Passos, C., Mergler, D., Larribe, F., 2004. Response to "Fruits, fish, and mercury: further considerations". Environ Res 96: 102-108.

134. Passos, CJS., Mergler, D., Fillion, M., Lemire, M., Mertens, F., Guimarães, JRD., Phillibert, A. 2007. Epidemiologic confirmation that fruit consumption influences mercury exposure in the Brazilian Amazon. Environ Res, in press.

135. Chapman, L., Chan, H. M., 2000. The influence of nutrition on methyl mercury Intoxication. Environ Health Perspect 108(suppl 1): 29-56.

136. Watanabe, C., 2002. Modification of mercury toxicity by selenium: practical importance? Tohoku J. Exp. Med. 196: 71-77.

137. Strain, JJ., Bonham, MP., Duffy, EM., Wallace, JMW., Robson, PJ., Clarkson, TW., Shamlaye, C., 2004. Nutrition and neurodevelopment: the search for candidate nutrients in the Seychelles Child Development Nutrition Study. Seychelles Med Dent J 7(1):77-83.

138. Campos, MS., Sarkis, JES., Muller, RCS., Brabo, ES., Santos, EO., 2002. Correlation between mercury and selenium concentrations in Indian hair from Rondônia State, Amazon region, Brazil. Sci Total Environ 287: 155-161.

139. Pinheiro, MCN., Muller, RCS., Sarkis, JE., Vieira, JLF., Oikawa, T., Gomes, MSV., Guimarães, GA., Nascimento, JLM., Silveira, LCL., 2005. Mercury and selenium

concentrations in hair samples of women in fertile age from Amazon riverside communities. Sci Total Environ 349: 284-288.

140. Lemire, M., Mergler, D., Fillion, M., Passos, CJS., Guimarães, JRD., Davidson, R., Lucotte, M., 2006. Elevated blood selenium levels in the Brazilian Amazon. Sci Total Environ 366: 101-111.

141. Feeney, MJ., 2004. Fruits and the prevention of lifestyle-related diseases. Clin Exp Pharmacol P 31: S11-S13.

142. Del Pozo-Insfran, D., Brenes, CH., Talcott, ST. 2004. Phytochemical composition and pigment stability of Açai (*Euterpe oleracea* Mart.). J Agri Food Chem 52: 1539-1545.

143. Del Pozo-Insfran, D., Percival, SS., Talcott, ST. 2006. Açai (*Euterpe oleracea* Mart.) polyphenolics in their glycoside and aglycone forms induce apoptosis of HL-60 leukemia cells. J. Agric. Food Chem. 54: 1222-1229.

144. Andrade, EH., Zoghbi, MGB., Maia, JGS., Fabricius, H., Marx, F. 2001. Chemical characterization of the fruit of *Annona squamosa* L. occurring in the Amazon. J Food Comp Anal 14: 227-232.

145. Lichtenthaler, R., Rodrigues, RB., Maia, GS., et al. 2005. Total oxidant scavenging capacities of *Euterpe oleracea* Mart. (Açai) fruits. Int J Food Sci Nut 56(1) : 53-64.

146. Santos, ECO., Jesus, IM., Brabo, ES., Loureiro, EB., Mascarenhas, AFS., Weirich, J., Câmara, VM., Cleary, D., 2000. Mercury exposures in riverside Amazon communities in Pará, Brazil. Environ Res Section A 84: 100-107.

147. Santos, ECO., Câmara, VM., Jesus, IM., Brabo, ES., Loureiro, ECB., Mascarenhas, AFS., Fayal, KF., Filho, GCS., Sagica, FES., Lima, MO., Higuchi, H., Silveira, IM., 2002. A contribution to the establishment of reference values for total mercury levels in hair and fish in Amazonia. Environ Res Section A 90: 6-11.

148. Santos, EO., Loureiro, ECB., Jesus, IM., Brabo, E., Silva, RSU., Soares, MCP., Câmara, VM., Souza, MRS., Branches, F., 1995. Diagnóstico das condições de saúde de uma comunidade garimpeira na região do Rio Tapajós, Itaituba, Pará, Brasil, 1992. Cad Saúde Pública 11(2): 212-225.

149. Dórea, JG., 2003. Fish are central in the diet of Amazonian riparians: should we worry about their mercury concentrations? Environ Res 92: 232-244.

150. Dórea, JG., 2004. Cassava cyanogens and fish mercury are high but safely consumed in the diet of native Amazonians. Ecotoxicol Environ Safety 57: 248-256.

151. Veiga, M., Baker, R., 2004. Protocols for environmental and health assessment of mercury released by artisanal and small-scale gold miners. UNIDO, 156 p.

152. Wotruba, H., 2003. Reducing mercury emissions in small scale mining-experiences from South America. TMS Annual Meetings, 185-193.

153. Spiegel, SJ., Veiga, MM., 2005. Building capacity in small-scale mining communities: health, ecosystem sustainability, and the Global Mercury Project. EcoHealth 2: 361-369.

154. Spiegel, SJ., Savornin, O., Shoko, D., Veiga, MM., 2006. Mercury reduction in Munhena, Mozambique: homemade solutions and the social context for change. Int J Occup Environ Health 12: 215-221.

155. Imperial College Consultants. Informal Gold Mining along the Amazon in Brazil – Full Report. **http://www.imperial-consultants.co.uk/example.php?id=24** (Website consulted on June the 1st 2007).

156. Cleary, D., 1996. Mercury contamination and health risk in the Brazilian Amazon: an ethical dilemma. Rev. Inst. Med. Trop. São Paulo 38(4): 247-248.

157. Veiga, MM., 2006. Unidades de demonstración y campaña de educación para mineros artesanales de oro. II Foro Internacional sobre la Mineria en Pequeña Escala, Estado de Bolívar, Venezuela. Mayo, 12 y 13, Puerto Ordaz.

158. Kligerman, DC., La Rovere, EL., Costa, MA., 2001. Management challenges on small-scale gold mining activities in Brazil. Environ Res Section A 87: 181-198.

159. Hilson, G., Hilson, CJ., Pardie, S., 2007. Improving awareness of mercury pollution in small-scale gold mining communities: challenges and ways forward in rural Ghana. Environ Res 103: 275-287.

160. Hilson, G., 2006. Abatement of mercury pollution in the small-scale gold mining industry: restructuring the policy and research agendas. Sci Total Environ 362: 1-14.

161. Global Mercury Project. Removal of Barriers to the Introduction of Cleaner Artisanal Gold Mining and Extraction Technologies. UNIDO/UNDP/GEF.

http://www.unites.uqam.ca/gmf/intranet/gmp/index_gmp.htm (Website consulted on June the 1st 2007).

162. Nepstad, DC., Stickler, CM., Almeida, OT., 2006. Globalization of the Amazon soy and beef industries: opportunities for conservation. Cons Biol 20(6): 1595-1603.

163. Soares-Filho, BS., Nepstad, DC., Curran, LM., Cerqueira GC., Garcia, RA., Ramos, CA., Voll, E., McDonald, A., Lefebre, P., Schlesinger, P., 2006. Modelling conservation in the Amazon basin. Nature 440: 520-523.

164. Duhaime, G., Chabot, M., Fréchette, P., Robichaud, V., Proulx, S., 2004. The impact of dietary changes among the Inuit of Nunavik (Canada): A socioeconomic assessment of possible public health recommendations dealing with food contamination. Risk Analysis 24(4): 1007-1018.

165. Levenson, CW., Axelrad, DM., 2006. Too much of a good thing? Update on fish consumption and mercury exposure. Nutrition Reviews 64(3): 139-145.

166. Marien, K., Stern, AH., 2005. An examination of the trade-offs in public health resulting from the use of default exposure assumptions in fish consumption advisories. Environ Res 98: 258-267.

167. Mozaffarian, D., Rimm, EB., 2006. Fish intake, contaminants, and human health: evaluating the risks and the benefits. JAMA 296(15): 1885-1899.

168. Swain, EB., Jakus, PM., Rice, G., Lupi, F., Maxson, PA., Pacyna, JM., Penn, A., Spiegel, SJ., Veiga, MM., 2007. Socioeconomic consequences of mercury use and pollution. Ambio 36(1): 45-61.

169. Mertens, F., Saint-Charles, J., Mergler, D., Passos, CJ., Lucotte, M., 2005. A network approach for analysing and promoting equity in participatory ecohealth research, EcoHealth 2: 113-126.

170. Saint-Charles, J., Mongeau, P., 2005. L'étude des réseaux humains de communication. In : Communication – Horizons de pratiques et de recherche, Saint-Charles J, Mongeau P (editors), Montréal : Presses de l'Université du Québec, pp 73-99.

171. Spiegel, SJ., Yassi, A., Spiegel, JM., Veiga, MM., 2005. Reducing mercury and responding to the global gold rush. The Lancet 366: 2070-2072.

172. Farella, N., Davidson, R., Lucotte, M., and S. Daigle., 2007. Nutrient and mercury variations in soils from family farms of the Tapajós region (Brazilian Amazon): recommendations for better farming. Agric Ecosyst Environ 120: 449-462.

173. Dórea, JG., Barbosa, AC., Ferrari, I., Souza, JR., 2003. Mercury in hair and fish consumed by riparian women of the Rio Negro, Brazil. Int J Environ Health Res 13(3): 239-248.

CHAPITRE II

EPIDEMIOLOGIC CONFIRMATION THAT FRUIT CONSUMPTION INFLUENCES MERCURY EXPOSURE IN RIPARIAN COMMUNITIES OF THE BRAZILIAN AMAZON

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RÉSUMÉ

L'identification de la déforestation comme élément contributeur à l'augmentation de la contamination mercurielle en Amazonie met en évidence que le problème d'exposition humaine au mercure est beaucoup plus répandu que l'on imaginait. Faisant suite à une étude exploratoire antérieure qui suggère que la consommation de fruits peut réduire l'exposition au mercure, la présente étude épidémiologique d'observation transversale, basée sur une enquête alimentaire pour la consommation de poissons et de fruits, a été réalisée auprès de 13 communautés riveraines. Des échantillons de cheveux de 449 personnes et des échantillons de sang d'un sous-groupe de 225 personnes ont été collectés afin de déterminer les teneurs en mercure total et inorganique, et ce par la méthode d'Absorption Atomique. En moyenne, les participants ont consommé 6.6 repas de poissons par semaine, et ils ont mangé 11 fruits/semaine. La teneur moyenne en mercure sanguin était 57.1 ± 36.3 µg/L (médiane: 55.1 µg/L), et la teneur moyenne en mercure dans les cheveux était 16.8 ± 10.3 µg/g (médiane: 15.7 µg/g). Il y avait une forte relation positive entre la fréquence de consommation de poissons et les teneurs en mercure sanguin ($r = 0.48$; $p < 0.0001$), et en mercure dans les cheveux ($r = 0.34$; $p < 0.0001$). La consommation de poissons ainsi que la consommation de fruits ont été significativement reliées à ces deux bioindicateurs d'exposition au mercure dans les modèles d'analyse multivariées : pour le sang (poissons: $\beta = 5.6$, $p < 0.0001$; fruit: $\beta = -0.5$, $p = 0.0011$; R^2 ajusté = 36.0%) et pour les cheveux (poissons: $\beta = 1.2$, $p < 0.0001$; fruit: $\beta = -0.2$, $p = 0.0002$; R^2 ajusté = 21.0%). Des modèles ANCOVA ont montré que pour le même nombre de repas de poissons, les personnes consommant des fruits plus fréquemment présentaient des teneurs en mercure significativement inférieures pour les deux bioindicateurs utilisés. Pour les faibles consommateurs de fruits, chaque repas de poisson augmentait le mercure sanguin de 9.8 µg/L comparé à seulement 3.3 µg/L pour les grands consommateurs de fruits. Ces nouvelles données montrent donc que la consommation de fruits peut effectivement influencer l'exposition au mercure chez des riverains amazoniens. Des stratégies de prévention qui cherchent à maintenir la consommation de poissons tout en réduisant l'exposition au mercure devraient être poursuivies.

Mots clés : Consommation de poissons et fruits, exposition au mercure, Amazonie, Brésil

ABSTRACT

Background: Since deforestation has recently been associated with increased mercury load in the Amazon, the problem of mercury exposure is now much more widespread than initially thought. A previous exploratory study suggested that fruit consumption may reduce mercury exposure. **Objectives**: To determine the effects of fruit consumption on the relation between fish consumption and bioindicators of mercury (Hg) exposure in Amazonian fish-eating communities. **Methods**: A cross-sectional dietary survey based on a seven-day recall of fish and fruit consumption frequency was conducted within 13 riparian communities from the Tapajós River, Brazilian Amazon. Hair samples were collected from 449 persons, and blood samples were collected from a subset of 225, for total and inorganic mercury determination by Atomic Absorption Spectrometry. **Results**: On average, participants consumed 6.6 fish meals/week and ate 11fruits/week. The average blood-Hg was 57.1 ± 36.3 μg/L (median: 55.1 μg/L), and the average hair-Hg was 16.8 ± 10.3 μg/g (median: 15.7 μg/g). There was a positive relation between fish consumption and blood-Hg ($r = 0.48$; $p < 0.0001$), as well as hair-Hg ($r = 0.34$; $p < 0.0001$). Both fish and fruit consumption entered significantly in multivariate models explaining blood-Hg (fish: $\beta = 5.6$, $p < 0.0001$; fruit: $\beta = - 0.5$, $p = 0.0011$; adjusted model $R^2 = 36.0\%$) and hair-Hg levels (fish: $\beta = 1.2$, $p < 0.0001$; fruit: $\beta = - 0.2$, $p = 0.0002$; adjusted model $R^2 = 21.0\%$). ANCOVA models showed that for the same number of fish meals, persons consuming fruits more frequently had significantly lower blood and hair Hg concentrations. For low fruit consumers, each fish meal contributed 9.8 μg/L Hg increase in blood compared to only 3.3 μg/L Hg increase for the high fruit consumers. **Conclusions**: These new data show that fruit consumption may indeed influence Hg exposure in Amazonian riparians. Prevention strategies that seek to maintain fish consumption while reducing Hg exposure in fish-eating communities should be pursued.

Key words: Fish consumption, fruit consumption, mercury exposure, Amazon, Brazil

Introduction

Over the last decades the presence of mercury (Hg) in the Amazon and its potential human health risks has given rise to much concern. During the 1970's, intense gold-mining activities were undertaken, with the arrival of thousands of gold miners coming from other regions of Brazil (Cleary 1990; Santos et al., 1992). Although elevated Hg levels found in the Amazonian environment were initially attributed to these gold-mining activities (Hylander 1994; Malm et al., 1990; Nriagu et al, 1992), more recent studies have shown high Hg concentrations both in fish and human tissues in regions where there has been no gold-mining (Guimarães et al., 1999; Silva-Forsberg et al., 1999; Dorea et al., 2003). Indeed, Amazonian soils constitute important reservoirs of Hg (Roulet et al., 1998; 1999, 2000; Fadini and Jardim, 2001), and a significant part of Hg contamination of the aquatic ecosystems is caused by erosion of such soils following deforestation for agriculture and/or cattle (Almeida et al., 2005; Farella et al., 2001, 2006; Roulet et al., 1999). Thus in the Amazonian environment, Hg from different sources is available for methylation processes contaminating the fish resources, which constitute a dietary mainstay for the large population living along the riverbanks (Dolbec et al., 2001; Guimarães, 2001; Lebel et al., 1997). Epidemiologic studies of riparian populations have shown dose-related associations between fish consumption, methyl mercury (MeHg) exposure, and early adverse health effects. Deficits in neurological and neuropsychological functions, as well as cytogenetic changes have been reported among adults and/or children from this area (Amorim et al., 2000; Cordier et al., 2002; Dolbec et al., 2000; Grandjean et al., 1999; Harada et al., 2001; Lebel et al., 1998, 1996; Yokoo et al., 2003). Additionally, recent exploratory studies in the Tapajós region suggest that Hg exposure may be associated with both increased blood pressure (Fillion et al., 2006) and autoimmune dysfunction (Silva et al., 2004).

There is a large variation in Hg levels in fish from the Tapajós region. A recent report indicated Hg concentrations above the recommended value of 0.5µg/g in 31% of predatory fish species (Silva et al., 2006). Another study presented high mean Hg levels for carnivorous species such as Dourada (*Brachyplatystoma flavicans*: 0.8µg/g), Surubim (*Pseudoplatystoma sp.*: 0.8 µg/g), Pescada (*Plagisocion squamosissimus:* 0.6 µg/g), and Sarda (*Pelona sp.*: 0.7µg/g), whereas low levels of Hg have been reported in herbivorous fish such as Aracu (*Leporinus sp.*: 0.07 μg/g), Pacu (*Mylossoma sp.*: 0.05 μg/g), and Tambaqui (*Colossoma macropomum*: 0.08 μg/g) (Santos et al., 2000). In the Tapajós region, fish appear to be the only food source for Hg. A recent study evaluating mercury pollution in cultivated and wild plant parts from the Tapajós region concluded that the translocation of Hg from soils throughout roots to aboveground is not significant (Egler et al., 2006). This is supported by European studies examining Hg levels in agricultural products of Hg-containing soils, which concluded that Hg intake through vegetables and fruits does not represent a health hazard for consumers (Ursinyová et al., 1997; Barghigiani and Ristori, 1994).

Since fish is a central and highly nutritious element in the Amazonian diet, some authors have minimized the importance of Hg exposure, suggesting that changes in fish consumption practices would necessarily have strong negative consequences for human health (Dorea, 2004; Dorea et al., 2005). An alternative public health approach would be to identify elements in the traditional diet that might influence Hg absorption and/or toxicity, thereby providing a way for this population to continue eating fish, while reducing Hg exposure. Despite the recognition that diet and nutrition can influence a population's vulnerability to the effects of MeHg (NRC 2000), dietary information has not been systematically collected in most epidemiologic studies examining the effects of MeHg exposure (Chapman and Chan 2000). Although a number of controlled experiments have estimated the effects of specific nutrients on Hg absorption and/or toxicity (Calabrese, 1978; Levander and Cheng, 1980; Imura and Naganuma, 1985; Whanger, 1992; Peraza et al., 1998; Lapina et al., 2000; Rao et al., 2001; Rao and Charma, 2001; Usuki et al., 2001; Affone et al., 2002), studies examining the role of diet in determining Hg concentrations in free-living populations are still scarce.

In a hypothesis-generating study of 26 adult women from a riparian village in the Brazilian Amazon, we examined the influence of the consumption of traditional foods on the relationship between fish consumption and Hg exposure (Passos et al., 2003). In that study, the women kept extensive food consumption frequency diaries, which included all food and beverages, for 12 months. The results of this food consumption survey revealed that the strong relationship between fish consumption and Hg exposure was significantly modified by fruit consumption.

The objective of the present study was to determine, in a large riparian population in the Brazilian Amazon, the effects of fruit consumption on the relation between fish consumption and bioindicators of Hg exposure, using an epidemiologic design. It is part of the CARUSO Project, a large interdisciplinary, ecosystemic study on Hg contamination and exposure in the region (CARUSO, 2007).

Methods

Study design and population

A cross-sectional dietary survey was undertaken among 13 riparian communities situated on the banks of the Tapajós River, a major tributary of the Amazon (Figure 1).

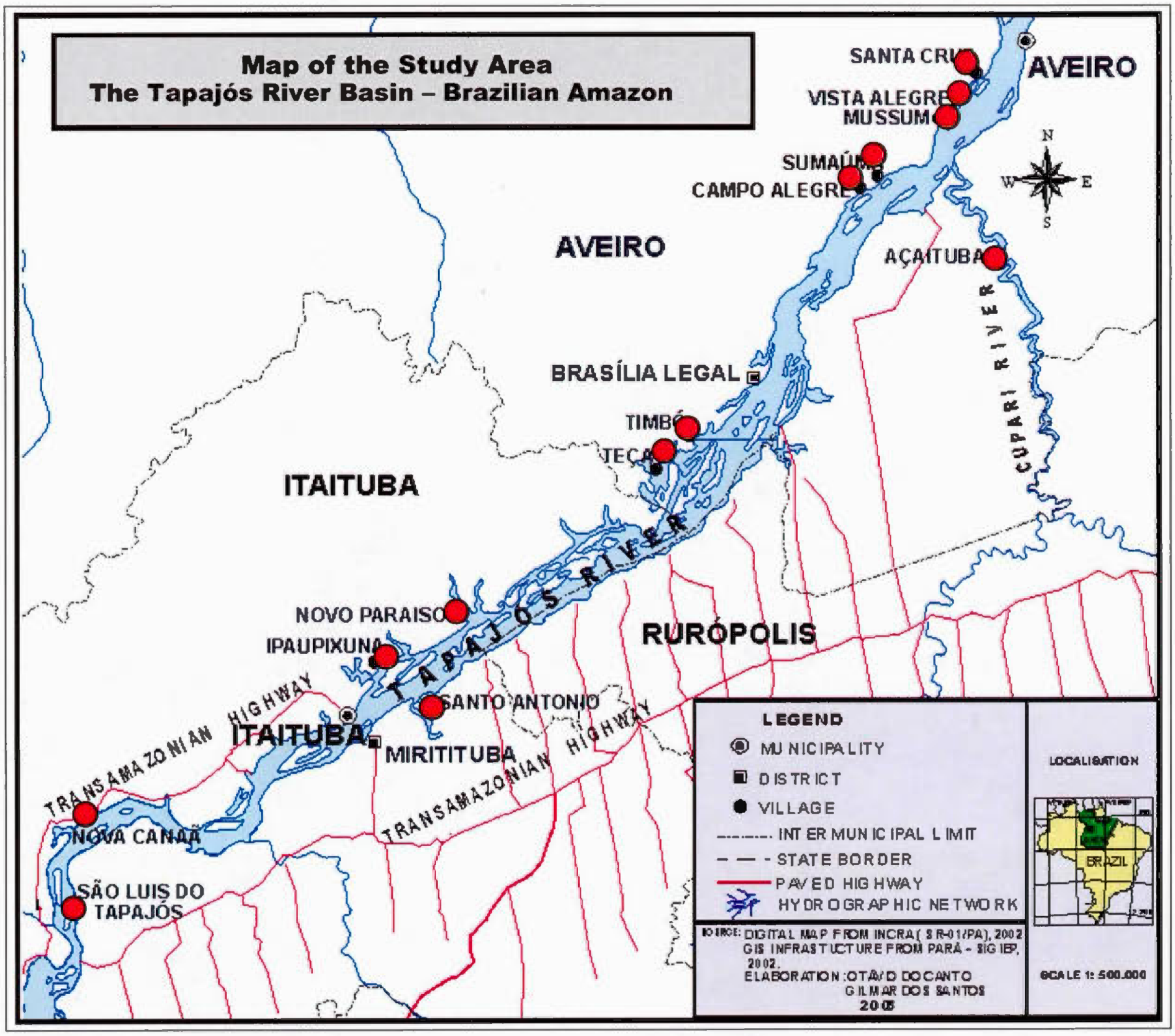


Figure 1: Map of the study area, the Tapajós River Valley. Participating communities are identified by a large dot.

These communities were chosen in order to represent the diversity created throughout the colonization process, as some of them were established after colonization began in the early 1960's, whereas others were established up to 100 years before. Because of the difficulties in applying a random sampling strategy in this setting, a convenience sample was used.

Age and sex distributions were then compared to the underlying population, which had previously been determined through a house-to-house survey, in each community (Table

1). During this survey the study was explained at each household and persons were invited to participate. Additionally, community meetings were conducted in each village in order to further explain the study.

Approval was obtained from Ethics Committees of the Federal University of Rio de Janeiro (Brazil) and the University of Quebec in Montreal (Canada). The study was explained individually, and persons agreeing to participate signed an informed consent form that was read to them.

Table 1: Age distribution and rates of participation in the study population

Age category	**Total adult population**	**Study population**	**% participation**
15 – 24	427	112	26.2
25 – 34	260	102	39.2
35 – 44	218	97	44.5
45 – 54	161	54	33.5
55 – 64	116	50	43.1
≥ 65	104	44	42.3
Total	**1286**	**459**	**35.7**

Assessment of fish and fruit consumption frequency

Because of important seasonal differences in the availability of fish species and types of fruit (Lebel et al., 1997; Dolbec et al., 2001; Passos et al., 2001), a seven-day dietary recall questionnaire (7-DDR) was used in order to determine recent fish and fruit consumption frequency. Development and validation studies of this instrument have shown that it is relatively easily administered and it constitutes a sensitive method to assess short-term food consumption (Hebert et al., 1997).

A list was prepared which included most of the fish and fruit species present in the region. In interviews performed over the months of June, July and August 2003, participants indicated the number of meals containing fish as well as the fish species that were consumed. As for fruits, the procedure was similar, but in this case, for each fruit species, the participant indicated the number of fruits that had been eaten each day over the preceding seven days, whether during a meal or not. Fish and fruit species that were not in the initial list were also recorded.

Sampling and analyses of bioindicators

Hair samples were collected from 449 persons (211 men and 238 women) and blood samples were collected from a subset of 225 persons (114 men and 111 women). Hair strands from the occipital region were cut at the root and then placed in plastic bags, with the root end stapled. The samples were analyzed at the Laboratory of Radioisotopes of the Federal University of Rio de Janeiro (Brazil), by Atomic Absorption Spectrometry with an AA 1475 Varian and a cold vapor generator accessory VGA-76 Varian. Mineralization of samples was done with mixtures of acids (HCl, HNO_3, and H_2SO_4) and oxidants ($KMnO_4$, $K_2S_20_7$, and H_2O_2), with techniques developed and adapted to the flow injection system vapor generator accessory (Malm et al., 1989). This laboratory participates regularly in inter-laboratory comparison programs for total and inorganic mercury analysis (Gill et al., 2002), and analytical quality control was ensured by the use of standard reference materials (Human Hair 085 and 086) provided by the International Atomic Energy Agency (IAEA).

Blood samples were collected by a nurse by venipuncture into 6 ml heparinized Becton Dickinson Vacutainer® (BD7863). All blood samples were kept frozen at –20° until analyzed. Total and inorganic mercury in blood were determined by Atomic Absorption Spectrometry at the laboratory of the Quebec Toxicology Center of the Quebec Public Health Institute (CTQ-INSPQ), Canada, according to the method described by Ebbestadt et al. (1975). The detection limit for blood mercury analysis was 0.2µg/L, and analytical quality control was ensured by the use of internal reference samples for blood analysis provided by the Inter-Laboratory Comparison Program conducted by the CTQ-INSPQ.

Statistical analysis

Descriptive statistics were used to describe the study population, Hg exposure as well as the results of fish and fruit consumption frequency. Correlation analyses were used to examine the relation between the frequency of consumption of specific fish species in relation to blood mercury (BHg) and hair mercury (HHg) concentrations. Where appropriate, nonparametric techniques were used for comparisons.

The associations between fish and fruit consumption frequency with respect to BHg and HHg levels were assessed using simple and multiple linear regression models. BHg and HHg levels were the dependent variables in separate linear regression models, which tested for the influence of overall fish and fruit consumption; the latter were included as continuous independent variables.

All pregnant women were excluded from the analyses, and potential covariates such as alcohol consumption, gender, age, schooling, and cigarette smoking were included in the models. Analysis of covariance (ANCOVA) was used to test interactions. Results were defined as statistically significant for a value of $P \leq 0.05$. Analyses were performed using Statview for Windows Version 5.0.1 and JMP 5.0.1a (SAS Institute Inc.).

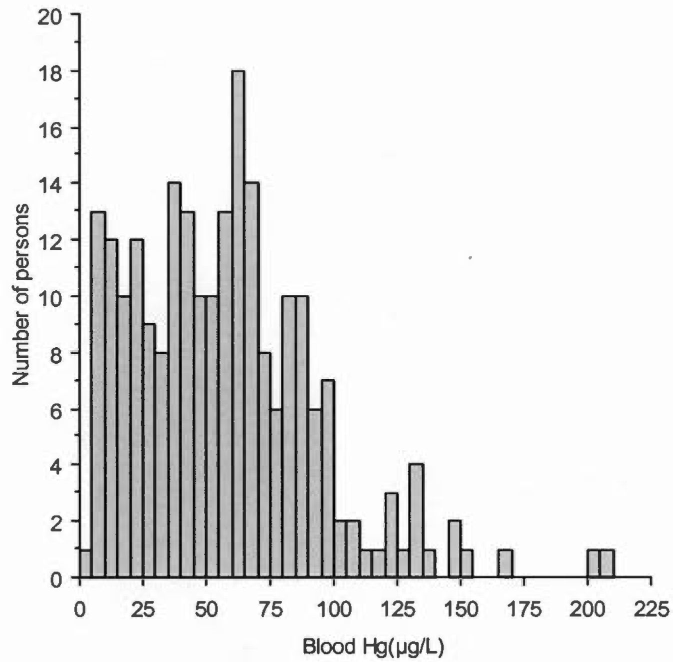
Results

Socio-demographic characteristics of the study population are shown in Table 2. Schooling varied between 0 and 12 years (mean 3.8 years ± 2.7), and the age range was 15-89 years (mean 38.6 years ± 17.2). Eighty-three percent (83%) of the participants were originally from the State of Pará, and 70% live on the Tapajós River banks, whereas 30% live on one of its tributaries. Figure 2 (A and B) presents the distribution of BHg and HHg levels, respectively. Overall, the average BHg was 57.1 ± 36.3μg/L (median: 55.1 μg/L, ranging from 4.8 to 205.4 μg/L), and the average HHg was 16.8 ± 10.3 μg/g (median: 15.7 μg/g, ranging from 0.2 to 58.3 μg/g).

The average percentage of MeHg was 86.8%, ranging from 75.2% to 94.3%. Men had significantly higher HHg levels (mean: 18.7 ± 11.2) than women (mean: 15.2 ± 9.1) (Mann-Whitney U, $p = 0.001$), but no significant difference was observed for BHg. There was a strong correlation between BHg and HHg concentrations ($r = 0.73$; $p < 0.0001$).

Table 2: Socio-demographic characteristics of the study population

	Women		Men	
Characteristics	n	%	n	%
Age				
15 to 24 y	61	25.1	51	23.6
25 to 34 y	58	23.9	45	20.8
35 to 44 y	51	21.0	47	21.8
45 to 54 y	27	11.1	25	11.6
55 to 64 y	23	9.5	27	12.5
≥ 65 y	23	9.5	21	9.7
Alcohol consumption				
Drinks	79	32.6	125	58.1
No longer drinks	33	13.6	45	20.9
Never drank	130	53.7	45	20.9
Smoking habits				
Smoker	51	21.1	74	34.4
No longer smokes	49	20.2	59	27.4
Never smoked	142	58.7	82	38.1
Education				
No formal education	21	8.7	29	13.6
Elementary school (1 to 8 years)	206	85.5	175	81.8
High school and more (≥9 years)	14	5.8	10	4.7
Born in Pará State	198	83.5	172	81.9
Location				
On the Tapajós River	172	70.8	144	66.7
On a tributary	71	33.3	72	33.2


Number of persons
0
2
4
6
8
10
12
14
16
18
20
0
25
50
75
100
125
150
175
200
225
Blood Hg(µg/L)

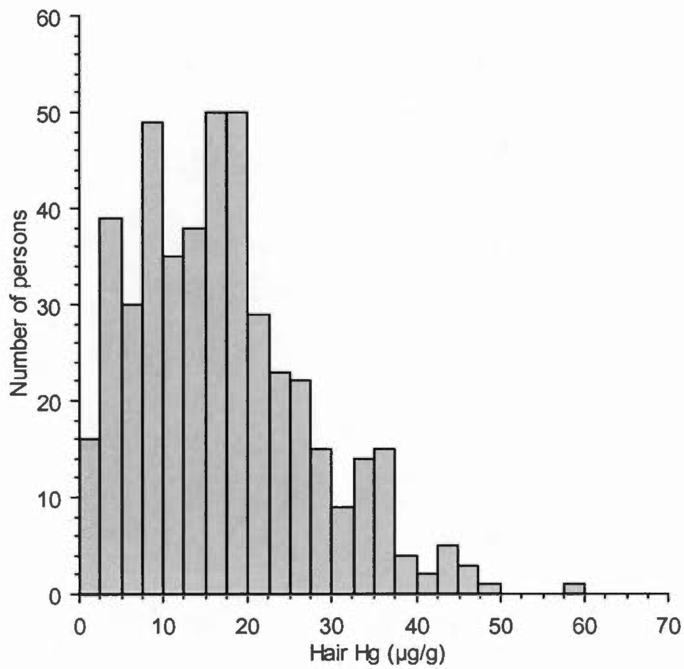

Number of persons
0
10
20
30
40
50
60
0
10
20
30
40
50
60
70
Hair Hg (µg/g)

Figure 2: Distribution of blood (A) and hair (B) total Hg concentrations for the study population

In this survey, 457 persons consumed at least one meal with fish over the preceding seven days, making up 99.6% of the study population. Of these, 345 persons consumed at least one meal containing a carnivorous species (75.2%), whereas 393 persons ate at least one fish meal containing a non-carnivorous species (85.6%). In all, participants had consumed an average of 6.6 fish meals/week, ranging from 0 to 19meals/week. Table 3 shows the fish species most frequently eaten over the preceding 7-day period.

Table 3: Frequency of reports for fish most frequently eaten over the preceding seven days

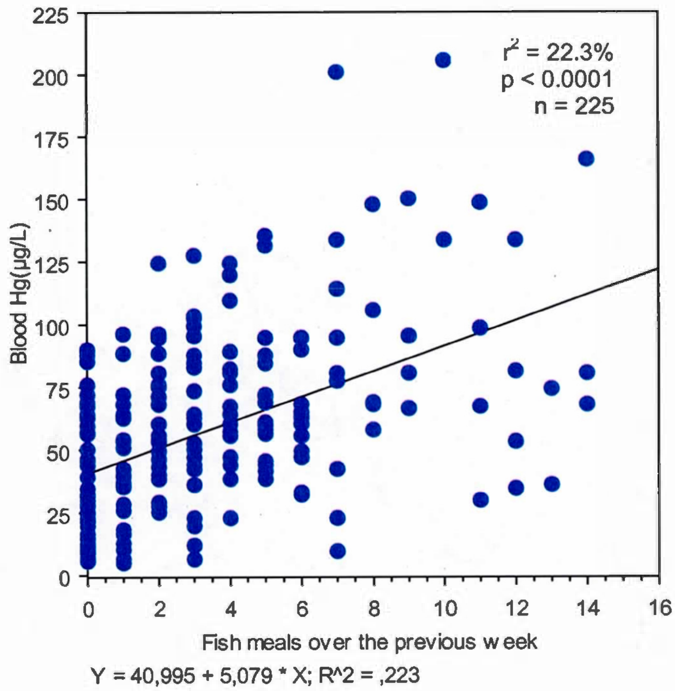
Fish species	**Feeding habits***	**Number of fish meals**	**%**
Aracu (*Shizodon sp.*)	n-c	696	23.0
Pescada (*Plagioscion sp.*)	c	602	19.9
Caratinga (*Geophagus sp.*)	n-c	375	12.4
Tucunaré (*Cichla sp.*)	c	291	9.6
Jaraqui (*Semaprochilodus sp.*)	n-c	160	5.3
Pacu (*Mylossoma sp.*)	n-c	155	5.1
Flexeira (*Hemiodus ocellatus*)	n-c	76	2.5
Branquinha (*Curimata amazonica*)	n-c	62	2.0
Piranha (*Sarrasalmus sp.*)	c	81	2.7
Others	-	**529**	**17.5**
Total	-	**3027**	**100**

* c, carnivorous ; n-c, non-carnivorous

Carnivorous fish made up an average of 43.5% of the fish diet, ranging from 0 to 100%. No associations were observed between total fish consumption and age, gender, schooling, cigarette smoking, and alcohol consumption. However, significant differences were observed between communities (Kruskal-Wallis, $p < 0.0001$), as well as between persons originally from the Tapajós region and immigrants from northeast Brazil (Mann-Whitney U, $p < 0.0001$). Those originally from the Tapajós region showed higher HHg levels (mean = 17.9 μg/g ± 10.1) compared to persons who had immigrated (mean = 12 μg/g ± 9.9).

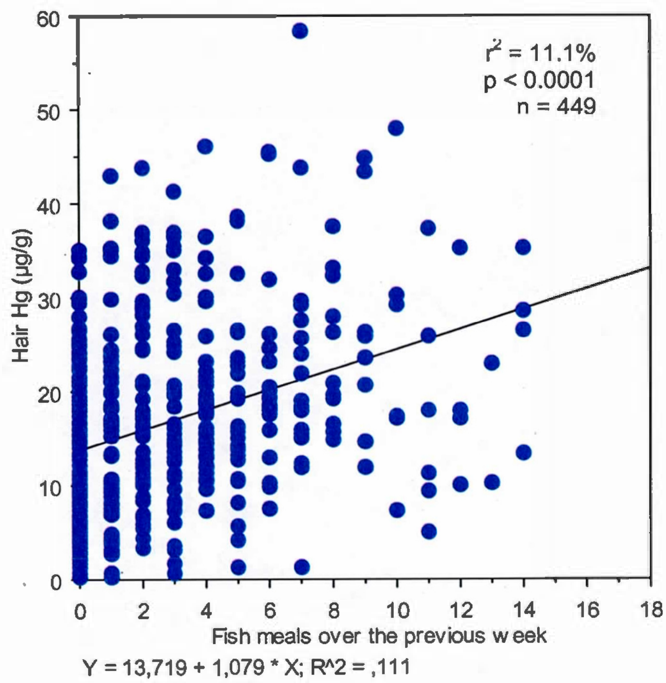
Figure 3 (A and B) shows the relationships between weekly fish consumption (meals/week), BHg and HHg, respectively. Partial correlation analyses of fish consumption, categorized by feeding habits and Hg levels, show that the frequency of consumption of carnivorous fish is significantly correlated to both BHg and HHg ($r = 0.48$, $p < 0.0001$ for BHg; $r = 0.34$, $p < 0.0001$ for HHg), whereas the frequency of consumption of non-carnivorous fish is not related to BHg ($r = 0.01$, $p = 0.15$), and weakly correlated to HHg ($r = 0.14$, $p = 0.002$). This is reflected in the individual species, with the highest correlations observed for large carnivorous fish such as *Pescada*, *Filhote* and *Piranha*. Despite its relatively high consumption, the carnivorous species *Tucunaré* was not significantly correlated to the bioindicators of Hg exposure, while *Aracu* and *Pacu* (non-carnivorous species) showed a weak correlation to HHg. These same relationships were observed when the fish were entered two-by-two into a multiple regression model.

A total of 40 fruit species were recorded during the survey, and 443 persons (96.5%) ate at least one of these fruits in the previous week. Three-hundred twenty-eight (328) persons (71.5%) reported eating bananas (*Musa spp*., Musaceae), the most consumed fruit, while 203 (44.2%) reported eating at least one orange (*Citrus spp*., Rutaceae). Table 4 summarizes the distribution of persons with respect to fruit species consumption, as well as the frequency of reports for fruits most frequently eaten over the previous seven days.



225
200
175
150
125
100
75
50
25
0
Blood Hg(µg/L)
0 2 4 6 8 10 12 14 16
Fish meals over the previous week
r² = 22.3%
p < 0.0001
n = 225
Y = 40,995 + 5,079 * X; R^2 = ,223

A



60
50
40
30
20
10
0
Hair Hg (µg/g)
0 2 4 6 8 10 12 14 16 18
Fish meals over the previous week
r² = 11.1%
p < 0.0001
n = 449
Y = 13,719 + 1,079 * X; R^2 = ,111

B

Figure 3: The relationship between fish consumption (number of meals over the previous week) and blood (A) and hair (B) total Hg concentrations

Because of the important biodiversity in the Amazon, most fruits are consumed by only a small percentage of the participants, whereas only a few fruits are widely consumed by significant portions of the population. On average, participants ate 11fruits/week, ranging from 0 to 62fruits/week. Although many types of fruit are seasonally available, the most frequently eaten are bananas and oranges. In this survey, we also observed a relatively high frequency of consumption of other regional fruits such as Tucumã (*Astrocaryum aculeatum*) and Jambo (*Eugenia spp.*), whereas Ingá (*Inga spp.*, Leguminosae-Mimosoideae) was hardly consumed in this season. Total fruit consumption was weakly correlated with fish consumption ($r = 0.1$; $p = 0.003$), and inversely correlated with age ($r = -0.1$; $p = 0.02$). It was also weakly correlated with schooling ($r = 0.1$; $p = 0.02$), but no relation was observed between fruit consumption and cigarette smoking or alcohol consumption. Similar to fish consumption, significant inter-village differences were observed (Kruskal-Wallis, $p = 0.004$). Villagers living close to Itaituba City, the only urban center of the upper and middle Tapajós, reported lower fruit consumption as compared to villagers living in the proximity of Aveiro, a small town in the lower Tapajós.

Table 4: Frequency of persons eating specific fruit and frequency of reports for fruit most frequently eaten over the previous seven days

Fruit	**Latin identification**	**Number of persons**	**Relative Frequency (%)**	**Number of fruits**	**% total fruits**
Bananas	*Musa paradisiaca*	328	71.5	1727	34.3
Oranges	*Citrus sp.*	203	44.2	973	19.3
Tucumã	*Astrocaryum aculeatum*	137	29.8	570	11.3
Guava	*Psidium guajava*	82	17.9	189	3.7
Passion fruit	*Passiflora sp.*	76	16.6	19	0.4
Jambo	*Eugenia sp.*	70	15.3	315	6.3
Avocado	*Persea americana*	69	15.0	109	2.2
Ingá	*Inga sp.*	49	10.7	82	1.6
Brazil Nuts	*Bertholletia excelsa*	37	8.1	202	4.0
Others	-	**300**	**65.4**	**845**	**16.8**
Total	-	**443**	**96.5**	**5031**	**100**

Both fish and fruit entered significantly into the multivariate models explaining BHg and HHg; the regression estimates are presented in Table 5 for both women and men. The inverse relationship between fruit consumption and Hg levels remained significant, even when carnivorous and non-carnivorous fish were included separately. In addition to the overall effect of fruit consumption, multivariate models showed that some individual fruits presented enhanced negative regression estimates. Table 6 shows regression estimates for frequency of specific fruit consumption in multiple linear models with fish consumption and bioindicators of Hg exposure. It is interesting to note that bananas are associated with HHg and Jambos are related to BHg, whereas oranges are associated with both bioindicators. Indeed, bananas are consumed throughout the year whereas Jambos and many other fruits present important seasonal variations (Passos et al., 2001), which might explain the relation between bananas and hair Hg since both reflect chronic exposure over time.

Figure 4 (A and B) illustrates the overall influence of these specific fruits (bananas, oranges, and jambos) on the relationship between fish consumption and Hg exposure. The regression lines are plotted for those with low fruit consumption (≤ 3 fruits/week; n = 64), medium fruit consumption (> 3 fruits/week ≤ 10 fruits/week; n = 86), and high fruit consumption (> 10 fruits/week, n = 75) in relation to BHg.

Table 5: Results of multiple regression analyses for fish and fruit consumption in relation to BHg (μg/L) and HHg (μg/g) concentrations

Biological indicator	**n**	**Regression estimates**		**Model R^2 (%)#**
Blood total mercury (μg/L)		**Carnivorous fish**	**Total fruit**	
Women	111	4.8 ($p < 0.0001$)	- 0.7 ($p = 0.0068$)	27.1
Men	114	6.8 ($p < 0.0001$)	- 0.4 ($p = 0.0417$)	46.3
Total	225	5.6 ($p < 0.0001$)	- 0.5 ($p = 0.0011$)	36.0
Hair total mercury (μg/g)				
Women	238	1.0 ($p < 0.0001$)	- 0.1 ($p = 0.0276$)	16.1
Men	211	1.4 ($p < 0.0001$)	- 0.2 ($p = 0.0058$)	21.6
Total	449	1.2 ($p < 0.0001$)	- 0.2 ($p = 0.0002$)	21.0

adjusted factors in the regression equation: age, cigarette smoking, alcohol and non-carnivorous fish consumption

Table 6: Regression estimates for frequency of specific fruit consumption (fruits/week) in multiple linear models with fish consumption (meals/week) as independent variable and bioindicators of Hg exposure

Biological indicator *Fruits*	**Regression estimates**		**Model R^2 (%)#**
Blood total mercury (µg/L) (n = 225)	**Carnivorous fish**	**Fruit**	
Oranges	5.3 ($p < 0.0001$)	- 1.6 ($p = 0.0006$)	36.2
Jambos	4.9 ($p < 0.0001$)	- 1.8 ($p = 0.0245$)	38.9
Hair total mercury (µg/g) (n = 449)			
Oranges	1.0 ($p < 0.0001$)	- 0.2 ($p = 0.0440$)	23.3
Bananas	1.0 ($p < 0.0001$)	- 0.2 ($p = 0.0246$)	23.0

adjusted factors in the regression equation: age, gender, cigarette smoking, alcohol and non-carnivorous fish consumption, community.

For HHg, the low consumption group comprises 177 persons, the medium 169 persons, and the high consumers include 113 persons. Analysis of covariance showed that the intercepts of the three regression lines were similar, but their slopes were significantly different (Interaction term for BHg: $F = 9.4$, $p = 0.0001$; for HHg: $F = 5.9$; $p = 0.0029$). Thus, for low fruit consumers, each fish meal contributed 9.8 µg/L Hg increase in blood compared to only 3.3 µg/L Hg increase for the high fruit consumers. Similarly, each fish meal contributed approximately 1.7 µg/g Hg increase in hair of low fruit consumers as opposed to 0.5 µg/g increase in hair of high fruit consumers.

Most sociodemographic features such as age, schooling, cigarette smoking, and alcohol consumption were similar between low and high fruit consumers, while some slight differences were observed for a limited number of variables (Table 7). It is interesting to note that high fruit consumers ate more carnivorous fish.

Table 7: Characteristics of fruit consumers according to their level of consumption

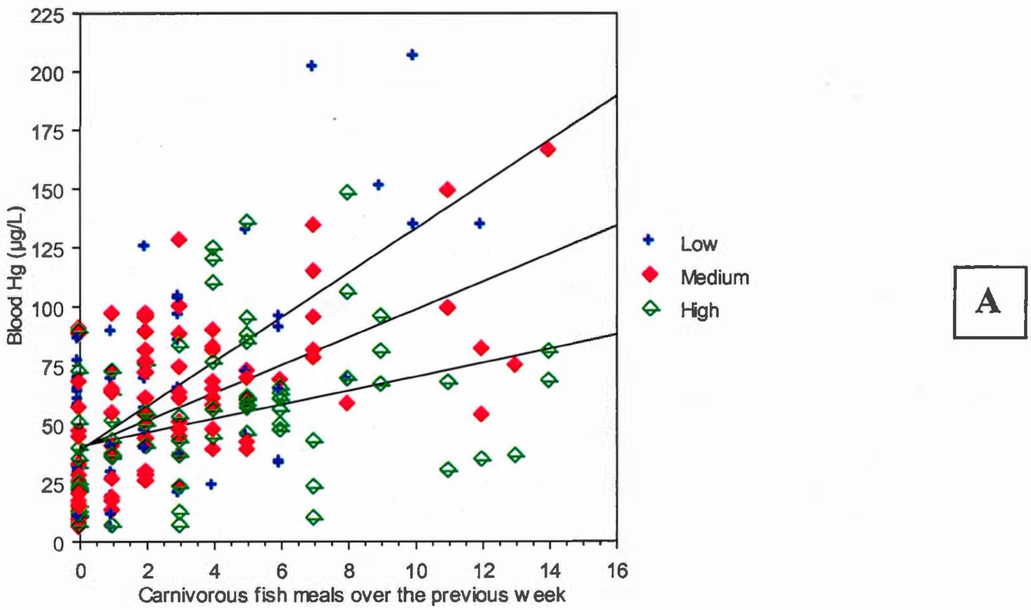
Characteristics	Low consumers*	Medium consumers*	High consumers*
	n = 177	n = 169	n = 113
Regional distribution			
Upriver (Itaituba)	97 (54.8)	78 (46.2)	38 (33.6)
Midriver (Brasília Legal)	37 (20.9)	18 (10.7)	6 (5.3)
Downriver (Aveiro)	43 (24.3)	73 (43.2)	69 (61.1)
Gender			
Women	98 (55.4)	97 (57.4)	47 (41.6)
Men	79 (44.6)	72 (42.6)	66 (58.4)
Fish consumption (meals/week)			
Carnivorous fish	2.1 ± 2.7	3.2 ± 3.2	3.8 ± 3.6
Non-carnivorous fish	3.8 ± 4.0	3.8 ± 3,5	3.2 ± 2.9
Hg levels			
Blood (μg/L)	61.7 ± 44.6	57.9 ± 33.1	52.3 ± 31.5
Hair (μg/g)	17.0 ± 11.2	17.4 ± 10.4	15.8 ±8.4

Data presented as mean ± standard deviation or number of persons (percentage).

Discussion

The results of the present study show a clear association between fruit consumption and lower Hg levels in this population, thus confirming the findings of our hypothesis-generating study conducted among 26 riparian women in the Amazon (Passos et al., 2003). This protective effect of fruit consumption against Hg exposure via dietary intake of fish is observed both for women and men; it is present in all categories of age and schooling, and occurs independently of other factors with a potential to influence Hg exposure, such as cigarette smoking and alcohol consumption.

A plausible explanation for the findings of this study is that the soluble dietary fiber content as well as other prebiotic nutrients of fruits could be interfering with absorption at the gastrointestinal tract. Indeed, demethylation of MeHg by microflora in the gut is a key and probably a rate-determining process in the removal of MeHg from the body, even though the microbes involved have not been identified nor have the biochemical mechanisms of cleavage of the carbon-mercury bond (Clarkson, 2002).



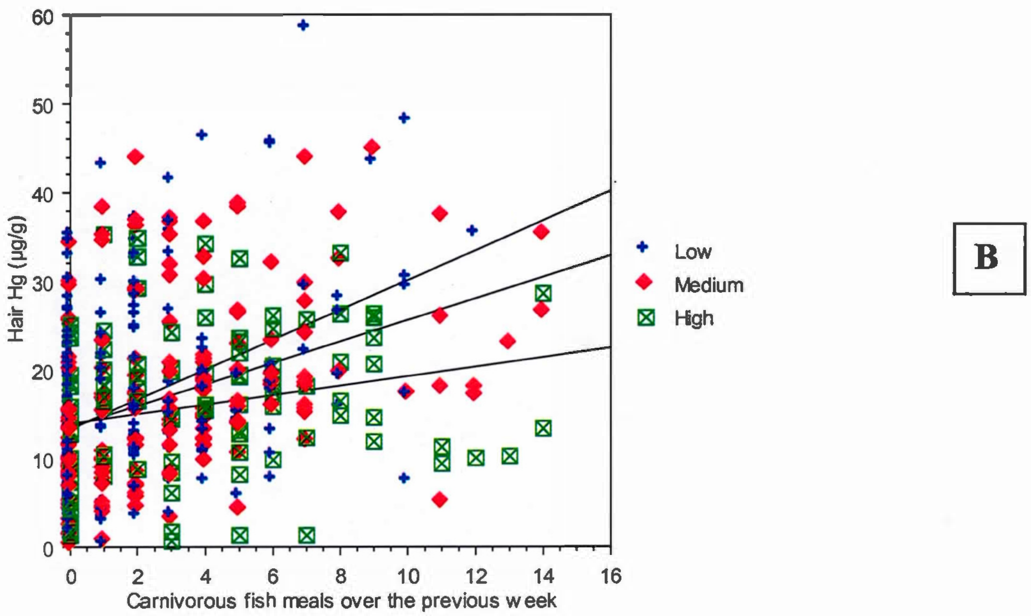


Figure 4: The influence of fruit consumption on the relationship between carnivorous fish consumption (meals over the previous week) and blood Hg levels (A), and hair Hg levels (B)

A number of studies have suggested that the demethylation process in the intestine might well constitute an important site for interaction between diet and MeHg accumulation in the body (Chapman and Chan, 2000), the fiber content of the diet having already been shown to affect the excretion rate of MeHg (Rowland et al., 1986). Dietary elements have important effects on the metabolic activity of the intestinal flora (Gibson et al., 2004; Rowland, 1988), including a number of the carbohydrates present in significant amounts in several fruits and vegetables, which are able to stimulate the growth and/or activity of intestinal bacteria associated with health and well-being (Roberfroid, 2005). The effect of fruit consumption on these processes might explain, at least in part, why there is such a broad range of biologic half-times reported for adults exposed to MeHg.

The substantial inverse relation between Hg levels and consumption of oranges, which are known to present high levels of ascorbic acid (vitamin C), is particularly interesting since the role of this nutrient on MeHg exposure and toxicity has been controversial. Although Vitamin C has been implicated in the enhancement of MeHg toxicity (Murray and Hughes, 1976; cited in NRC, 2000), because of its strong reducing capacity, it is supposed to have potent detoxifying properties and has been used in cases of intoxication by heavy metals, including Hg. Sharma and colleagues (1982) demonstrated that ascorbic acid mediated a small but significant degradation of MeHg to inorganic mercury. Also, a more recent study concluded that ascorbic acid prevents mercury induced-genotoxicity in blood cultures due to its probable nucleophilic and detoxifying nature (Rao et al., 2001). In addition to ascorbic acid, oranges are also excellent sources of flavonoids and soluble dietary fiber.

Despite a positive relation between cigarette smoking and Hg levels observed in this population, the influence of fruit consumption remained unchanged. It is known that smokers have lower antioxidant status than nonsmokers, but fruit consumption leads to a higher antioxidant status (Dietrich et al., 2003), which might explain the unchanged effect of fruit consumption. Indeed, one of the properties of several antioxidants particularly abundant in fruits is that they can form complexes with reactive metals, thus reducing their absorption (Bravo, 1998). Furthermore, the effect of fruit consumption also remained unchanged despite inter-village differences in terms of fruit consumption. Such regional differences probably reflect the fact that villagers near Itaituba City often buy fruit in the market, whereas those in more remote villages in lower Tapajós acquire fruit more often from their own home gardens.

Over these last years, diet of fish-eating communities has been the subject of much debate because of concerns about the potential health risks of MeHg exposure and, on the other hand, the public health implications of a diminished fish consumption (Arnold et al., 2005; Egeland and Middaugh, 1997; Myers et al., 2000; Weihe and Grandjean, 1998). Indeed, decreases in traditional food use has already been shown to affect diet quality and even to contribute to a number of diet-related health problems in indigenous peoples of Arctic Canada (Receveur et al., 1997). It is interesting that until recently the on-going birth cohort studies of heavy fish consumers of the Seychelles Islands in the Indian Ocean did not reveal adverse effects of MeHg, and some results even indicated beneficial outcomes that correlate with Hg levels during pregnancy; the authors suggest a potential role of micronutrients in fish as a possible explanation for such findings (Clarkson and Strain, 2003). The importance of maintaining fish consumption when intervening to reduce Hg exposure in fish-eating populations was stressed by the Joint Expert Committee on Food Additives and Contaminants (JECFA) under the Food and Agriculture Organization (FAO) and the World Health Organization in their recent recommendations for tolerable daily maximum intake for Hg in pregnant/childbearing age women (WHO, 2003).

In the Amazon, recent reports have criticized any eventual suggestion to restrict fish consumption in traditional populations, which rely on fish as the main source of animal protein and other essential nutrients, suggesting that despite high concentrations of MeHg in fish, daily consumption of this food in large amounts poses no health hazards (Dórea, 2003, 2004). Although these reports rightfully point out the public health issues involved in diminished fish consumption, a more comprehensive approach, which takes into account the different sources of pollution as well as the socio-cultural and economic aspects of agriculture and diet, is needed in order to achieve a viable risk management challenge in this region. In particular, deforestation should be better controlled, thereby limiting Hg leaching from soils. It will also be necessary to better understand the dynamics involved in methylation in the areas of fish capture and to improve knowledge on the role of other foods able to influence Hg absorption and metabolism.

In this context, the challenge to maintain fish consumption while reducing Hg exposure remains. The encouraging results of a first intervention, which aimed at shifting towards consumption of less contaminated fish species and its impact in lowering exposure in a village on the Tapajós river have been presented elsewhere (Mertens et al., 2005; Bahia et al., 2004; Mergler et al., 2001). Indeed, through education based on posters showing the status of Hg contamination in relation to the fish species, the change in diet habits resulted in a reduction of close to 40% of HHg levels (Lucotte et al., 2004). The findings of the present study confirm a relevant avenue that deserves to be further explored as a potential additional intervention strategy aimed at achieving the short-term challenge of maintaining fish consumption while reducing Hg exposure in this Amazonian setting.

In public health, it is well known that fruits contain a variety of compounds that may slow or prevent chronic diseases through several possible mechanisms. Components in fruits thought to be associated with the reduction of these conditions include soluble and insoluble dietary fiber, antioxidant nutrients (vitamins C, E, selenium, β-carotene), as well as other phytonutrients including polyphenols, flavonoids, anthocyanins and carotenoids (Feeney, 2004). Our findings indicate that fruit consumption may also be protective against the bioaccumulation of Hg in human populations exposed via dietary intake of fish.

Certain methodological issues of the present study need to be considered. First, there is always a trade-off between the amount of data that can be collected and the size of the population. In the Passos et al. (2003) study, we opted for a large amount of chronological data collected through food diaries (written record of the foods as they are eaten, thus minimizing under- or over-reporting due to recall bias), and sequential hair Hg analyses from a small female population in order to identify the relevant food items that could then be used in a study with a much larger population (Passos et al., 2004). For the present study, we used a cross-sectional design on a convenience sample of men and women villagers from numerous riparian communities, assessing fish and fruit consumption frequency through a 7-DDR, and measuring Hg levels both in recent and chronic bioindicators of exposure. While the 7-DDR has been shown to constitute a sensitive method to assess short-term food consumption (Hebert et al., 1997), because of its retrospective nature there might have been some level of under- or over-reporting due to recall bias, especially for food items only moderately consumed (Pereira and Koifman, 1999). In addition, although data collection on convenience samples has been shown to appropriately represent the underlying population in other settings (Kelly et al., 2002; Zelinski et al., 2001), this sampling strategy may have introduced some selection bias in the present study. We did, however, achieve a participation rate of 35.7% in this adult population, well represented in most age categories. Moreover, most characteristics of fruit consumers were well distributed in the three categories of fruit consumption.

Another limitation of the present study is that it did not allow us to examine some of the possible physiologic events that may be involved in the interactions between fruit nutrients and MeHg. Studies examining the use of chelating agents as an intervention strategy to reduce blood lead levels raised questions about whether the process of chelation causes potentially dangerous redistribution of lead to susceptible organs from those less susceptible to lead toxicity (Goyer et al., 1995). Further studies should therefore examine the effect of fruit consumption from a toxicokinetic viewpoint.

Conclusion

Despite some limitations, this study constitutes strong evidence that fruit consumption provides a protective effect against Hg exposure in Amazonian riparian communities, whose traditional diet is based on daily consumption of Hg-containing freshwater fish. The results of this epidemiologic study are consistent with our previous findings (Passos et al., 2003) in which 26 riparian women presented lower hair Hg levels associated with consumption of regional fruit. Even though we did not measure toxicological outcomes in this study, it is reasonable to hypothesize that villagers consuming fruit regularly would be less vulnerable to neurological and/or cardiovascular risks linked to chronic Hg exposure. Future studies should be conducted to identify the specific nutrients responsible for this protective effect and examine the pharmacokinetics involved in these relations.

Acknowledgments

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References

Afonne, OJ., Orisakwe, OE., Obi, E., Dioka, CE., Ndubuka, GI., 2002. Nephrotoxic actions of low-dose mercury in mice: protection by zinc. Arch Environ Health 57(2): 98-102.

Almeida, MD., Lacerda, LD., Bastos, WR., Herrmann., 2005. Mercury loss from soils following conversion from forest to pasture in Rondônia, Western Amazon, Brazil. Environ Pollut 137: 179-186.

Amorim, M.I.M., Mergler, D., Bahia, M.O., Dubeau, H., Miranda, D., Lebel, J., Burbano, R.R., Lucotte, M., 2000. Cytogenetic damage related to low levels of methyl mercury contamination in the Brazilian Amazon. An. Acad. Bras. Ci. 72(4): 497-507.

Arnold, SM., Lynn, TV., Verbrugge, LA., Middaugh, JP., 2005. Human biomonitoring to optimize fish consumption advice: reducing uncertainty when evaluating benefits and risks. Am J Public Health 95(3): 393-397.

Bahia, MO., Corvelo, TC., Mergler, D., Burbano, RR., Lima, PDL., Cardoso, CS., Lucotte, M., Amorim, MIM., 2004. Environmental biomonitoring using cytogenetic endpoints in a population exposed to mercury in the Brazilian Amazon. Environ Mol Mutagen 44: 346-349.

Barghigiani, C., Ristori, T., 1994. Mercury levels in agricultural products of Mt. Amiata (Tuscany, Italy). Arch. Environ. Contam. Toxicol. 26: 329-334.

Bravo, L., 1998. Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. Nutr Rev 56(11): 317-333.

Calabrese, E.J., 1978. Nutrition and Environmental Health. New York (NY): John Wiley and Sons.

Caruso, 2007. Mercury exposure and ecosystem health in the Amazon. http://www.unites.uqam.ca/gmf/caruso/caruso.htm

Clarkson, TW., 2002. The three modern faces of mercury. Environ Health Perspect 110 (suppl 1): 11-23.

Clarkson, TW., Strain, JJ., 2003. Nutritional factors may modify the toxic action of methyl mercury in fish-eating populations. J Nutr. 133: 1539S-1543S.

Cleary, D., 1990. Anatomy of the Amazon Gold Rush. Iowa City: University of Iowa Press, 245 p.

Chapman, L., Chan, H. M., 2000. The influence of nutrition on methyl mercury Intoxication. Environ Health Perspect. 108(suppl 1): 29-56.

Cordier, C., Garel, M., Mandereau, L., Morcel, H., Doineau, P., Gosme-Seguret, S., Josse, D., White, R., Amiel-Tison, C., 2002. Neurodevelopmental Investigations among Methylmercury-Exposed Children in French Guiana. Environ Res. 89: 1-11.

Dietrich, M., Block, G., Norkus, EP., Hudes, M., Traber, MG., Cross, CE., Pacher, L., 2003. Smoking and exposure to environmental tobacco smoke decrease some plasma antioxidants and increase γ-tocopherol in vivo after adjustment for dietary antioxidant intakes. Am J Clin Nutr 77: 160-166.

Dolbec, J., Mergler, D., Passos, C.J.S., Morais, S.S., Lebel, J., 2000. Methyl mercury exposure affects motor performance of a riverine population of the Tapajós River, Brazilian Amazon. Int Arch Occup Environ Health 73: 195-203.

Dolbec, J., Mergler, D., Larribe, F., Roulet, M., Lebel, J., Lucotte, M., 2001. Sequential analysis of hair mercury levels in relation to fish diet of an Amazonian population, Brazil. Sci Total Environ 271: 87-97.

Dórea, JG., Barbosa, AC., Ferrari, I., Souza, JR., 2003. Mercury in hair and in fish consumed by riparian women of the Rio Negro, Amazon, Brazil. In J Environ Health Res 13(3): 239-248.

Dórea, JG., 2003. Fish are central in the diet of Amazonian riparians: should we worry about their mercury concentrations? Environ Res. 92: 232-244.

Dórea, JG., 2004. Cassava cyanogens and fish mercury are high but safely consumed in the diet of native Amazonians. Ecotoxicol Environ Safety 57: 248-256.

Dórea, JG., de Souza, JR., Rodrigues, P., Ferrari, I., Barbosa, AC., 2005. Hair mercury (signature of fish consumption) and cardiovascular risk in Munduruku and Kayabi Indians of Amazonia. Environ Res. 97: 209-219.

Ebbestadt V, Gunderson and Torgrimsen TA., 1975. Simple method for the determination of inorganic mercury and methylmercury in biological samples by flameless atomic absorption. Atom Absorption Newsletter 14(6):142-143.

Egeland, GM., Middaugh, JP., 1997. Balancing fish consumption benefits with mercury exposure. Science, 278 (5345): 1904-1905.

Egler, SG., Rodrigues-Filho, S., Villas-Bôas, RC., Beinhoff, C., 2006. Evaluation of mercury pollution in cultivated and wild plants from two small communities of the Tapajós gold mining reserve, Pará State, Brazil. Sci total Environ 368:424-433.

Fadini, PS., Jardim, WF., 2001. Is the Negro River Basin (Amazon) impacted by naturally occurring mercury? Sci Total Environ 275: 71-82.

Farella N, Lucotte M, Louchouarn P and Roulet M., 2001. Deforestation modifying terrestrial organic transport in the Rio Tapajós, Brazilian Amazon. Org Geochem 32: 1443-1458.

Farella, N., Lucotte, M., Davidson, R., Daigle, S., 2006. Mercury release from deforested soils triggered by base cation enrichment. Sci Total Environ 368: 19-29.

Feeney, MJ., 2004. Fruits and the prevention of lifestyle-related diseases. Clin Exp Pharmacol P 31: S11-S13.

Fillion, M., Mergler, D., Passos, CJS., Larribe, F., Lemire, M., Guimarães, JRD., 2006. A preliminary study of mercury exposure and blood pressure in the Brazilian Amazon. Environ Health, 5:29

Gibson, GR., Probert, HM., Loo, JV., Rastall, RA., Roberfroid MB., 2004. Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. Nutr Res Rev 17: 259-275.

Gill, US., Schwartz, HM., Bigras, L., 2002. Results of multiyear international inter-laboratory comparison program for mercury in human hair. Arch Environ Contam Toxicol 43:466-472.

Goyer, RA., Cherian, MG., Jones, MM., Reigart, JR., 1995. Meeting Report: Role of chelating agents for prevention, intervention, and treatment of exposures to toxic metals. Environ Health Perspec 103(11).

Grandjean, P., White, RF., Nielsen, A., Cleary, D., Santos, ECO., 1999. Methyl mercury neurotoxicity in Amazonian children downstream from gold mining. Environ Health Perspect 107(7): 587-591.

Guimarães, JRD., Fostier, AH., Forti, MC., et al., 1999. Mercury in human and environmental samples from two lakes in Amapá, Brazilian Amazon. Ambio 28(4): 296-301.

Guimarães, JRD., 2001. Les processus de méthylation du mercure en milieu amazonien. *In*: Carmouze JP, Lucotte M and Boudou A (coord.), Le mercure en Amazonie: Rôle de l'homme et de l'environnement, risques sanitaires. IRD Éditions, Paris.

Harada, M., Nakanishi, J., Yasoda, E., Pinheiro, M.C.N., Oikawa, T., Guimarães, G.A., Cardoso, B.S., Kisaki, T., Ohno, H., 2001. Mercury pollution in the Tapajós River Basin, Amazon: mercury level of haid hair and health effects. Environ Int. 27: 285-290.

Hebert, JR., Ockene, IS., Hurley, TG., Luippold, R., Well, AD., Harmatz, MG., et al., 1997. Development and testing of a seven-day dietary recall. J Clin Epidemiol 50(8): 925-937.

Hylander, LDH., Silva, EC., Oliveira, LJ., Silva, SA., Kuntze, EK., Silva, DX., 1994. Mercury levels in Alto Pantanal: a screening study. Ambio 23: 478-484.

Imura, N., Naganuma, A., 1985. Mode of modifying action of selenite on toxicity and behavior of mercury and other metals. Nutr Res (suppl 1): 499-507.

Kelly, H., Riddell, MA., Gidding, HF., Nolan, T., Gilbert, GL., 2002. A random cluster survey and a convenience sample give comparable estimates of immunity to vaccine preventable diseases in children of school age in Victoria, Australia. Vaccine 20: 3130-3136.

Lapina, VA., Sheshko, PM., Dontsov, AE., 2000. Phytosorbent prepared from sunflower seed husks prevents mercuric chloride accumulation in kidney and muscle of adult rabbits. Arch Environ Health 55(1): 48-50.

Lebel, J., Mergler, D., Lucotte, M., Amorim, M., Dolbec, J., Miranda, D., Arantes, G., Rheaulth, I., Pichet, P., 1996. Evidence of early nervous system dysfunctions in Amazonian populations exposed to low-level of methyl mercury. Neurotoxicology 17(1): 157-168.

Lebel, J., Roulet, M., Mergler, D., Lucotte, M., Larribe, F., 1997. Fish diet and mercury exposure in a riparian Amazonian population. Water Air Soil Poll. 97: 31-44.

Lebel, J., Mergler, D., Branches, F., Lucotte, M., Amorim, M., Larribe, F., Dolbec, J., 1998. Neurotoxic effects of low-level methyl mercury contamination in the Amazonian Basin. Environ Res 79: 20-32.

Levander, O.A., Cheng, L., 1980. Micronutrient interactions, vitamins, minerals, and hazardous elements. Ann NY Acad Sci 1, 355-372.

Lucotte, M., Davidson, R., Mergler, D., St-Charles, J., Guimarães, JRD., 2004. Human exposure to mercury as a consequence of landscape management and socio-economic behaviors. Part I: The Brazilian Amazon Case Study. *In*: Proceedings of the 7th International Conference on Mercury as a Global Pollutant. RMZ-M&G: 51: 668-672.

Malm, O., Pfeiffer, WC., Bastos, WR., Souza, CMM., 1989. Utilização do acessório de geração de vapor frio para investigação do mercúrio em amostras ambientais por espectrofotometria de absorção atômica [In Portuguese]. Ci Cult 41: 88-92.

Malm, O., Pfeiffer, W.C., Souza, C.M.M., Reuther, R., 1990. Mercury pollution due to gold mining in the Madeira river basin, Brazil. Ambio 19, 11-15.

Mergler, D., Boischio, AA., Branches, F., Morais, S., Passos, CJ., Gaspar, E., Lucotte, M., 2001. Neurotoxic sequelae of methyl mercury exposure in the Brazilian Amazon: a follow-up study. In: Proccedings of the 6th International Conference on Mercury as a Global Pollutant. October 2001, Minamata, Japan, 15 – 19.

Mertens, F., Saint-Charles, J., Mergler, D., Passos, CJ., Lucotte, M., 2005. A network approach for analysing and promoting equity in participatory ecohealth research, EcoHealth 2: 113-126.

Murray, DR., Hughes, RE., 1976. The influence of dietary ascorbic acid on the concentration of mercury in guinea-pig tissues. Proc. Nutr. Soc. 35(3): 118A-119A.

Myers, GJ., Davidson, PW., Cox, C., Shamlaye, C., Cernichiari, E., Clarkson, TW., 2000. Twenty-seven years studying the human neurotoxicity of methyl mercury exposure. Environ Res 83: 275-285.

National Research Council, 2000. Toxicological effects of methyl mercury. National Academy Press, Washington DC, 344 pp.

Nriagu, J.O., Pfeiffer, W.C., Malm, O., Sousa, C.M.M., Mierle, G., 1992. Mercury pollution in Brazil. Nature 356: 389.

Passos, CJ., Mergler, D., Gaspar, E., Morais, S., Lucotte, M., Larribe, F., de Grosbois, S., 2001. Characterization of the diet of a riverside population in the Brazilian Amazon [In Portuguese]. Rev Saúde Ambiente, 4(1/2): 72-84.

Passos, C.J., Mergler, D., Gaspar, E., Morais, S., Lucotte, M., Larribe, F., Davidson, R., de Grosbois, S., 2003. Eating tropical fruit reduces mercury exposure from fish consumption in the Brazilian Amazon. Environ Res 93: 123-130.

Passos, C., Mergler, D., Larribe, F., 2004. Response to "Fruits, fish, and mercury: further considerations". Environ Res 96: 102-108.

Peraza, M.A., Ayala-Fierro, F., Barber, D.S., Casares, E., Rael, L.T., 1998. Effects of micronutrients on metal toxicity. Environ Health Perspect 106, Suppl 1: 203-216.

Pereira, RA., Koifman, S., 1999. Using food frequency questionnaire in past dietary intake assessment [In Portuguese]. Rev. Saúde Pública 33(6): 610-621.

Rao, MV., Chinoy, NJ., Suthar, MB., Rajvanshi, MI., 2001. Role of ascorbic acid on mercuric chroride-induced genotoxicity in human blood cultures. Toxicol in Vitro 15: 649-654.

Rao, MV., Sharma, PSN., 2001. Protective effect of vitamin E against mercuric chloride reproductive toxicity in male mice. Reprod Toxicol 15: 705-712.

Receveur, O., Boulay, M., Kuhnlein, HV., 1997. Decreasing traditional food use affects diet quality for Adult Dene/Métis in 16 communities of the Canadian Northwest Territories. J Nutr. 127: 2179-2186.

Roberfroid, MB., 2005. Introducing inulin-type fructans. Brit J Nutr 93(suppl 1): S13-S25.

Roulet M, Lucotte M, Saint-Aubin A, Tran S, Rhéault I, Farella N, De Jesus da Silva E; Dezencourt J, Passos CJS, Santos Soares G, Guimarães JRJ, Mergler D. and Amorim M., 1998. The geochemistry of Hg in Central Amazonian soils developed on the Alter-do-Chão formation of the lower Tapajós river valley, Pará state, Brazil. Sci Total Environ 223: 1-24.

Roulet M, Lucotte M, Farella N, Serique G, Coelho H, Passos CJS, de Jesus da Silva E, de Andrade PS, Mergler D, Guimaraes JRD, Amorim M., 1999. Effects of recent human colonization on the presence of mercury in Amazonian ecosystems. Water Air Soil Poll. 112: 297-313.

Roulet, M., Lucotte, M., Canuel, R., Farella, N., Courcelles, M., Guimarães, JRD., Mergler, D., Amorim, M., 2000. Increase in mercury contamination recorded in lacustrine sediments following deforestation in the central Amazon. Chem Geol 165: 243-266.

Rowland, I.R., Mallett, A.K., Flynn, J., Hargreaves, R.J., 1986. The effect of various dietary fibers on tissue concentration and chemical form of mercury after methylmercury exposure in mice. Arch Toxicol 59: 94-98.

Rowland, IR., 1988. Factors affecting metabolic activity of the intestinal microflora. Drug Metab Rev. 19 (3-4): 243-261.

Santos, E.C.O., Rosa, J.F.T., Jesus, I.M., Loureiro, E.C.B., 1992. A saúde das populações da Amazônia Brasileira. *In*: Enfoque Integral de la Salud Humana em la Amazônia. L. Yarzabal, C. Espinal y L.E. Aragon (Eds). Unamaz: p. 95-156.

Santos, LSN., Muller, RCS., Sarkis, JES., Alves, CN., Brabo, ES., Santos, EO., Bentes, MHS., 2000. Evaluation of total mercury concentrations in fish consumed in the municipality of Itaituba, Tapajós River Basin, Pará, Brazil. Sci Total Environ 261:1-8.

Sharma, DC., Davis, PS., Sharma, PK., 1982. Effect of ascorbic acid on biotransformation and modification of the toxicity of mercurials in goldfish (*Carassius auratus*). Experientia 38 (5): 565-567.

Silva, DS., Lucotte, M., Roulet, M., Poirier, H., Mergler, D., Crossa, M., 2006. Mercury in fish of the Tapajós River in the Brazilian Amazon. INTERFACEHS 1(1), art 6.

Silva, IA., Nyland, JF., Gorman, A., Perisse, A., Ventura, AM., Santos, ECO., Souza, JM., Burek, CL., Rose, NR., Silbergeld., 2004. Mercury exposure, malaria, and serum antinuclear/antinucleolar antibodies in Amazon populations in Brazil: a cross-sectional study. Environ Health 3:11.

Silva-Forsberg, MC., Forsberg, BR., Zeidemann, VK., 1999. Mercury contamination in humans linked to river chemistry in the Amazon basin. Ambio 28(6): 519-521.

Ursinyová, M., Hladiková, V., Uhnák, J., Kovacicová, J., 1997. Toxic elements in environmental samples from selected regions in Slovakia. Bull Environ Contam Toxicol 58: 985-992.

Usuki, F., Yasutake, A., Umehara, F., Tokunaga, H., Matsumoto, M., Eto, K., Ishiura, S., Higuchi, I., 2001. In vivo protection of a water-soluble derivative of vitamin E, Trolox, against methyl mercury-intoxication in the rat. Neurosci Lett 304: 199-203.

Weihg, P., Grandjean, P., 1998. Methyl mercury risks. Science 279 (5351): 635.

Whanger, P.D., 1992. Selenium in the treatment of heavy metal poisoning and chemical carcinogenesis. J Trace Elem Electrol Health Dis 6: 209-221.

WHO. 2003. World Health Organization, Joint FAO\WHO Expert Committee on Food Additives JECFA/61/SC.

Yokoo, EM., Valente, JG., Grattan, L., Schimidt, SL., Platt, I., Silbergeld, EK., 2003. Low-level methyl mercury exposure affects neuropsychological functions in adults. Environ Health 2: 8.

Zelinski, EM., Burnight, KP., Lane CJ., 2001. The relationship between subjective and objective memory in the oldest old: comparisons of findings from a representative and a convenience sample. J Aging Health 13(2): 248-266.

CHAPITRE III

DAILY MERCURY INTAKE IN FISH-EATING POPULATIONS IN THE BRAZILIAN AMAZON

This paper is dedicated to the memory of our dear and always CARUSO Project fellow, Dr. Marc Roulet, who made the fascinating discovery of deforestation and soil erosion as the major source of mercury into the riparian Tapajós region, and opened a new era of mercury biogeochemistry research in the Amazon. To this outstanding scientist, all our respect and acknowledgments.

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RÉSUMÉ

Malgré la consommation élevée de poissons et malgré les niveaux élevés d'exposition humaine au mercure en Amazonie, peu est connu sur les taux d'ingestion quotidienne de ce contaminant. À l'aide d'une approche écosystémique on a mesuré la fréquence de consommation de poissons et de fruits auprès de 256 personnes vivant dans 6 villages dans le bassin du Tapajós. Les quantités de poissons par repas ont été évaluées. De plus, nous avons eu accès aux données sur les concentrations de mercure dans 1,123 spécimens de poissons locaux. L'ingestion quotidienne de mercure (µg/kg/jour) a été estimée pour les hommes et pour les femmes de chaque village à partir des moyennes de concentrations de mercure dans les poissons capturés dans leurs zones de pêche, la quantité moyenne de poissons par repas, la fréquence de consommation des différentes espèces de poissons et le poids corporel des participants. La concentration moyenne de mercure dans les poissons était 0.33µg/g ± 0.33, et l'ingestion quotidienne de mercure variait entre 0 et 11.8 µg/kg/jour (moyenne : 0.92µg/kg/jour ± 0.89), et a fluctué selon le genre et le village. Les concentrations moyennes de mercure sanguin et dans les cheveux étaient 60.1 ± 38.4µg/L et 17.9 ± 11.5µg/g, respectivement. On observe une forte relation positive entre le mercure sanguin et l'ingestion quotidienne de mercure, avec une relation inverse par rapport à la consommation de fruits et la scolarité des individus. Des variations significatives ont été observées selon le statut d'immigration régionale et entre les différents villages. Le mercure dans les cheveux a été directement associé à l'ingestion quotidienne de mercure et inversement relié à la scolarité ainsi qu'à la consommation de fruits. La consommation de fruits a été la seule variable qui modifiait la relation entre l'ingestion quotidienne de mercure et les teneurs sanguines du métal : pour une même ingestion quotidienne de mercure, les personnes mangeant plus de fruits ont des concentrations inférieures de mercure sanguin. Ces résultats révèlent des niveaux élevés d'ingestion quotidienne de mercure. Des études rigoureuses pour le développement de doses de référence basées sur l'analyse du risque toxique devraient être menées afin d'orienter des stratégies viables de gestion du risque, et ainsi de réduire l'exposition humaine, tout en respectant les avantages et la nécessité du régime alimentaire riche en poissons pour ces populations.

Mots clés : Consommation de poissons, apport quotidien en mercure, bioindicateurs d´exposition, ingestion de fruits, évaluation du risque, gestion du risque, Amazonie.

ABSTRACT

Although high levels of fish consumption and bioindicators of Hg exposure have been reported for traditional populations in the Amazon, little is known about their actual daily intake of Hg. **Objectives:** Using an ecosystem approach, calculate daily Hg-intake in adult fish-eaters, examine the relations between Hg-intake and bioindicators of exposure and the factors that influence these relations. **Methods:** A cross-sectional dietary survey on fish and fruit consumption frequency was carried out with 256 persons from 6 villages of the Tapajós River. Fish portion per meal was determined. Mercury concentration was determined for 1,123 local fish specimens. Daily Hg-intake (μg/kg/d) was determined for men and women from each village using the average fish-Hg concentration for the fish caught in their fishing zone, the average quantity of fish per meal, fish-species frequency consumption and participants' body weight. **Results**: Fish-Hg averaged 0.33μg/g ± 0.33. Daily Hg-intake varied between 0 and 11.8μg/kg/d, (mean 0.92μg/kg/d ± 0.89) and varied by gender and village. Mean blood and hair mercury were 60.1 ± 38.4μg/L and 17.9 ± 11.5μg/g, respectively. There was a strong and positive relation between blood-Hg and daily Hg-intake, with an inverse relation for fruit intake and schooling; significant variations were observed with immigrant status, and among villages. Hair-Hg was directly associated with daily Hg-intake and inversely related to schooling and fruit consumption. Fruit consumption modified the relation between daily Hg-intake and blood-Hg levels: for the same daily Hg-intake, persons eating more fruit had lower blood-Hg concentrations (ANCOVA Interaction term: $F = 10.9$, $p < 0.0001$). The median difference of the ratio of blood-Hg to daily Hg-intake between low and high fruit consumers was 26, representing a 26.3% reduction of the input of ingested Hg into the blood stream. **Conclusion**: These findings reveal high levels of daily Hg-intake. Rigorous studies for developing risk-based reference doses in the Amazon should be undertaken to orient viable risk-management strategies to reduce exposure, while maintaining fish diet.

Key words: Fish consumption, daily mercury intake, bioindicators of exposure, fruit intake, risk-assessment, risk-management, Amazon

Introduction

Although gold mining activities were considered the unique source of mercury pollution into the Amazon for many years (Malm, 1998), pioneering studies in the Tapajós River Basin in Brazil have shown that the mercury content in the water column is influenced by the amount of particulate matter, independently from upstream gold mining activities (Roulet et al., 1998a), and regional variations of mercury concentrations and burdens in soil compartments cannot be explained by the presence of gold mining centers in the region (Roulet et al., 1998b). In fact, removal of forest cover and destruction of root systems subsequent to deforestation and 'slash and burn' agricultural practices deplete the soil, releasing naturally occurring Hg into the water systems (Roulet et al., 1999). Roulet et al. (2000), who examined mercury contamination in lacustrine sediments following deforestation, suggest that the environmental changes initiated between 1950 and 1970, which coincide with the first colonizing projects in the Amazon, probably correspond to the beginning of mercury leaching into the rivers. The resulting mercury pollution of the aquatic environment is taken up by the halieutic resources (Roulet et al., 2001; Farella et al., 2001). These important pathways of exposure, later confirmed for other watersheds of the Amazonian Basin (Fostier et al., 2000; Lechler et al., 2000; Fadini and Jardim, 2001; Wasserman et al., 2003; Lacerda et al., 2004; Almeida et al., 2005; Farella et al., 2006, 2007; Mainville et al., 2006, Bastos et al., 2006), constitute a continuous source of mercury exposure for the large number of traditional and immigrant communities of the region that rely on fish consumption as a dietary mainstay.

Despite many years of research and biomonitoring, adult traditional populations in the Amazon continue to be chronically exposed to high levels of mercury through their fish diet (Bastos et al., 2006, Pinheiro et al., 2006). In these populations, there is strong evidence of early nervous system dysfunction and, to a less extent, immunologic and cardiovascular effects among both adults and children (Lebel et al., 1996, 1998; Grandjean et al., 1999; Amorim et al., 2000; Dolbec et al., 2000; Harada et al., 2001; Cordier et al., 2002; Mergler, 2002; Yokoo et al., 2003; Silbergeld et al., 2005; Fillion et al., 2006).

During the last decade a large number of exposure assessments of fish-eating populations have been conducted in different countries of the Amazon (Bastos et al., 2006; Pinheiro et al., 2006, Dórea et al., 2005; Gonçalves and Gonçalves, 2004; Webb et al., 2004;

Passos et al., 2003a; Santos et al., 2003; Dolbec et al., 2001; Cordier et al., 1998; Barbosa et al., 1997; Lebel et al., 1997). However, little attention has been given to the importance of mercury intake in these populations, although a limited number of studies have tried to estimate the rates of mercury intake (Boischio and Henshel, 1996, 2000; Bidone et al., 1997; Hacon et al., 1997; Kehrig et al., 1998; Fréry et al., 2001). Daily oral exposures are used in many countries as the basis for risk assessment (Clarkson and Magos, 2006). In the Amazon, mercury daily dose is particularly difficult to estimate due to the diversity of fish and ecosystems, as well as seasonal changes in fish availability. Furthermore, studies in this region have shown that fruit consumption influences the relation between fish consumption and mercury concentrations both in blood and hair (Passos et al., 2003b, 2004, 2007a, b), but we do not know how it affects mercury intake and exposure.

To determine oral doses, one requires not only information on human eating habits and bioindicators of exposure, but also a considerably large database on mercury concentrations in fish from different local ecosystems. As part of the Caruso Project (CARUSO, 2007), which has been studying mercury sources, its pathways through the ecosystem, human exposure and effects, there is extensive data on mercury concentrations in a large number of local fish species. The aims of the present study were to i) estimate mercury intake in communities along the Tapajós River of the Brazilian Amazon; (ii) analyze the relations between mercury intake and bioindicators of exposure; (iii) examine the factors that influence the relation between mercury intake and bioindicators of exposure.

Methods

Study design and population

We conducted a cross-sectional study including six riparian communities situated in the Tapajós region, one of the most important tributaries of the Amazon River. Two of these communities are located on small tributaries of the Tapajós (villages of Açaituba and Santo Antonio), while the communities of São Luis do Tapajós and Nova Canaã are located on the east and west banks of the main channel of southern Tapajós, respectively. The villages of Vista Alegre and Mussum are located on the west bank of the lower Tapajós, near the small town of Aveiro (Figure 1). These traditional communities originated from the miscegenation of old indigenous populations with European colonizers and, to a less extent, with African slaves during the 18th and 19th centuries (Lima, 1992, Murrieta, 2001).

In this area of the Tapajós, the only source of methyl mercury exposure for these remote riparian communities is the consumption of fish, many of which can have high levels of Hg. The villagers are mostly involved in subsistence fishing, agriculture and, to a lesser extent, cattle-raising. Fish are obtained from the river and fluvial lakes close to the villages, without significant cross over between communities, which are relatively far away from each other. Most villages can only be reached by boat.

Recruitment was performed during a house-to-house survey, which also served to estimate the socio-demographic characteristics of the total population. The study was explained at each household and persons were invited to participate on a voluntary basis. In addition, community meetings were held in each village to further explain the study. A total of 256 villagers, 15 years and older (121 women and 135 men), making up 38.2% of the underlying population of 670 adults, participated in the present study, which was carried out during the descending water season of 2003 (June and July).

All human and fish data were collected at the same period. Two interview-administered questionnaires were used. One targeted socio-demographic information (age, educational level, place of birth, length of time in the region, subsistence activities, as well as specific questions about working in gold mining and exposure to mercury through burning amalgam or other contaminants). The second was a food frequency questionnaire (described below). Hair and blood samples were taken and fish sampling was carried out.

The research protocol was approved by the Institutional Review Board of the Federal University of Rio de Janeiro (Brazil) and of the University of Quebec in Montreal (Canada). The study was explained individually, and persons agreeing to participate signed an informed consent form that was read to them.

Assessment of fish-Hg concentrations

Fish, from local village fishing sites, were captured with gill nets, and a piece of muscle tissue devoid of skin and bones was taken and frozen individually for future mercury analysis. The analyzed species included the following: Aracu (*Schizodon sp.*), Pescada (*Plagioscion squamosissimus*), Tucunaré (*Cichla monoculus* and *Cichla temensis*), Caratinga

(*Geophagus proximus*), Pacu (*Mylossoma sp.*), Jaraqui (*Semaprochilodus insignis*), Piranha (*Pygocentrus sp.*), Branquinha (*Curimata inordata*), Acari (*Liposarcus pardallis*), Charuto (*Hemiodus unimaculatus*), Mandi (*Auchenipterus nuchalis*), Peixe-Cachorro (*Raphiodus vulpinus*), Surubim (*Pseudoplatystoma sp.*), Traira (*Hoplias malabaricus*), Sarda (*Pellona castelnaeana*), Curimata (*Prochilodus nigricans*), Tambaqui (*Colossoma macropomum*), Matrinxã (*Brycon cephalus*), and Curvina (*Pachypops furcraeus*). A total of 1,339 fish were captured and analyzed. Here we use the average mercury concentrations for the fish species that were effectively eaten in each community, comprising 1,123 fish specimens. The species included here constitute 99.8% of the total fish diet as recorded in the dietary survey described below.

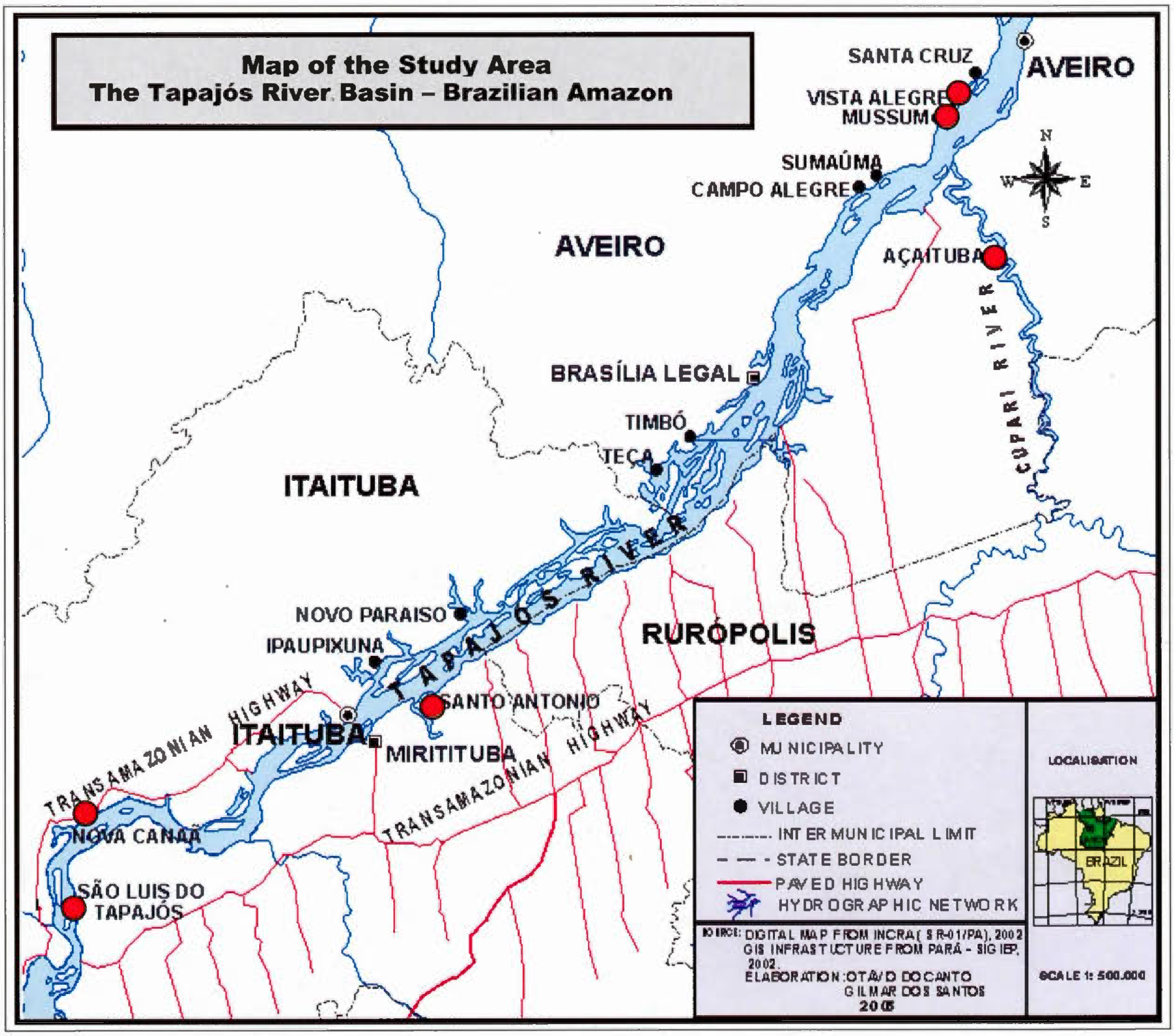

Figure 1: Map of the study area, the Tapajós River Valley. Participating communities are identified by a large red dot

Total mercury concentrations in fish flesh were determined in the laboratory of the Environmental Research Chair-GEOTOP at the University of Quebec in Montreal, according to the method described by Pichet et al. (1999). A wet weight ranging from 100 to 200 mg was removed from each specimen for the acid digestion process. The digest was brought to a final volume of 30 ml with water, and then it was analyzed by measuring the atomic fluorescence (CVAFS) of the liberated mercury following its reduction by Sn (II). The system has a detection limit of 0.002μg/g for 1 mg of sample. This laboratory participates annually in the Mercury Quality Assurance Program of the Canadian Agency for Food Inspection, as well as twice a year in the Inter-Laboratory Comparison Program of Health Canada. The accuracy of the method was verified using the TORT-2 certified standard (lobster hepatopancreas reference material from NRC). During the evaluation period, results averaged 284 ± 8ng/g, which falls well within the certified value range of 272 ± 60ng/g. For samples analyzed in duplicate, the average standard deviation was 4 ± 3%.

Fish and fruit intake

In the Amazon, water levels vary by 6 m up to 20 m between the high water and low water seasons causing important seasonal changes in fish habitat and behaviour (Goulding et al., 1996; Bastos et al., 2007) and thus in the availability of different fish species for the riverside populations (Lebel et al., 1997; Dolbec et al., 2001; Passos et al., 2001). The rain cycle and local conditions likewise affect seasonal fruit availability (Passos et al., 2001). Because of these seasonal variations, we focused on recent consumption practices and an interview-administered 7-day recall food frequency questionnaire was used for fish and fruit consumption. For fish, a list was prepared which included most of the fish species present in the region. Participants indicated, for each day, the number of meals containing fish as well as the fish species that were consumed.

In addition, in each of the villages, the quantity of fish (g) eaten during a meal was determined in a subset of 72 villagers. The fish meal, after cooking and before serving, was weighed using a Digital Scale Tanita™ (Model KD-200, 2 kg capacity, Min. grad 0-2000g, d=2g). Data were analyzed separately for men and women and averaged for each village.

As for fruits, for each fruit species, the participant indicated the number of fruits that had been eaten each day over the preceding seven days, whether during a meal or not. Fish and fruit species that were not in the initial list were also recorded.

Estimate of mercury intake

Estimated mercury intake was determined for men and women from each village using the average fish mercury concentration for the fish caught in their fishing zone, and the average gender-specific quantity of fish eaten at each meal. In the case of fish species for which there were no mercury measurements (95 fish meals (5.3% of total fish meals)), we used the average regional mercury concentration of the fish's feeding habit (carnivorous or non-carnivorous). The product of fish species-specific mercury (µg/g) and the weight of the average meal (g) was then multiplied by the number of reported meals of the specific species over the last seven days. These were then summed to provide the total intake over the past week. Total daily intake per body weight was then determined by dividing the weekly intake by 7 days and the person's weight, according to the formula contained in Figure 2:

Weekly Hg intake =

Fish quantity per meal (g) * (Fish $species_1$-Hg-concentration (µg/g) * Number of fish meals of $species_1$) + (Fish $species_2$-Hg-concentration (µg/g) * Number of fish meals of $species_2$) + … (Fish $species_i$-Hg-concentration (µg/g) * Number of fish meals of $species_i$) = *µg Hg/week*

Daily Hg intake per body weight = Weekly Hg intake (µg)/body weight/7days (kg) = *µg/kg/d*

Figure 2: Formula for the calculation of weekly and daily mercury intakes.

Sampling and analyses of bioindicators of exposure

Total mercury concentrations in whole blood or scalp hair are the indicator media of choice in adults for methyl mercury exposure from fish consumption (Cernichiari et al., 1995; Clarkson and Magos, 2006; Mergler et al., 2007).

Blood samples were collected by a nurse by venipuncture into 6 ml heparinized Becton Dickinson Vacutainer® (BD7863). All blood samples were kept frozen at –20° until analyzed. Total and inorganic mercury in blood were determined by Atomic Absorption Spectrometry at the laboratory of the Quebec Toxicology Center of the Quebec Public Health Institute (CTQ-INSPQ), Canada, according to the method described by Ebbestadt et al. (1975). The detection limit for blood mercury analysis was 0.2µg/L, and analytical quality control was ensured by the use of internal reference samples for blood analysis provided by the Inter-Laboratory Comparison Program conducted by the CTQ-INSPQ. The CTQ is accredited ISO 17025 and analytical performance for mercury analysis in the Inter-Laboratory Comparison Program for Metals in Biological Media was 36/36 for precision and 6/6 for reproducibility.

Hair strands from the occipital region were cut at the root and then placed in plastic bags, with the root end stapled. The first centimeter of each sample was analyzed at the

Radioisotopes Laboratory of the Federal University of Rio de Janeiro (Brazil), by Atomic Absorption Spectrometry with an AA 1475 Varian and a cold vapor generator accessory VGA-76 Varian. Mineralisation of samples was done with mixtures of acids (HCl, HNO_3, and H_2SO_4) and oxidants ($KMnO_4$, $K_2S_2O_7$, and H_2O_2), with techniques developed and adapted to the flow injection system vapor generator accessory (Malm et al., 1989). This laboratory participates regularly in inter-laboratory comparison programs for total and inorganic mercury analysis (Gill et al., 2002), and analytical quality control was ensured by the use of standard reference materials (Human Hair 085 and 086) provided by the International Atomic Energy Agency (IAEA).

Statistical analyses

Fish mercury levels, fruit and fish intake, mercury daily intake, mercury exposures and socio-demographics were characterized using descriptive statistics. Inter-group comparisons were made using parametric or nonparametric techniques, depending on data distribution. The relations between socio-demographic variables and daily mercury intake were examined using multivariate regression models. Where possible, continuous variables were used (age, years of schooling, number of fish meals, number of fruits). Smoking and alcohol consumption status, and villages were included as categorical variables. The influence of fruit consumption frequency on the relations between daily mercury intake and bioindicators of exposure was examined using analysis of covariance. For these analyses all pregnant women were excluded. Results were defined as statistically significant for a value of $P \leq 0.05$. Analyses were performed using Statview for Windows Version 5.0.1 and Jump 5.0.1a (SAS Institute Inc.).

Results

Table 1 shows mean mercury concentrations for the fish species captured and effectively eaten in each community. Mercury concentrations averaged 0.33µg/g for the 1,123 fish samples, of which the 702 non-carnivorous specimens presented a mean concentration of 0.11µg/g, while the 421 carnivorous specimens averaged 0.52 µg of Hg per gram of fish tissue. No significant inter-community variations in fish mercury levels were observed (Kruskal-Wallis, $p = 0.5$).

Table 2 presents the participants' socio-demographic characteristics. The overall level of education was low (mean: 3.6 ± 2.6 schooling years), ranging from 0 – 11 years, and the age range was 15 to 89 years (mean 35.3 years ± 15.9). Eighty-one percent (81%) of the participants were originally from the Tapajós region, and 73.4% lived on the Tapajós River banks, while the others lived on one of its tributaries.

In this season, 92.5% of the participants consumed at least one meal with fish over the preceding seven days. On average, villagers had consumed 7 fish meals over the last week, with carnivorous fish constituting an average of 45% of the fish diet (ranging from 0 to 100%). The gender-differentiated rates of fish ingestion as well as anthropometric data for each community are summarized in Table 3. In all, participants consumed an average of 141 grams of fish per meal, with a significant difference between women (mean: 124g ± 65.9) and men (mean: 189.7g ± 105.5) (Mann-Whitney U, $p = 0.0014$). Although no significant inter-community differences were observed (Kruskal-Wallis, $p = 0.1$), when villagers were grouped by village location, persons living on the shores of the Tapajós ate larger portions (mean: 155.3 ± 38 grams of fish) compared to those living on its tributaries (mean: 121.5 ± 36.3 grams of fish) (Mann-Whitney U, $p < 0.0001$). Persons born in the Tapajós region consumed significantly higher quantities of fish (151.1 ± 40.8 grams of fish) than immigrants from northeast Brazil (129.8 ± 35.1 grams of fish) (Mann-Whitney U, $p = 0.003$). Multiple regression analysis confirmed the relation of fish ingestion rates with gender ($\beta = 31.5$; $p < 0.0001$) and village ($\beta = 36.8$; $p < 0.0001$), but showed no relation with schooling or immigrant status.

The overall mean body weight was 57 ± 10.6 kg, which significantly differed between women and men (means: 52.9 ± 9.5 and 60.8 ± 10.1, respectively) (Unpaired t-test, $p < 0.0001$). Although body weight did not significantly differ between communities (ANOVA, $p = 0.7$) and did not vary with villages' location (Unpaired t-test, $p = 0.2$), multiple regression analysis revealed a negative relation for body weight with fish consumption frequency ($\beta = -0.28$; $p = 0.05$), a direct link with the quantity (g) of fish ingested ($\beta = 0.11$; $p < 0.0001$), and a weak but significant positive association with age ($\beta = 0.08$; $p = 0.04$).

Table 1: Mean Hg concentrations (μg/g) in fish species captured and eaten in the participating communities

		Mean ± S.D.					
Fish species	**Feeding habits**	**SLT***	**NC***	**SA***	**VA***	**MU***	**AÇ***
Pescada (*Plagioscion sp.*)	carnivorous	0.65 ± 0.33	0.57 ± 0.23	0.60 ± 0.28	0.45 ± 0.17	0.45 ± 0.17	0.39 ± 0.23
Aracu (*Schizodon sp.*)	non-carnivorous	0.20 ± 0.14	0.17 ± 0.11	0.13 ± 0.11	0.10 ± 0.06	0.10 ± 0.06	0.14 ± 0.10
Caratinga (*Geophagus sp*)	non-carnivorous	0.18 ± 0.12	0.18 ± 0.11	0.14 ± 0.06	0.07 ± 0.03	0.07 ± 0.03	0.11 ± 0.02
Tucunare (*Cichla sp.*)	carnivorous	2.3 ± -	0.60 ± 0.55	0.39 ± 0.15	0.52 ± 0.36	0.52 ± 0.36	0.19 ± 0.13
Sarda (*Pellona sp*)	carnivorous	0.43 ± 0.11	0.61 ± 0.22	0.66 ± -	0.77 ± 0.24	0.77 ± 0.24	0.66 ± 0.30
Piranha (*Serrasalmus sp.*)	carnivorous	0.40 ± 0.26	0.57 ± 0.40	0.40 ± 0.21	0.40 ± 0.19	0.40 ± 0.19	0.31 ± 0.21
Branquinha (*Curimata sp.*)	non-carnivorous	0.19 ± 0.06	0.17 ± 0.09	0.10 ± 0.03	0.06 ± 0.02	0.06 ± 0.02	0.07 ± 0.05
Charuto (*Hemiodus sp.*)	non-carnivorous	0.09 ± 0.08	0.08 ± 0.06	0.14 ± 0.10	0.05 ± 0.05	0.05 ± 0.05	0.07 ± 0.03
Mandi (*Auchenipterus sp.*)	carnivorous	0.46 ± 0.38	0.33 ± 0.15	0.41 ± 0.19	0.69 ± -	0.69 ± -	0.20 ± 0.09
Jaraqui (*Semaprochilodus sp.*)	non-carnivorous	0.06 ± 0.02	0.09 ± 0.05	-	-	-	0.05 ± 0.002
Surubim (*Pseudoplatystoma sp.*)	carnivorous	0.28 ± 0.16	0.27 ± 0.12	-	-	-	0.31 ± 0.12
Peixe-cachorro (*Raphiodus sp)*	carnivorous	0.63 ± 0.52	0.58 ± 0.25	0.91 ± 1.2	-	-	0.42 ± 0.13
Matrinxã (*Brycon sp.*)	non-carnivorous	0.1 ± -	-	-	-	-	-
Pacu (*Mylossoma sp.*)	non-carnivorous	0.02 ± -	0.2 ± -	-	-	-	0.2 ± -
Tambaqui (*Colossoma sp.*)	non-carnivorous	-	-	-	-	-	0.04 ± -
Curvina (*Pachypops sp.*)	non-carnivorous	-	0.1 ± -	-	-	-	-
Traira (*Hoplias sp.*)	carnivorous	-	0.3 ± -	-	-	-	0.4 ± -

Curimata (*Prochilodus sp.*)	non-carnivorous	-	-	-	-	-	0.1
Sardinha (*Triportheus sp.*)	non-carnivorous	-	0.02 ± 0.1	-	-	-	0.03 ± 0.2
Aruanã (*Osteoglossum*	carnivorous	0.2 ± -	-	0.8 ± -	-	-	0.3 ± -
bicirrhosum)	non-carnivorous	-	0.1 ± -	0.1 ± -	-	-	0.04 ± -
Acari (Liposarcus pardallis)	non-carnivorous	0.1 ± -	0.2 ± -	-	0.3 ± -	0.3 ± -	-
Mapará (*Hypophthalmus sp.*)	carnivorous	-	-	-	-	-	0.2 ± -
Cujuba (*Pseudodoras sp.*)	carnivorous	-	0.5 ± -			-	-
Jacundá (*Crenicichla sp.*)							

* SLT : São Luis do Tapajós; NC: Nova Canaã; SA : Santo Antonio; VA : Vista Alegre; MU : Mussum; AÇ : Açaituba

Ninety-one percent (91%) of participants ate at least one fruit over the seven-day survey, with an average consumption of 8 fruits/week, ranging from 0 - 39 fruits/week. Fruit consumption was slightly but significantly different between women (mean: 7.1 ± 7.5 fruits/week) and men (mean: 9.6 ± 8.7 fruits/week) (Mann-Whitney U; $p = 0.03$). Significant differences were also observed among communities (Kruskal-Wallis, $p < 0.0001$), with villagers living in the southern Tapajós (São Luis do Tapajós, Nova Canaã, and Santo Antonio) presenting lower rates of consumption compared to villagers situated in communities close to the small town of Aveiro. The intake of fruit was also weakly but directly and significantly associated with years of schooling (Spearman = 0.15; $p = 0.02$).

Table 2: Socio-demographic characterization of the sampled population

	Women		**Men**	
Characteristics	n	%	n	%
Age categories				
15 to 24 y	33	27.9	39	29.4
25 to 34 y	30	24.6	29	21.3
35 to 44 y	24	19.7	32	23.5
45 to 54 y	13	10.7	16	11.8
55 to 64 y	14	11.5	11	8.1
≥ 65 y	7	5.7	8	5.9
Current alcohol consumers	29	23.9	67	49.6
Current Smokers	27	22.3	50	37.0
Schooling				
No formal education	13	10.7	23	17.0
Elementary school (1 to 8 years)	100	82.6	107	79.3
High school and more (≥9 years)	8	6.6	2	1.5
Village location				
On the Tapajós River banks	93	76.9	91	67.4
On an affluent	29	23.9	45	33.3

Estimated daily mercury intake varied between 0 and 11.8μg/kg/d, with a mean of 0.92 and median of 0.59μg/kg/d. There was one extreme value of 11.8 in the village of São Luis do Tapajós, which was excluded from the present analyses. Figure 2 shows the distribution of the daily intake for the entire population, without the above-mentioned outlier.

Nonparametric comparisons showed no differences with respect to gender or smoking status, but a weak difference was observed between alcohol drinkers and non-drinkers, with the latter presenting lower rates of mercury intake (Mann-Whitney, $p = 0.04$). Also, daily mercury intake was twice as high for persons born in the Tapajós region compared to immigrants (means: 0.97 ± 0.92 μg/kg/d and 0.48 ± 0.61 μg/kg/d, respectively) (Mann-Whitney, $p < 0.0001$). Pronounced and significant differences among communities were observed, as shown in Figure 3 (Kruskal-Wallis, $p < 0.0001$). Multivariate regression analyses showed that the daily mercury intake is gender-related, with men presenting higher intake rates than women ($\beta = 0.1$; $p < 0.05$); there was a tendency towards an inverse and weak association with age ($\beta = -0.01$, $p = 0.07$), as well as strong variations between villages ($\beta = 0.4$; $p < 0.0001$).

Table 3: Mean values for the amounts of fish ingested during one meal, and weight information of study participants

	Mean fish consumption (g)		Mean weight (kg)	
Communities	Women	Men	Women	Men
São Luis do Tapajós	147.3 ± 72.0	206.7 ± 71.1	54.4 ± 8.9	60.5 ± 11.1
Nova Canaã	101.3 ± 44.2	132.5 ± 38.9	51.5 ± 8.8	62.1 ± 11.7
Santo Antonio	81.0 ± 32.5	165.0 ± 40.9	55.8 ± 13.7	55.1 ± 8.5
Vista Alegre	122.1 ± 70.2	200.6 ± 150.7	52.3 ± 8.3	64.1 ± 9.8
Mussum	122.1 ± 70.2	200.6 ± 150.7	50.9 ± 8.1	62.6 ± 7.5
Açaituba	80.3 ± 25.1	133.1 ± 30.2	48.5 ± 7.6	60.2 ± 7.4

Mean blood total mercury for the whole cohort was 60.1 ± 38.4 μg/L (median: 57.1 μg/L, ranging from 4.8 to 205 μg/L), of which 86.8% was in the form of methyl mercury, ranging from 75.2% to 94.3%. Inorganic mercury averaged 7.6 ± 4.5μg/L, with a maximum of 24μg/L. Mean total hair mercury concentration was 17.9 ± 11.5μg/g (median: 16.5, range

0.2 – 58.3μg/g). Bioindicators of exposure differed significantly by gender and village, with men presenting higher concentrations than women (mean blood Hg: 65 ± 40.9 μg/L and 55 ± 35 μg/L; mean hair Hg: 19.3 ± 11.9 μg/g and 16.3 ± 11 μg/g, respectively), and São Luis do Tapajós and Santo Antonio representing the most and the least exposed communities, respectively (Kruskal-Wallis, $p < 0.0001$).

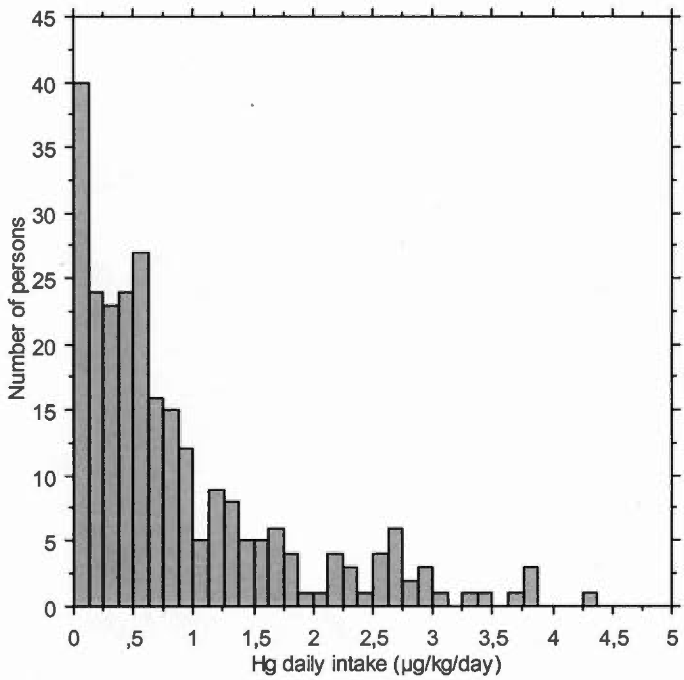

Figure 3: Overall distribution of the daily Hg intake for the entire population

When total blood and hair mercury levels were examined as dependent variables in separate simple regression models, the daily mercury intake explained 28% and 9% of their variations, respectively. Table 4 shows the set of covariates explaining mercury variations in blood and hair. There was a strong and positive relation between daily mercury intake and blood total mercury levels, with an inverse relation for blood mercury with fruit intake. We likewise observed a negative relation for blood mercury with years of schooling, a tendency for differences between men and women, and significant variations with smoking and immigrant status, as well as between villages. Blood organic mercury concentration was also strongly related to the daily mercury intake (β = 17.2; $p < 0.0001$) and inversely associated with fruit consumption (β = - 0.5; $p < 0.05$). It was inversely related to years of schooling (β = - 2.8; p = 0.001), and varied with both immigrant status (β = -5.7; $p < 0.05$; Tapajós natives presenting higher levels than immigrants) and village (β = 12.2; p = 0.002). In turn, blood inorganic mercury was associated with daily mercury intake from fish (β = 1.9; $p < 0.0001$), and a tendency of inverse relation with fruit intake was observed (β = - 0.05; $p < 0.09$). Hair mercury was directly associated with daily mercury intake and inversely related to years of schooling. It was related to gender, with men presenting higher levels compared to women, and varied with villages as well as immigrant status. An inverse relation with fruit consumption was likewise observed.

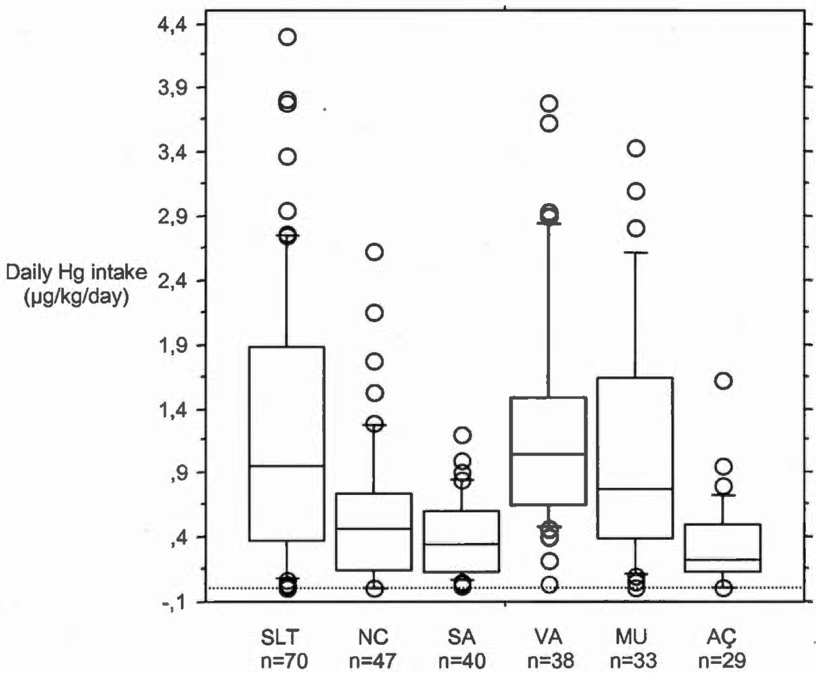

Figure 4: Percentile distributions of daily Hg intake for the six participating communities (SLT – São Luis do Tapajós; NC: Nova Canaã; SA – Santo Antonio; VA: Vista Alegre; MU: Mussum; AÇ: Açaituba)

The influence of fruit consumption on the relation between daily mercury intake from fish and blood total mercury levels was further examined through analysis of covariance, as illustrated in Figure 4. The regression lines are drawn for those with low fruit consumption ($\leq$ 3 fruits/week; n = 64), medium fruit consumption (> 3 fruits/week $\leq$ 10 fruits/week; n = 90), and high fruit consumption (> 10 fruits/week, n = 79) in relation to blood mercury concentrations.

Table 4: Beta (β) coefficients and significance levels for covariates explaining blood and hair total mercury concentrations in multiple stepwise regression models.

	Bioindicators of exposure (n = 256 persons, 121 women and 135 men)	
Covariates	*β coefficients*	*β coefficients*
	Blood T-Hg (μg/L)	**Hair T-Hg (μg/g)**
Hg daily intake (μg/kg/d)	19.7 ($p < 0.0001$)	3.4 ($p = 0.0001$)
Fruit intake (fruits/week)	- 0.56 ($p = 0.0357$)	- 0.19 ($p = 0.0353$)
Gender (woman/man)	- 3.6 ($p = 0.0880$)	- 1.3 ($p = 0.05$)
Schooling (years)	- 2.8 ($p = 0.0028$)	- 0.7 ($p = 0.0102$)
Smoking (smoker/non-smoker)	- 5.3 ($p = 0.0266$)	ns
Village location (Tapajós/tributary)	- 5.5 ($p = 0.0323$)	- 3.5 ($p = 0.0002$)
Immigrant status(native/immigrant)	- 6.9 ($p = 0.0104$)	- 1.8 ($p = 0.0429$)
	Model R^2 = 0.40	**Model R^2 = 0.23**

ns: non-significant

The intercepts of the three regression lines are similar, but their slopes are significantly different (ANCOVA Interaction term: $F = 10.9$, $p < 0.0001$), indicating that for the same daily mercury intake, persons eating fruit more frequently have lower blood mercury concentrations. The difference of mean mercury concentrations between high and low fruit consumers is 9.3μg/L, corresponding to 14.4% reduction in mean blood mercury (Figure 5). In addition, the median difference of the ratio of blood mercury to daily mercury intake between low and high fruit consumers was 26, representing a reduction in the order of 26.3% in terms of the input of ingested mercury into the blood stream.

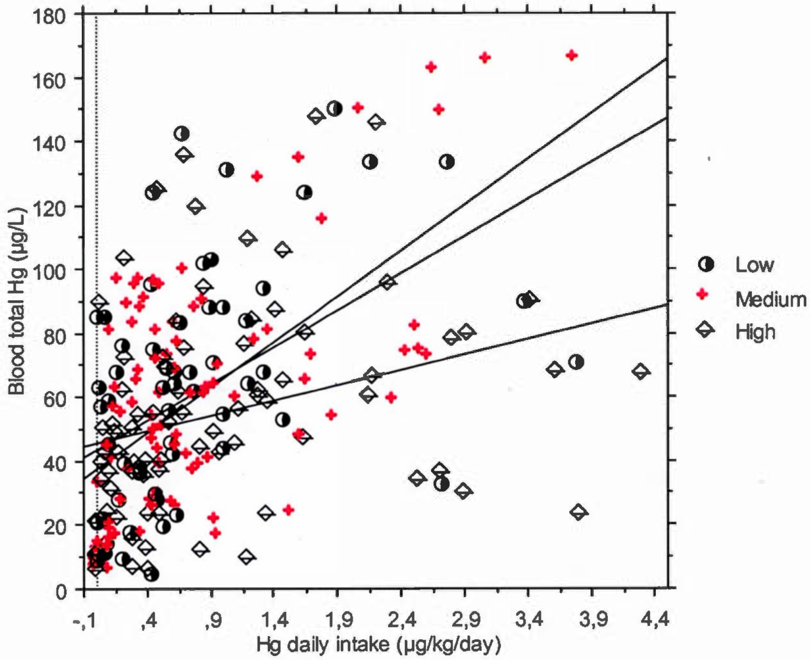

Figure 5: The influence of fruit intake on the relation between daily Hg intake and blood mercury levels.

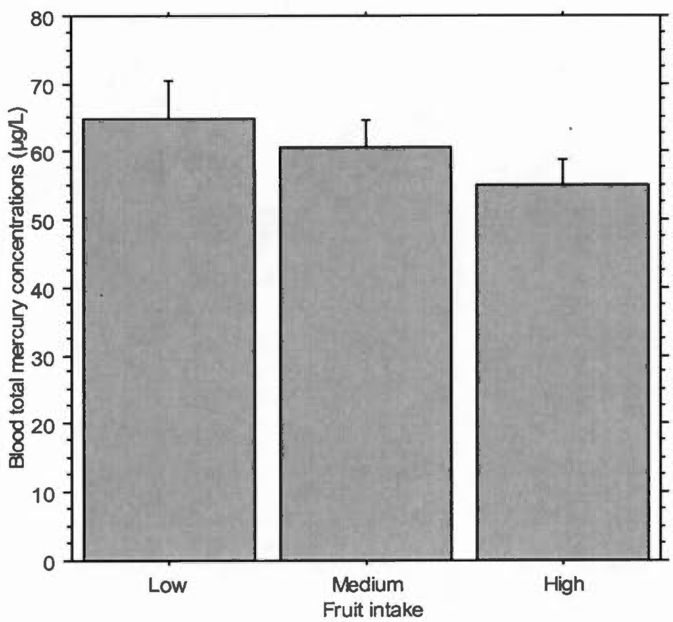

Figure 6: Mean blood total mercury concentrations according to the levels of fruit intake (Low fruit consumption: ≤ 3 fruits/week; n = 64; Medium fruit consumption: > 3 fruits/week ≤ 10 fruits/week; n = 90; High fruit consumption: > 10 fruits/week, n = 79)

Discussion

This study shows high rates of daily mercury intake in riparian communities of the Amazonian Tapajós region, who rely heavily on fish consumption. The calculations, based on measured concentrations of mercury for local fish species consumed by the different communities and weighed meal quantities, provide a high level of precision. Bioindicators of exposure were highly correlated to daily mercury intake averaged over the past seven days, with blood, an indicator of current exposure, showing a much stronger correlation than hair. Fruit consumption significantly modified these relations.

Regulatory guidelines to limit environmental mercury exposures have been developed by governmental agencies throughout the world (Adams and Schantz, 2006). Reference doses (RfD) are derived from a no observed adverse effect level (NOAEL) (the highest dose at which no adverse effects are identified), or a lowest observed adverse effect level (LOAEL) (the lowest dose at which adverse effects are observed), typically identified in animal studies (Rice et al., 2003). The RfD can also be derived from a benchmark (BMD) analysis, which is based on the dose associated with a specified measure or change of a biological effect (Rice et al., 2003).

Based primarily on the results of large longitudinal neurodevelopmental toxicity studies conducted in New Zealand, the Seychelles and the Faroe Islands, and using varying interpretations of the epidemiological evidence, recommended intake without appreciable risks currently range from 0.1 to 0.3 μg/kg/d (Wijngaarden et al., 2006; Innis et al., 2006; Clarkson and Magos, 2006). In comparison, daily mercury intake of the Amazonian population in the present study averaged 0.92μg/kg/d, ranging from 0 to 4.3μg/kg/d, with an outlier whose intake was 11.8μg/kg/d. In this population, 86.3% of participants presented daily intake above 0.1μg/kg/d and 72.4% above 0.3μg/kg/d. Thus, the large majority of participants surpassed all recommended reference doses (*RfD*), which have been derived by various agencies. The *RfD* is "an estimate of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious effects during a lifetime" (U.S. EPA, 1998). Even if for some authors, exceeding these guidelines by a small margin is meaningless in terms of actual risk because of so many uncertainties (Clarkson and Magos, 2006), given the wide range of variation of the daily intake in this Amazonian cohort, there is reason for concern about the potential for deleterious health effects that could be caused by high mercury intake. Indeed, almost 50% of the population surpassed the Benchmark dose (BMD) for blood mercury (58µg/L) that was used to calculate the U.S. NAS/NRC reference dose (NRC, 2000), and over 50% surpassed the BMD for hair mercury (14µg/g) used to determine the World Health Organization reference dose (WHO, 2003).

There are important inter-village differences in mercury intake, varying from a median of 0.22µg/kg/d to 1.05µg/kg/d. Daily mercury intake was much higher among persons born in the Tapajós region compared to immigrants, and for persons living on the shores of the Tapajós as opposed to those residing in the tributaries. This probably reflects the fact that those born on the Tapajós have a longer tradition of eating fish and those that live directly on the Tapajós eat fish more frequently and consume bigger portions. In addition, environmental mercury levels around the villages, differences in methylation rates (Miranda et al., 2004; Guimarães, 2001; Roulet et al., 2001) or simply changes in the availability of the different fish species present in the various ecosystems can also contribute to inter-village differences (Dolbec et al., 2001).

Men presented higher intakes compared to women, who consumed significantly lesser quantities of fish than men, even when considering their lower body weight. Men also showed higher mercury intake rates despite higher self-reported alcohol consumption as compared to women. Even though the inhibition of mercury absorption by dietary ethanol in dentists has recently been suggested (Martin and Naleway, 2004), to date only one study showed such evidence in a fish-eating population (Grandjean et al., 1992), which although involving a large number of participants was only able to report that blood mercury levels were slightly lower if the mother had occasionally ingested alcohol beverages. In the current study, multivariate analyses did not reveal any effect of alcohol consumption on mercury exposure.

In the present study, there were substantial inter-village differences in mean fish portion size per meal, ranging from 171 grams in São Luis do Tapajós to 115 grams in Açaituba. Pronounced variations have also been reported for different areas of the Amazon basin. For example, Boischio and Henshel (1996) reported fish portions of 200 grams for the riverside population of the Madeira River Basin, while Hacon et al. (1997) presented an average fish ingestion rate of 110 grams per day for fishermen of the Alta Floresta region. A daily consumption of about 110 grams has likewise been reported for the villagers of the Balbina reservoir (Kehrig et al., 1998), whereas Fréry et al. (2001) showed that in periods of abundance the amount of fish consumption may reach over 600 grams per day and that in times of scarcity these amounts may be relatively low. These latter authors report an average daily consumption surrounding 340 grams for adult men of native Amerindian communities in French Guiana. Although there are variations in portion size, depending probably on the size and amount of fish captured and the availability of different fish species, throughout the Amazon, large amounts of fish are consumed daily.

In the present study, several factors were predictive of blood and hair mercury levels. As expected, the strongest associations were observed with the daily mercury intakes. The intake of fruit was associated with lower mercury concentrations in both bioindicators, corroborating findings of recent studies of fish eaters in the Tapajós basin, which indicate that individuals consuming fresh fruit more frequently have much lower blood and hair mercury concentrations for the same level of fish consumption, as compared to those who consume fruit less frequently (Passos et al., 2003b, 2004, 2005, 2007a, b). In an exploratory study, which covered a large variety of dietary information such as the intake of vegetables and many other foods, only fruit consumption was related to bioindicators of mercury exposure, when fish consumption frequency was considered (Passos et al., 2001; Passos et al 2003b). In the present study, fruit consumption was the only element that modified the relation between mercury intake and bioindicators of exposure.

There has been much debate about how to address the risks of mercury exposure and the benefits of fish consumption with questions raised about the suitability of regulating human risk based either on controlling average daily intake, as recommended by the EPA, or excluding fish with high mercury levels from the diet as put forward by FDA (Clarkson and Magos, 2006; Stern et al., 2004). In the Amazon, because fish is a dietary mainstay and there is high mercury intake, there is a need for a suitable strategy that would reduce mercury exposure while maintaining fish consumption.

Some researchers have proposed that fish intake in the Amazon should be limited. Kehrig et al. (1998) suggested that pregnant women and women of childbearing age should consume less fish in order to limit their exposure to potential sources of methyl mercury, while Boischio and Henshel (2000) put forward that risk communication for this exposed population could be addressed using fish advisories for consumers, fishermen and retailers, which would recommend an acceptable number of fish meals to be consumed according to species. While both these studies used predictive models to provide important insights on the potential risks to human health, their recommendations are limited by their methodological approach. For example, despite some inaccuracies (Passos and Mergler, 2006), a recent study which reconstructs methyl mercury intakes in indigenous and riparian populations from biomarker data, indicates that notwithstanding the chronic exposure scenario in the Amazon, steady-state assumptions are not in order because of the strong seasonal fluctuations in methyl mercury exposure (Gosselin et al., 2006). These findings, coupled to the results of the present study which show regional ecosystem variations in mercury concentrations, as well as inter-community differences in fish portions, and considering the socio-economic conditions as well as the dietary and cultural background of these communities (Passos et al., 2003a, 2001; Mertens et al., 2005), suggest that a risk management strategy that focuses solely on limiting the number of fish meals might deprive this population of an important food source. In French Guyana, Fréry et al. (2001) encouraged close collaboration between local populations, public health authorities, and scientists in order to develop concrete recommendations and adopt measures to reduce the environmental sources of this high exposure. A community-based campaign carried out in a village on the Tapajós, which focused on "eating more fish that don't eat other fish" was successful in reducing mercury exposure by close to 40% while maintaining the same quantity of fish consumption (Mertens et al., 2005; Lucotte et al., 2004; Bahia et al., 2004; Mergler et al., 2001). Further interventions, focusing on agro-forestry practices that would limit deforestation and/or reduce soil erosion, would serve to reduce the source of mercury contamination in these ecosystems.

Some authors have recently minimized the importance of mercury exposure in the Amazonian region, suggesting that despite high concentrations of methyl mercury in fish, daily consumption of large amounts of this central and highly nutritious food over the course of a lifetime, poses no health hazards for Amazonians (Dórea, 2003, 2004; Dórea et al., 2005). There has been the suggestion that based on the general health status of the population, "normal" values higher than those proposed by WHO could be established in the Amazon (Santos et al., 2002). However, another Amazonian study concludes that differences in exposed and non-exposed populations in this region would argue against an Amazonian reference level, particularly since levels surpass those of WHO (Pinheiro et al., 2006). Despite attempts to establish reference values and background parameters as well as eliminate uncertainties about the reference dose in the Amazonian context, few studies have adopted a rigorous procedure for developing risk-based reference doses (Gaylor and Kodell, 2002; Rice, 2005). Dietary and genetic factors (Passos et al., 2003b, 2004, 2005, 2007a,b; Klautau-Guimarães et al., 2005) as well as other specificities of the Amazonian traditional populations, make it difficult to evaluate the suitability of using current reference doses for methyl mercury exposure in the Amazon. Despite this difficulty, allegations that daily consumption of mercury-contaminated fish in large amounts poses no health hazards, based on the absence of mercury-induced clinical effects and the existence of general health problems (Dórea, 2003, 2004), do not take into account the importance of changes in biological functions on the population level and their usefulness for risk assessment (Bellinger, 2004; Rice, 2005). Simplistic allegations may be misleading and do not contribute to the establishment of scientifically-based guidelines able to effectively orient a viable risk management strategy in the Amazon.

The influence of fruit consumption on the relation between fish consumption and mercury exposure in the present study are consistent with previous investigations undertaken in this same region (Passos et al., 2003b, 2004, 2005, 2007a,b), corroborating a relevant avenue that should be further explored as a potential additional short-term intervention strategy, while mercury levels in the environment are brought under control through other intervention projects aiming to reduce the release of mercury from soils toward aquatic ecosystems, based on better farm land-use management strategies (Farella et al., 2006, 2007). The data from the present study suggest that reference doses for mercury in fish-eating populations where fruit is an important component of diet could be considerably different from those without daily access to fresh fruit. These findings further underline the importance of considering the influence of dietary factors in risk assessment.

Conclusion

The present results for riparian populations of the Tapajós River Basin indicate that a variety of factors determine both the rates of mercury daily intake and their relations with bioindicators of exposure. The findings support the need to conduct rigorous studies for developing risk-based reference doses adapted to the Amazonian context. In particular, future studies examining early neurotoxic and cardiovascular effects as well as immunologic outcomes associated with mercury exposure should attempt to determine benchmark doses, which could form the basis for the establishment of reference doses suitable to orient viable risk management strategies for these populations. Meanwhile, the high level of daily mercury intake points out the need for intervention projects, which should not only seek to reduce the degree of exposure in the human populations while maintaining fish consumption, but also re-establish the integrity of the ecosystems.

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References

Almeida, MD., Lacerda, LD., Bastos, WR., Herrmann., 2005. Mercury loss from soils following conversion from forest to pasture in Rondônia, Western Amazon, Brazil. Environ Poll 137: 179-186.

Adams, J., Schantz, SL., 2006. Editors' note: Methyl mercury 2006. Neurotoxicol Teratol 28: 527-528.

Amorim, M.I.M. et al., 2000. Cytogenetic damage related to low levels of methyl mercury contamination in the Brazilian Amazon. An Acad Bras Ci 72(4): 497-507.

Bahia, MO., et al. 2004., Environmental biomonitoring using cytogenetic endpoints in a population exposed to mercury in the Brazilian Amazon. Environ Mol Mutagen 44: 346-349.

Barbosa, AC., Garcia, AM., Souza, JR., 1997. Mercury contamination in hair of riverine populations of Apiacás reserve in the Brazilian Amazon. Water Air Soil Poll 97: 1-8.

Bastos, WR., et al., 2006. Mercury in the environment and riverside population in the Madeira River Basin, Amazon, Brazil. Sci Total Environ 368: 344-351.

Bastos, WR., Almeida, R., Dórea, JG., Barbosa, AC., 2007. Annual flooding and fish-mercury bioaccumulation in the environmentally impacted Rio Madeira (Amazon). Ecotoxicol 16: 341-346.

Bellinger, DC., 2004. What is an adverse effect? A possible resolution of clinical and epidemiological perspectives on neurobehavioral toxicity. Environ Res 95: 394-405.

Bidone, ED., Castilhos, ZC., Santos, TJS., Souza, TMC., Lacerda, LD., 1997. Fish contamination and human exposure to mercury in Tartarugalzinho River, Amapá State, Northern Amazon, Brazil. A screening approach. Water Air Soil Poll 97: 9-15.

Boischio, AAP., Henshel, DS., 1996. Risk assessment of mercury exposure through fish consumption by the riverside people in the Madeira Basin, Amazon, 1991. Neurotoxicol 17(1): 169-176.

Boischio, AAP., Henshel, D., 2000. Fish consumption, fish lore, and mercury pollution – risk communication for the Madeira River people. Environ Res Section A 84: 108-126.

CARUSO. Mercury exposure and ecosystem health in the Amazon. 2007. http://www.unites.uqam.ca/gmf/caruso/caruso.htm

Cernichiari, E., et al., 1995. Monitoring methyl mercury during pregnancy: maternal hair predicts fetal brain exposure. Neurotoxicol 16(4), 705-710.

Clarkson, TW., Magos, L., 2006. The toxicology of mercury and its chemical compounds. Crit Rev Toxicol 36: 609-662.

Cordier, S., Grasmick, C., Paquier-Passelaigue, M., Mandereau, L., Weber, J-P., Jouan, M., 1998. Mercury exposure in French Guiana: levels and determinants. Arch Environ Health 53(4): 299-305.

Cordier, C., et al., 2002. Neurodevelopmental investigations among methyl mercury-exposed children in French Guiana. Environ Res 89: 1-11.

Dolbec, J., Mergler, D., Passos, C.J.S., Morais, S.S., Lebel, J., 2000. Methyl mercury exposure affects motor performance of a riverine population of the Tapajós River, Brazilian Amazon. Int Arch Occup Environ Health 73: 195-203.

Dolbec, J., Mergler, D., Larribe, F., Roulet, M., Lebel, J., Lucotte, M., 2001. Sequential analysis of hair mercury levels in relation to fish diet of an Amazonian population, Brazil. Sci Total Environ 271: 87-97.

Dórea, JG., 2003. Fish are central in the diet of Amazonian riparians: should we worry about their mercury concentrations? Environ Res 92: 232-244.

Dórea, JG., 2004. Cassava cyanogens and fish mercury are high but safely consumed in the diet of native Amazonians. Ecotoxicol Environ Safety 57: 248-256.

Dórea, JG., Souza, JR., Rodrigues, P., Ferrai, I., Barbosa AC., 2005. Hair mercury (signature of fish consumption) and cardiovascular risk in Mundurucu and Kayabi Indians of Amazonia. Environ Res 97:209-219.

Ebbestadt V, Gunderson and Torgrimsen TA., 1975. Simple method for the determination of inorganic mercury and methyl mercury in biological samples by flameless atomic absorption. Atom Absorption Newsletter.14(6):142-143.

Fadini, PS., Jardim, WF., 2001. Is the Negro River Basin (Amazon) impacted by naturally occurring mercury? Sci Total Environ 275: 71-82.

Farella N, Lucotte M, Louchouarn P and Roulet M., 2001. Deforestation modifying terrestrial organic transport in the Rio Tapajós, Brazilian Amazon. *Org Geochem* 32: 1443-1458.

Farella, N., Lucotte, M., Davidson, R., Daigle, S., 2006. Mercury release from deforested soils triggered by base cation enrichment. Sci Total Environ 368: 19-29.

Farella, N., Davidson, R., Lucotte, M., and S. Daigle., 2007. Nutrient and mercury variations in soils from family farms of the Tapajós region (Brazilian Amazon): recommendations for better farming. Agric Ecosyst Environ 120: 449-462.

Fillion, M., Mergler, D., Passos, CJS., Larribe, F., Lemire, M., Guimarães, JRD., 2006. A preliminary study of mercury exposure and blood pressure in the Brazilian Amazon. Environ Health 5:29.

Fostier, AH., et al., 2000. Mercury fluxes in a natural forested Amazonian catchment (Serra do Navio, Amapá State, Brazil). Sci Total Environ 260: 201:211.

Fréry, N., Maury-Brachet, R., Maillot, E., Deheeger, M., Mérona, B., Boudou, A., 2001. Gold mining activities and mercury contamination of native Amerindian communities in French Guiana: key role of fish in dietary uptake. Environ Health Perpect 109: 449-456.

Gaylor, DW., Kodell, RL., 2002. A procedure for developing risk-based reference doses. Regul Toxicol Pharmacol 35: 137-141.

Gill, US., Schwartz, HM., Bigras, L., 2002. Results of multiyear international Inter-laboratory comparison program for mercury in human hair. Arch Environ Contam Toxicol 43:466-472.

Gonçalves, A., Gonçalves, NNS., 2004. Human exposure to mercury in the Brazilian Amazon: a historical perspective. Pan Am J Public Health 16(6): 415-419.

Gosselin, NH., Brunet, RC., Carrier, G., Bouchard, M., Feeley, M., 2006. Reconstruction of methyl mercury intakes in indigenous populations from biomarker data. J Exp Sci Environ Epidemiol 16: 19-29.

Goulding, M., Smith, N.J.H., Mahar, D.J. 1996. Floods of fortune. New York (NY): Columbia University Press.

Grandjean, P., et al., 1992. Impact of maternal seafood diet on foetal exposure to mercury, selenium, and lead. Arch Environ Health 47: 185-195.

Grandjean, P., White, RF., Nielsen, A., Cleary, D., Santos, ECO., 1999. Methyl mercury neurotoxicity in Amazonian children downstream from gold mining. Environ Health Perspect 107(7): 587-591.

Guimarães, JRD., 2001. Les processus de méthylation du mercure en milieu amazonien. *In*: Carmouze JP, Lucotte M and Boudou A (coord.), Le mercure en Amazonie: Rôle de l'homme et de l'environnement, risques sanitaires. IRD Éditions, Paris, 494 p.

Hacon, S., Rochedo, ER., Campos, R., Rosales, G., Lacerda, LD., 1997. Risk assessment of mercury in Alta Floresta. Amazon Basin – Brazil. Water Air Soil Poll 97: 91-105.

Harada, M., et al., 2001. Mercury pollution in the Tapajós River Basin, Amazon: mercury level of haid hair and health effects. Environ Int 27: 285-290.

Innis, SM., Palaty, J., Vaghri, Z., Lockitch, G., 2006. Increased levels of mercury associated with high fish intakes among children from Vancouver, Canada. J Pediatr 148: 759-763.

Kehrig, HA., Malm., O., Akagi, H., Guimarães, JRD., Torres, JPM., 1998. Methyl mercury in fish and hair samples from the Balbina Reservoir, Brazilian Amazon. Environ Res 77: 84-90.

Klautau-Guimarães, MN., et al., 2005. Analysis of genetic susceptibility to mercury contamination evaluated through molecular biomarkers in at-risk Amazon Amerindians populations. Gen Molec Biol 28(4): 827-832.

Lacerda, LD., Souza, M., Ribeiro, MG., 2004. The effects of land use change on mercury distributions in soils of Alta Floresta, Southern Amazon. Environ Poll 129: 247-255.

Lebel, J., et al., 1996. Evidence of early nervous system dysfunctions in Amazonian populations exposed to low-level of methyl mercury. Neurotoxicol 17(1): 157-168.

Lebel, J., Roulet, M., Mergler, D., Lucotte, M., Larribe, F., 1997. Fish diet and mercury exposure in a riparian Amazonian population. Water Air Soil Poll 97: 31-44.

Lebel, J., et al., 1998. Neurotoxic effects of low-level methyl mercury contamination in the Amazonian Basin. Environ Res 79: 20-32.

Lechler, PJ., et al., 2000. Elevated mercury concentrations in soils, sediments, water, and fish of the Madeira River basin, Brazilian Amazon: a function of natural enrichments? Sci Total Environ 260: 87-96.

Lima, DM., 1992. The social category caboclo: history, social organization, identity and outsider's social classification of the rural population of an Amazonian region. Doctoral dissertation, Cambridge, King's College.

Lucotte, M., Davidson, R., Mergler, D., St-Charles, J., Guimarães, JRD., 2004. Human exposure to mercury as a consequence of landscape management and socio-economic behaviors. Part I: The Brazilian Amazon Case Study. *In*: Proceedings of the 7th International Conference on Mercury as a Global Pollutant. RMZ-M&G: 51: 668-672.

Mainville, N., et al., 2006. Decrease of soil fertility and release of mercury following deforestation in the Andean Amazon, Napo River Valley, Ecuador. Sci Total Environ 368: 88-98.

Malm, O., Pfeiffer, WC., Bastos, WR., Souza, CMM., 1989. Utilização do acessório de geração de vapor frio para investigação do mercúrio em amostras ambientais por espectrofotometria de absorção atômica. Ci Cult 41: 88-92.

Malm, O., 1998. Gold mining as a source of mercury exposure in the Brazilian Amazon. Environ Res Section A 77: 73-78.

Martin, MD., Naleway, C., 2004. The inhibition of mercury absorption by dietary ethanol in humans: cross-sectional and case-control studies. Occup Environ Med 61: 8.

Mergler, D., 2002. Review of neurobehavioral deficits and river fish consumption from the Tapajós (Brazil) and St. Lawrence (Canada). Environ Toxicol Pharmacol 00: 1-7.

Mergler, D., Anderson, HA., Chan, LHM., Mahaffey, KR., Murray, M., Sakamoto, M., Stern, AH., 2007. Methyl mercury exposure and health effects in humans: a worldwide concern. Ambio 36(1): 3-11.

Mergler, D., et al., 2001. Neurotoxic sequelae of methyl mercury exposure in the Brazilian Amazon: a follow-up study. *In*: Proceedings of the 6th International Conference on Mercury as a Global Pollutant. October 2001, Minamata, Japan, 15 – 19.

Mertens, F., Saint-Charles, J., Mergler, D., Passos, CJ., Lucotte, M., 2005. A network approach for analysing and promoting equity in participatory ecohealth research, EcoHealth 2: 113-126.

Miranda, MR., et al., 2004. Mercury methylation and bacterial activity in macrophyte-associated periphyton in floodplain lakes of the Amazon basin. Proceedings of the 7th International Conference on Mercury as a Global Pollutant, 27 June – 02 July, Ljubljana, Slovenia.

Murrieta, RSS., Dialética do sabor: alimentação, ecologia e vida cotidiana em comunidades ribeirinhas da Ilha do Ituqui, Baixo Amazonas, Pará. Rev Antropologia, São Paulo, USP: 44(2): 39-88.

National Research Council, 2000. Toxicological effects of methyl mercury. National Academy Press, Washington DC, 344 pp.

Passos, CJ., et al., 2001. Characterization of the diet of a riverside population in the Brazilian Amazon. Rev Saúde & Ambiente 4(1/2): 72-84 (In Portuguese).

Passos, CJ., et al., 2003a. Socioeconomic conditions and mercury exposure through fish consumption: a case study in Santarém, Pará, Brazil. Rev Saúde & Ambiente 6(1/2): 3-11.

Passos, C.J., et al., 2003b. Eating tropical fruit reduces mercury exposure from fish consumption in the Brazilian Amazon. Environ Res 93: 123-130.

Passos, C., Mergler, D., Larribe, F., 2004. Response to "Fruits, fish, and mercury: further considerations". Environ Res 96: 102-108.

Passos, CJS., Mergler, D., 2006. Data use in a toxicokinetic model to reconstruct methyl mercury intake. J Exp Sci Environ Epidemiol 16, 299.

Passos, CJS., Mergler, D., Fillion, M., Lemire, M., Mertens, F., Guimarães, JRD., Phillibert, A. 2007a. Epidemiologic confirmation that fruit consumption influences mercury exposure in the Brazilian Amazon. Environm. Res., doi: 10.1016/j.envres.2007.01.012.

Passos, CJS., Mergler, D., Lemire, M., Fillion, M., Guimarães, JRD., 2007b. Fish consumption and bioindicators of inorganic mercury exposure. Sci Total Environ 373: 68-76.

Pichet, P., et al., 1999. Analysis of total mercury and methyl mercury in environmental samples. *In*: Lucotte, M., Schetagne, R., Thérien, N., Langlois C., Tremblay, A. (eds), Mercury in the biogeochemical cycle: Natural environments and hydroelectric reservoirs of Northern Québec, Springer, 41-52.

Pinheiro, MCN., et al., 2006. Comparative study of human exposure to mercury in riverside communities in the Amazon region. Braz J Med Biol Res 39: 411-414.

Rice, DC., Schoeny, R., Mahaffey, K., 2003. Methods and rationale for derivation of a reference dose for methyl mercury by the U.S. EPA. Risk Analysis 23(1): 107-115.

Rice, DC., 2004. The US EPA reference dose for methyl mercury: sources of uncertainty. Environ Res 95: 406-413.

Rice, DC., 2005. Assessing the effects of environmental toxicant exposure in developmental epidemiological studies: issues for risk assessment. Neurotoxicol 25: 483-489.

Roulet, M., et al., 1998a. Distribution and partition of total mercury in waters of the Tapajós River Basin, Brazilian Amazon. Sci Total Environ 213: 203-211.

Roulet M., et al., 1998b. The geochemistry of Hg in Central Amazonian soils developed on the Alter-do-Chão formation of the lower Tapajós river valley, Pará state, Brazil. Sci Total Environ 223: 1-24.

Roulet, M., et al., 1999. Effects of recent human colonization on the presence of mercury in Amazonian ecosystems. Water Air Soil Poll 112: 297-313.

Roulet, M., et al., 2000. Increase in mercury contamination recorded in lacustrine sediments following deforestation in the central Amazon. Chem Geol 165: 243-266.

Roulet, M., Guimarães, JRD., Lucotte, M., 2001. Methyl mercury production and accumulation in sediments and soils of an Amazonian floodplain – effect of seasonal inundation. Water Air Soil Poll 128: 41-60.

Santos, ECO., et al., 2002. A contribution to the establishment of reference values for total mercury levels in hair and fish in Amazonia. Environ Res Section A 90: 6-11.

Santos, ECO., et al., 2003. Exposure to mercury and arsenic in Amazon States: a summary of studies by Evandro Chagas Institute/FUNASA. Rev Bras Epidemiol 6(2): 171-185.

Silbergeld, EK., Silva, IA., Nyland, JF., 2005. Mercury and autoimmunity: implications for occupational and environmental health. Toxicol Appl Phamacol 207: S282-S292.

Stern, AH., Jacobson, JL., Ryan, L., Burke, T., 2004. Do recent data from the Seychelles Islands alter the conclusions of the NRC Report on the toxicological effects of methyl mercury? Environ Health 3: 2.

U.S. EPA/630/R-95/001F., 1998. Guidelines for neurotoxicity risk assessment. Washington, DC, 79 p.

Wasserman, JC., Hacon, S., Wasserman, MA., 2003. Biogeochemistry of mercury in the Amazonian environment. Ambio 32(5): 336-342.

Webb, J., et al., 2004. Mercury in Fish-eating Communities of the Andean Amazon, Napo River Valley, Ecuador. EcoHealth 1(suppl 2): 59-71.

WHO., 2003. World Health Organization, Joint FAO\WHO Expert Committee on Food Additives JECFA/61/SC.

Wijngaarden, EV., et al., 2006. Benchmark concentrations for methyl mercury obtained from the 9-year follow-up of the Seychelles Child Development Study. Neurotoxicol 27: 702-709.

Yokoo, EM., Valente, JG., Grattan, L., Schimidt, SL., Platt, I., Silbergeld, EK., 2003. Low level methyl mercury exposure affects neuropsychological functions in adults. Environ Health 2: 8.

CHAPITRE IV

FISH CONSUMPTION AND BIOINDICATORS OF INORGANIC MERCURY EXPOSURE

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RÉSUMÉ

La relation directe et étroite entre la consommation de poissons et les teneurs en mercure sanguin et dans les cheveux est bien connue, mais l'influence de la consommation de poissons sur les teneurs en mercure inorganique dans le sang et dans l'urine n'est pas claire. Cette étude examine la relation entre la consommation de poissons et les teneurs en mercure total, inorganique et organique dans le sang et dans l'urine. Une étude transversale a été menée auprès de 171 personnes de sept villages riverains de la région du Tapajós. Ces personnes n'avaient pas d'histoire d'exposition aux vapeurs de mercure en milieu de travail, et elles ne possédaient pas des amalgames dentaires. Pendant la période de montée des eaux en 2004, les personnes ont participé à une enquête alimentaire basée sur la fréquence de consommation de poissons et des fruits, et des renseignements sociodémographiques ont été fournis. Des échantillons de sang et d'urine ont été collectés, et les teneurs en mercure total, organique, et inorganique tout comme le mercure urinaire ont été déterminées par Spectrométrie d'Absorption Atomique. En moyenne, les participants ont consommé 7.4 repas de poissons par semaine, et 8.8 fruits par semaine. La teneur moyenne en mercure total dans le sang a été 38.6 ± 21.7 µg/L, et le pourcentage moyen de mercure inorganique sanguin était 13.8%. La valeur moyenne de mercure organique (méthylmercure) a été 33.6 ± 19.4 µg/L, alors que celle de mercure inorganique était 5.0 ± 2.6 µg/L. La teneur moyenne en mercure urinaire était 7.5 ± 6.9 µg/L, avec 19.9% des participants ayant des niveaux de mercure urinaire au-dessus de 10 µg/L. Le mercure inorganique sanguin a été hautement et significativement relié au nombre de repas de poissons carnivores, mais aucune relation n'a été observée avec les poissons non-carnivores. Le mercure inorganique sanguin était aussi négativement relié à la fréquence de consommation de fruits et positivement associé à l'âge. De plus, il variait selon les communautés et était plus élevé parmi les personnes nées dans la région du Tapajós. Le mercure urinaire a également été associé à l'ingestion de poissons carnivores, et il a montré une tendance vers une relation négative avec la consommation de fruits; il était plus élevé chez les hommes que chez les femmes et supérieur parmi les participants natifs de la région. Le mercure urinaire a été fortement relié au mercure inorganique et organique et total dans le sang. Le rapport de cotes (OR) pour des teneurs en mercure urinaire supérieures à 10µg/L chez les participants consommant plus que 4 repas de poissons carnivores par semaine est 4.00 [1.83 – 9.20]. Les résultats de cette étude appuient une relation directe entre la consommation de poissons et les teneurs en mercure inorganique sanguin et urinaire. Il se peut que le mercure inorganique soit absorbé des poissons carnivores ou qu'il provienne de la déméthylation du méthylmercure.

Mots clés : Consommation de poissons, exposition, mercure inorganique, déméthylation, Amazonie.

ABSTRACT

Background: The direct and close relationship between fish consumption and blood and hair mercury (Hg) levels is well known, but the influence of fish consumption on inorganic mercury in blood (B-IHg) and in urine (U-Hg) is unclear.

Objective: Examine the relationship between fish consumption, total, inorganic and organic blood Hg levels and urinary Hg concentration. **Methods**: A cross-sectional study was carried out on 171 persons from 7 riparian communities on the Tapajós River (Brazilian Amazon), with no history of inorganic Hg exposure from occupation or dental amalgams. During the rising water season in 2004, participants responded to a dietary survey, based on a seven-day recall of fish and fruit consumption frequency, and socio-demographic information was recorded. Blood and urine samples were collected. Total, organic and inorganic Hg in blood as well as U-Hg were determined by Atomic Absorption Spectrometry. **Results**: On average, participants consumed 7.4 fish meals/week and 8.8 fruits/week. Blood total Hg averaged $38.6 \pm 21.7 \mu g/L$, and the average percentage of B-IHg was 13.8%. Average organic Hg (MeHg) was $33.6 \pm 19.4 \mu g/L$, B-IHg was $5.0 \pm 2.6 \mu g/L$, while average U-Hg was $7.5 \pm 6.9 \mu g/L$, with 19.9% of participants presenting U-Hg levels above $10 \mu g/L$. B-IHg was highly significantly related to the number of meals of carnivorous fish, but no relation was observed with non-carnivorous fish; it was negatively related to fruit consumption, increased with age, was higher among those who were born in the Tapajós region, and varied with community. U-Hg was also significantly related to carnivorous but not non-carnivorous fish consumption, showed a tendency towards a negative relation with fruit consumption, was higher among men compared to women and higher among those born in the region. U-Hg was strongly related to I-Hg, blood methyl Hg (B-MeHg) and blood total Hg (B-THg). The Odds Ratio (OR) for U-Hg above $10\mu g/L$ for those who ate > 4 carnivorous fish meals/week was 4.00 [1.83 – 9.20].

Conclusion: This study adds further evidence to a positive relation between fish consumption and IHg in both blood and urine, which may result from absorption of IHg from fish or from demethylation of MeHg. The findings support the importance of assessing IHg exposure in fish-eating communities. Further studies should examine the potential toxicity of IHg in heavy fish consumers.

Keywords: Fish consumption, inorganic mercury exposure, demethylation, Amazon

Introduction

It is well known that both freshwater and sea fish consumption are directly and closely related to increases of blood and hair mercury (Hg) concentrations (Clarkson, 2002; UNEP, 2002), whereas the influence of fish consumption on urinary mercury levels (U-Hg) is unclear. Recent studies of fish consumers in Europe and North America have revealed significant associations between fish consumption and U-Hg levels (Carta et al., 2002; Apostoli et al., 2002; Levy et al., 2004; Johnsson et al., 2005), and most of these authors suggest that demethylation of methyl mercury (MeHg) in the body may be responsible for these U-Hg levels. This hypothesis is supported by several human and animal studies, as well as recent MeHg toxicokinetic modeling (Bjorkman et al., 1995; Vahter et al., 1995, 2000; Carrier et al., 2001a, b; Young et al., 2001).

In the Amazon, Hg exposure through fish consumption has been the subject of much concern over the last two decades. A large number of exposure assessments of fish-eating populations have been conducted in many parts of the huge Amazon region, using blood and/or hair as biomarkers to study communities environmentally exposed solely through their fish diet (Pinheiro et al., 2006, Dórea et al., 2005; Gonçalves and Gonçalves, 2004; Webb et al., 2004; Passos et al., 2003; Santos et al., 2003; Dolbec et al., 2001; Boischio and Henshel, 2000; Cordier et al., 1998; Lebel et al., 1997). These have consistently shown elevated blood and/or hair Hg concentrations, strongly related to fish consumption. Recent studies indicate that fruit consumption may modulate this relation, with those who eat more fruit presenting lower hair Hg levels for the same amount of fish consumption (Passos et al., 2003, 2004).

While, on average, the major part of total Hg content in fish is in the form of MeHg, the proportion of this chemical species may range from 57% to 100% (Ikingura and Akagi, 2003; Maurice-Bourgoin et al., 2000; Kehrig et al., 1998; Holsbeek et al., 1997; Akagi et al., 1995). Moreover, in fish-eating humans the proportion of MeHg in blood and hair varies between 20% and 100% (Lebel et al., 1996; Lodenius and Malm, 1998; Maurice-Bourgoin et al., 2000; Pinheiro et al., 2000; Barbosa et al., 2001; Dolbec et al., 2001). Despite these variations, most authors gloss over the amount of inorganic Hg or use total Hg as a proxy for MeHg.

The objective of the present study was to examine blood inorganic Hg (B-IHg) and U-Hg and the factors that influence their concentration, among fish-eating communities of the Brazilian Amazon.

Methods

Study design and population

This cross-sectional study was conducted over the months of January and February 2004 (rainy season), among 7 riparian communities situated on the banks of the Tapajós River, a major tributary of the Amazon (Figure 1). The communities were selected in order to represent the diversity created throughout the colonization process in this region, as some of them were established after colonization began in the early 1960's, whereas others were established up to 100 years previously.

In this area of the Amazon, it is difficult to apply a random sampling strategy. Boats are the only means of transport from one village to another and villagers are spread out over large areas, which require small crafts and/or several hours of walking inland to reach. In addition, in many villages there are evangelical groups whose members systemically refuse to participate. Thus, a convenience sample was used and recruitment was carried out through village meetings and door-to-door invitation. Inclusion criteria for the present study were ≥15 years of age, no dental amalgams, not pregnant and no history of exposure to inorganic Hg in gold-mining. Age and sex distributions were then compared to the underlying population, which had previously been determined through a house-to-house survey in each community. Of the 216 who agreed to participate, 171 adults (79.2%) met the criteria. The age distributions and participation rates with respect to the underlying population are presented in Table 1. Distributions were similar for most categories, except for those under 24 and over 65 years, whose rates of participation were relatively lower.

Approval was obtained from Ethics Committees of the Federal University of Rio de Janeiro (Brazil) and the University of Quebec in Montreal (Canada). The study was explained individually, and persons agreeing to participate signed an informed consent form. This study is part of a larger interdisciplinary and ecosystemic investigation examining Hg dynamics in the environment, human exposure and health effects in the Tapajós region (CARUSO, 2007).

Assessment of fish and fruit consumption

Because of important seasonal differences in the availability of fish species in this region (Lebel et al., 1997; Dolbec et al., 2000; Passos et al., 2001), a seven-day dietary recall questionnaire (7-DDR) was used to determine current fish and fruit consumption frequency. Development and validation studies of this instrument have shown that it is easily administered and constitutes a sensitive method to assess short-term food consumption (Hebert et al., 1997). Our previous studies concur with this assessment. Using this tool, we have found good concordance between seasonal bioavailability of fish species and reported consumption of those fish species (Lebel et al., 1997), as well as between fish consumption frequency and bioindicators of exposure (Dolbec et al., 2001).

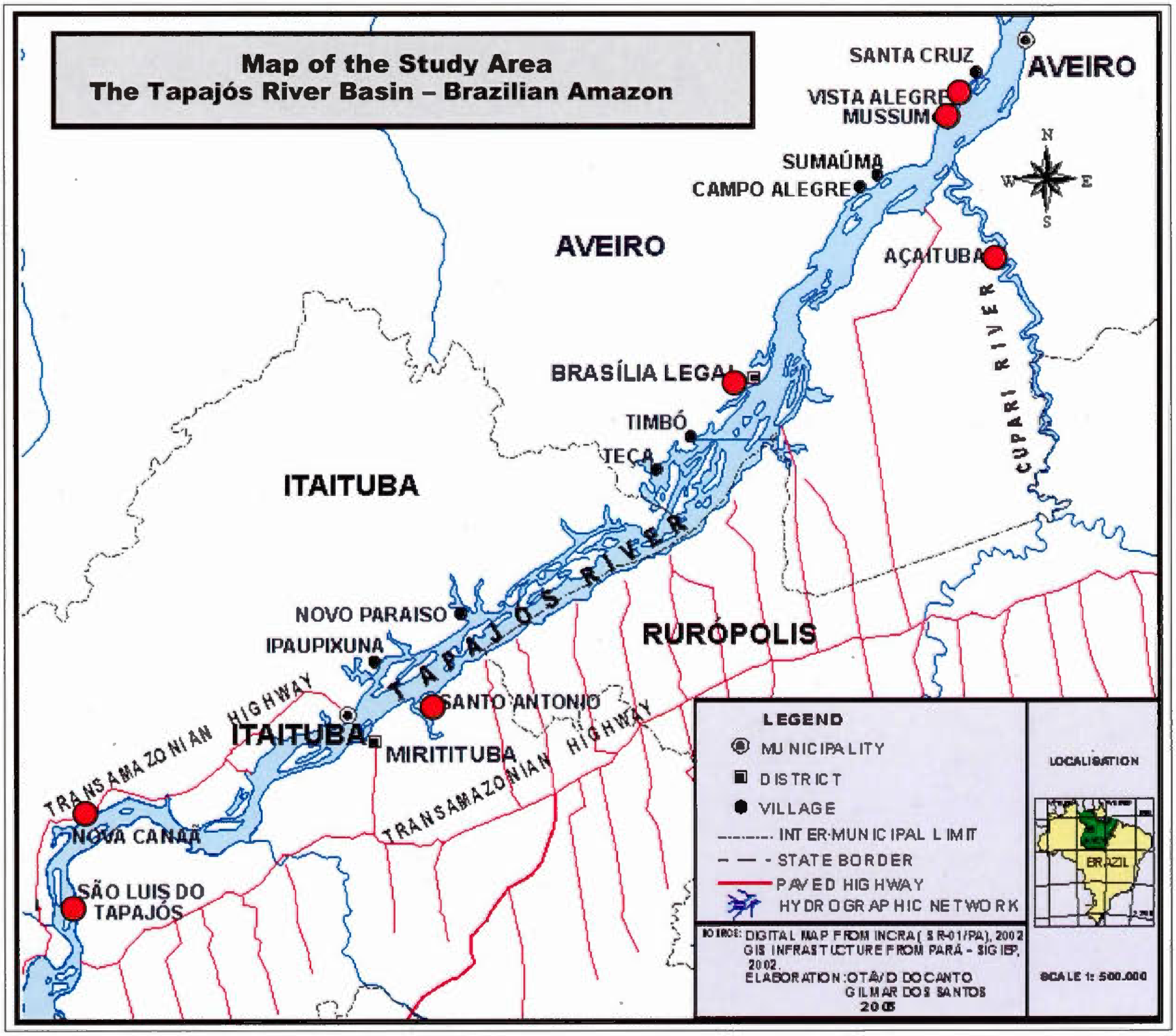


Figure 1: Map of the study area. Participating communities are identified by a large red dot

Table 1: Age distribution and rates of participation in the study population

Age category	Total adult population	Study population	% participation
15 – 24	308	25	8.1
25 – 34	214	39	18.2
35 – 44	174	36	20.7
45 – 54	132	31	23.5
55 – 64	95	30	31.6
≥ 65	79	10	12.7
Total	**1000**	**171**	**17.1**

A list was prepared which included most of the fish species present in the region. Participants indicated for each day the number of meals containing fish, as well as the name of the fish species that were consumed. Based on the dietary habits of the fish species and the trophic classification proposed by Ferreira et al. (1998), fish were then grouped into carnivorous and non-carnivorous species. As for fruits, the procedure was similar, but in this case, for each fruit species, the participant indicated the number of fruits that had been eaten each day over the preceding seven days, whether during a meal or not. Here, total fruit consumption over the previous 7 days is used. Fish and fruit species that were not in the initial list were also recorded.

Sampling and analyses of bioindicators

Urine samples were collected into polypropylene bottles (Nalgene 125 mL # 2104-0004) and then transferred to screw cap tubes with conical base (RPK PPGWB 15 mL, SARSTEDT™) for transport purposes. Samples were kept frozen until analyzed. U-Hg was determined by Cold Vapour Atomic Absorption Spectrometry. Following an acidic mineralization of the Hg present in urine at 50°C, the resulting solution was diluted and analyzed on the FIMS 100 module from Perkin Elmer (M-568). Urinary Hg concentrations were adjusted for urine density.

Blood samples were collected by a nurse by venipuncture into 6 ml heparinized Becton Dickinson Vacutainer® (BD7863). All blood samples were kept frozen at –20° C until analyzed. Total and inorganic Hg in blood were determined by Atomic Absorption

Spectrometry according to the method described by Ebbestadt et al. (1975), with a detection limit of 0.2 µg/L. B-MeHg was estimated as the difference between T-Hg and B-IHg.

All urine and blood analyses were conducted at the laboratory of the Quebec Toxicology Center of the Quebec Public Health Institute (CTQ-INSPQ), Canada. Analytical quality control was ensured by routine checks of accuracy and precision, using reference materials from CTQ-INSPQ Inter-Laboratory Comparison Program. The CTQ is accredited ISO 17025 and analytical performance for Hg analysis in the Inter-Laboratory Comparison Program for Metals in Biological Media was 36/36 for precision and 6/6 for reproducibility.

Statistical analyses

Descriptive statistics were used to characterize the study population, fish consumption, as well as Hg exposure. Inter-group comparisons were performed using parametric or nonparametric techniques, depending on data distribution. The relation between fish consumption, fruit intake, socio-demographic variables and the bioindicators of Hg exposure were examined using linear multiple regression models. Where possible, continuous variables were used (age, years of education, number of fish meals, number of fruits). Alcohol consumption, smoking and immigrant status were included as categorical variables. The relation between the bioindicators of exposure was examined using correlational statistics (Spearman's rho), and the risk for having U-Hg levels above 10µg/L was analysed using logistic regression analyses. Results were defined as statistically significant for a value of $P \leq 0.05$. Analyses were performed using Statview for Windows Version 5.0.1 and Jump 5.0.1a (SAS Institute Inc.).

Results

Participants' age ranged from 15 to 77 years (mean 40.2 years ± 15.1) with the distribution presented in Table 1. Other socio-demographic characteristics are shown in Table 2. Formal education was low, varying between 0 and 11 years (mean: 4.0 years ± 2.7). Seventy-one percent (71%) of the participants were originally from the Tapajós region, and 72.5% lived on the Tapajós River banks, while the others lived on one of its tributaries.

In this rainy period, 99.5% of the study population consumed at least one meal with fish over the preceding seven days. On average, participants consumed 7.4 fish meals/week, ranging from 0 to 24meals/week; carnivorous fish made up an average of 65.2% of the fish diet, ranging from 0 to 100%.

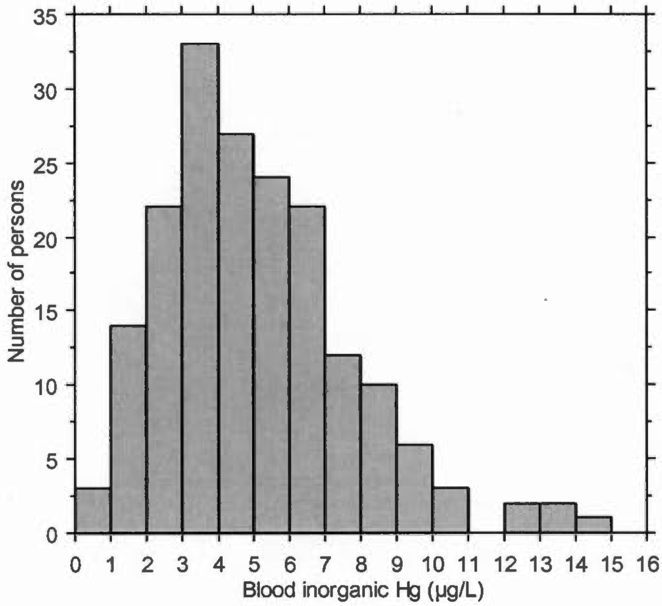
Figure 2 presents the distributions of B-IHg and U-Hg. Blood total Hg averaged 38.6 ± 21.7µg/L (median: 34.6 µg/L, ranging from 4.2 to 114µg/L). The average percentage of B-IHg was 13.8%, ranging from 7.4% to 23.4%. Average B-IHg was 5.0 ± 2.6µg/L (median: 4.7µg/L, ranging from 0.4 to 14.8µg/L).

Table 2: Socio-demographic characteristics of the study population

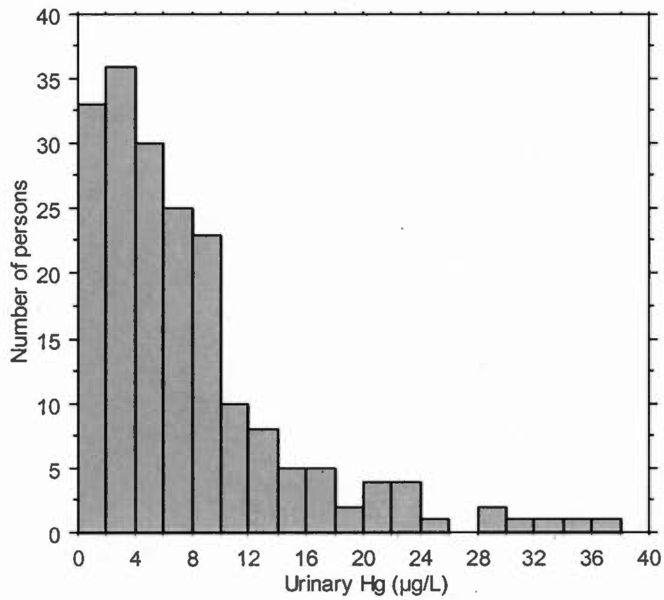
	Women		**Men**	
	n	**%**	**n**	**%**
Schooling				
No formal education	7	7.4	16	21.1
Elementary school (1 to 8 years)	72	75.8	51	67.1
High school and more (≥9 years)	16	16.8	9	11.4
Born in the region (State of Pará)	72	75.8	51	67.1
Village location				
On the Tapajós River	72	75.8	52	68.4
On an affluent	23	24.2	24	31.6
Consumes alcohol	36	37.9	39	51.3
Current smokers	21	22.1	40	52.6

B-MeHg and B-IHg were highly correlated ($r = 0.84$; $p < 0.0001$). The percentage of B-IHg was not correlated to B-IHg levels ($p > 0.1$), but was inversely related to B-THg ($r = -0.1$; $p < 0.0001$). Average U-Hg was 7.5 ± 6.9 µg/L (median: 5.6 µg/L, ranging from 0.2 to 36.1 µg/L), with 19.9% of participants presenting U-Hg levels above 10 µg/L, the upper limit for unexposed populations (WHO, 1991).

Table 3 presents the predictive factors for B-IHg, B-MeHg and B-THg. Carnivorous fish frequency consumption was highly significantly related to all three blood bioindicators, while non-carnivorous fish consumption was related to B-MeHg and B-THg, but not B-IHg. Fruit consumption frequency was negatively related to all of these bioindicators. Age was only related to B-IHg. There were significant differences between communities and immigrant status; those who were not born in the region had significant lower levels of all of these bioindicators of exposure. Gender did not enter into any of the models, nor did smoking, alcohol or education (not shown here).



A



B

Figure 2: Distribution of blood inorganic Hg (A) and urinary Hg (B) in the study population

Table 3: Results of regression analyses for blood bioindicators of Hg exposure

	B-IHg R^2 of model : 0.36		B-MeHg R^2 of model : 0.52		B-THg R^2 of model : 0.50	
	β coeff	p	β coeff	p	β coeff	p
Carnivorous fish	0.37	<0.001	2.25	<0.001	2.62	<0.001
Non-carnivorous	0.10	ns	0.93	0.04	1.03	0.04
Fruit consumption	-0.03	0.05	-0.18	0.04	-0.20	0.04
Age	0.03	0.05	0.05	ns	0.08	ns
Gender	0.05	ns	1.84	ns	1.79	ns
Immigrant	-0.91	<0.01	-7.77	<0.001	8.67	<0.001
Community		0.05		<0.001		<0.001

ns: non-significant

Urinary Hg was positively related to carnivorous fish consumption (β coeff: 0.39; p<0.01) and negatively to fruit consumption (β coeff: -0.07; p = 0.05). Immigrants had lower U-Hg with respect to non-immigrants (β coeff: –1.76; p = 0.02) and women had higher U-Hg compared to men (β coeff: 1.14; p = 0.03). Since the blood bioindicators were highly inter-correlated, for the models of U-Hg each one was examined separately with potential covariables. Only gender entered significantly in all models. Figure 3 (A, B, and C) shows the relations between density-adjusted U-Hg and B-IHg, B-MeHg and B-THg for men and women.

A total of 34 persons (19.9%) had U-Hg levels above 10μg/L, adjusted for urinary density. Logistic regression analyses for U-Hg levels above 10μg/L, with respect to fish consumption, fruit consumption and socio-demographic co-variables showed that the only variable that entered significantly into the model was consumption of carnivorous fish. A total of 70 persons (40.9%) ate more than 4 carnivorous fish meals over the past 7 days. The Odds Ratio (OR) for having U-Hg above 10μg/L for those who ate more than 4 carnivorous fish meals was 4.00 [1.83 – 9.20], with a Chi square p < 0.001. Those with U-Hg levels above 10μg/L had mean B-IHg concentrations of 7.71μg/L ± 2.67 (median: 6.81) while those with lower U-Hg concentrations had a mean B-IHg of 4.38μg/L ± 2.18 (median: 4.00). Although the differences in IHg were highly significant (t-test; p < 0.001), there was no difference between the 2 groups in the percentage of B-IHg in total B-Hg. (13.1% for those with higher U-Hg concentrations and 13.9% for those with lower levels).

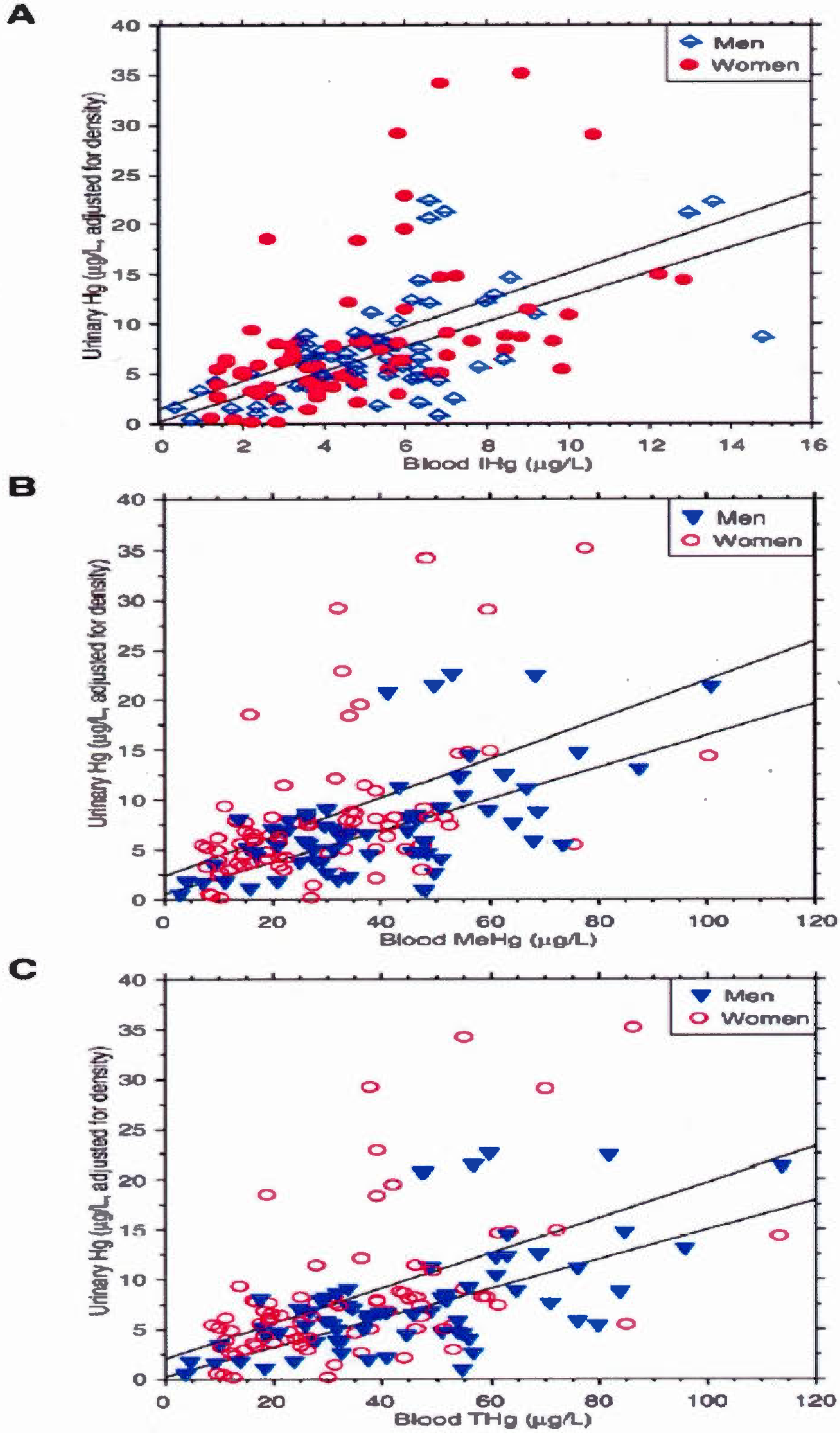

A
Men
Women
Urinary Hg (µg/L, adjusted for density)
Blood IHg (µg/L)
B
Men
Women
Urinary Hg (µg/L, adjusted for density)
Blood MeHg (µg/L)
C
Men
Women
Urinary Hg (µg/L, adjusted for density)
Blood THg (µg/L)

Figure 3: Relations between density-adjusted U-Hg concentrations with respect to blood bioindicators of mercury exposure

Discussion

The present study shows high levels of IHg in a fish-eating population who have not been occupationally exposed to Hg vapours and without dental amalgams. For 19.9% of the participants, U-Hg concentrations surpass 10μg/L, the upper limit for unexposed populations (WHO, 1991). IHg levels were directly associated with fish consumption and influenced by a number of socio-demographic factors.

Few studies have examined the association between fish consumption and IHg exposure in human populations. In Europe, an Italian polycentric study provided important insight on the relevance of assessing IHg exposure in fish consumers (Carta et al., 2002; Apostoli et al., 2002). These authors found a significant relation between the number of fish meals consumed weekly and U-Hg levels; they concluded that the organic compounds absorbed by usual sea fish consumption may be partially demethylated, thus increasing the IHg concentration in the kidney, and consequently its urinary excretion. More recently, two Swedish studies have corroborated these findings. Johnsson et al. (2005) reported a significant relationship between freshwater fish consumption and U-Hg levels, the latter being 15-fold higher in frequent fish consumers as compared to low consumers. The authors also suggested that demethylation of MeHg in the human body may explain such relations. A study examining the inter-individual variations of human biomarkers of Hg exposure, reported an average percentage of 6.8% for IHg in red blood cells, which increased with increasing consumption of fish, but not with increasing number of dental amalgam fillings (Berglund et al., 2005). A further study in Canada on U-Hg excretion in children (Levy et al., 2004) reported that, unexpectedly, children with higher levels of fish consumption excreted significantly elevated amounts of Hg in urine.

In the present study, the relation between fish intake and B-IHg and U-Hg concentrations was observed only with carnivorous fish consumption, suggesting that carnivorous fish may have higher levels of IHg or that when a bolus dose of MeHg is ingested from carnivorous fish, some may be converted to IHg. It is unlikely that this relation is due to a higher quantity of carnivorous fish consumption, since in these communities there is no difference between the preparation of carnivorous and non-carnivorous fish. Moreover, carnivorous fish can be large like Tucunaré (*Cichla sp.)* or small like Piranha (*Serrasalmus sp.*), the same being true for herbivorous fish which can be large like Tambaqui (*Collossoma macropomum)* or small like Pacu (*Mylossoma sp*). Based on different studies, on average people eat approximately 163 grams of fish per day (Kehrig et al., 1998; Boischio & Henshel, 2000, Yokoo et al., 2001; Dórea et al., 2005). On the other hand, the conversion of MeHg into IHg in the body has long been proposed (Kozak and Forsberg, 1979). Demethylation of MeHg by microflora in the human gut is considered to be a key and probably a rate-determining process in the removal of MeHg, even though the microbes involved have not been identified nor have the biochemical mechanisms of cleavage of the carbon-mercury bond (Clarkson, 2002). This biotransformation has recently been confirmed through the use of biologically- and physiologically-based toxicokinetic models, which describe the deposition kinetics of MeHg and its inorganic metabolites both in animals and humans (Carrier et al., 2001a, b; Young et al., 2001).

Apart from the demethylation of MeHg in the human body, the IHg fraction of fish Hg may provide another explanation of the relation between fish consumption and IHg concentrations. Despite the general assumption that MeHg constitutes the major part of the Hg content in fish, recent studies have shown substantial variations of the IHg fraction in fish flesh, both in the Amazon and elsewhere. For example, in a study on Hg speciation and accumulation in freshwater and anadromous fish from Bangladesh, Holsbeek et al. (1997) reported an accumulation mechanism reflected by increasing levels of IHg combined to low and constant MeHg levels, leading to a relative decrease of the MeHg fraction with age. According to the authors, this unexpected pattern had only been reported in cases of some marine species where it seemed to be linked to demethylation mechanisms or regional influences on fish Hg levels. An intermediate accumulation pattern with increasing concentrations of both MeHg and IHg fraction with age was found in one bottom dwelling species. In the Amazon, Maurice-Bourgoin et al. (2000) reported IHg fractions in fish varying from 2% to 27% of the total Hg in fish of the Madeira River. In a more recent investigation, analyses of total Hg and MeHg levels in fish from hydroelectric reservoirs in Tanzania show that approximately 0 – 44% of the total Hg in these fish was IHg (Ikingura and Akagi, 2003). There is thus reason to believe that IHg ingested from fish may contribute to IHg in the human body, which is reflected in the blood and urinary Hg levels. Although in humans IHg is less well absorbed by the gastro-intestinal tract than MeHg, at rates of about 7% and 95% respectively (Rahola et al., 1973; Aberg et al., 1969; cited in Berglund et al., 2005), the findings of the present study suggest that even this relatively low absorption rate of IHg can result in substantial blood and urinary IHg levels in heavy fish consumers.

Another interesting finding in the present study was that both B-IHg concentrations and the percentage of B-IHg were positively associated to the age of participants. This association might be explained by increasing rates of IHg accumulation with time, under a chronic exposure scenario. Indeed, physiologically-based toxicokinetic models which extrapolate experimental data to much longer periods of exposure, suggest that the concentrations of IHg can surpass those of MeHg overtime, thus potentially reflecting increased blood and urinary IHg levels (Young et al., 2001).

Even when fish consumption frequency was taken into account, villagers who were born in the Tapajós region presented significantly higher B-IHg and B-MeHg than immigrants from northeast Brazil. Those who were born in the Tapajós region have had a fish-based diet throughout their life, while those who were arrived later consumed much less fish prior to their arrival in the region. Over the last years there has been growing interest in evaluating the potential of perinatal and lifetime exposures to determine different MeHg pharmacokinetic patterns during adulthood, since chronic and low-level exposures to environmental pollutants often begin prenatally and then continue through the lifetime (Stern et al., 2001). In experimental conditions which sought to obtain basic information about blood and brain Hg levels in mice under conditions of chronic lifetime exposure, these authors observed that both brain and blood Hg levels rose significantly between 14 and 26 months, suggesting an interaction between dose and age. Lifetime exposure might, therefore, explain the higher blood Hg levels observed in persons born in the Tapajós region, when compared to persons who arrived in this region during adolescence or even adulthood.

In this study a convenience sample was used. Convenience samples have been shown to appropriately represent underlying populations in different settings (Kelly et al., 2002; Zelinski et al., 2001) and in the present study, 17.1% of the population of these villages surveyed well represented most age categories. Although this sampling strategy may have introduced some selection bias it would probably not affect the relation between fish consumption and blood or urinary inorganic Hg.

In conclusion, the present results support the need to measure and better understand the role of the different chemical forms of Hg from fish consumption, since Hg chemical species present different toxicological properties. Given the degree of environmental exposure in the present cohort, attention should be paid to the potential of toxicity of IHg, since inorganic metabolites may, in part, be responsible for some of the neurotoxic effects induced by MeHg (Charleston et al., 1994; Vahter et al., 1994, 1995), even if the high load of IHg in the kidneys may leave less IHg circulating in blood and available for transfer to the brain (Carrier et al., 2001b). Future studies should examine the potential toxicity of IHg in heavy fish consumers.

Acknowledgments

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References

Aberg, B., Ekman, L., Falk, R., Greitz, U., Persson, G., Snihs, JO. Metabolism of methyl mercury (203Hg) compounds in man. Arch Environ Health 1969; 19: 478-484.

Akagi, H., Malm, O., Branches, FJP., Kinjo, Y., Kashima, Y., Guimarães, JRD., Oliveira, RB., Haraguchi, K., Pfeiffer, WC., Takizawa, Y., Kato, H. Human exposure to mercury due to goldmining in the Tapajós River Basin, Amazon, Brazil: speciation of mercury in human hair, blood and urine. Water Air Soil Poll. 1995; 80: 85-94.

Apostoli, P., Cortesi, I., Mangili, A., Elia, G., Drago, I., Gagliardi, T., Soleo, L., Valente, T., Sciarra, GF., Aprea, C., Ronchi, A., Minoia, C. Assessment of reference values for mercury in urine : the results of an Italian polycentric study. Sci Total Environ 2002; 289-13-24.

Barbosa, AC., Jardim, W., Dórea, JG., Forsberg, B., Souza, J. Hair Mercury Speciation as a Function of Gender, Age, and Body Mass Index in Inhabitants of the Negro River Basin, Amazon, Brazil. Arch. Environ. Contam. Toxicol. 2001; 40: 439-444.

Berglund, M., Lind, B., Bjornberg, A., Palm, B., Einarsson, O., Vahter, M. Inter-individual variations of human mercury exposure biomarkers: a cross-sectional assessment. Environ Health 2005; 4:20.

Bjorkman, L., Mottet, K., Nylander, M., Vahter, M., Lind, B., Friberg, L. Selenium concentrations in brain after exposure to methylmercury: relations between the inorganic mercury fraction and selenium. Arch Toxicol. 1995; 69(4): 228-234.

Boischio, AAP., Henshel, DS. Linear Regression Models of Methyl Mercury Exposure during Prenatal and Early Postnatal Life among Riverside People along the Upper Madeira River, Amazon. Environ Res 2000; 83: 150-161.

Carrier, G., Brunet, RC., Caza, M., Bouchard, M. A Toxicokinetic Model for Predicting the Tissue Distribution and Elimination of Organic and Inorganic Mercury Following Exposure to Methyl Mercury in Animals and Humans. I. Development and Validation of the Model Using Experimental Data in Rats. Toxicol Appl Pharmacol 2001; 171: 38-49.

Carrier, G., Bouchard, M., Brunet, RC., Caza, M. A Toxicokinetic Model for Predicting the Tissue Distribution and Elimination of Organic and Inorganic Mercury Following Exposure to Methyl Mercury in Animals and Humans. II. Application and Validation of the Model in Humans. Toxicol Appl Pharmacol 2001; 171: 50-60.

Carta, P., Flore., C., Ibba, A., Tocco, M., Aru, G., Mocci, F., Randaccio, FS. Urinary and blood levels of mercury among chloralkali workers and non-occupationally exposed subjects : relationship with amalgam fillings and fish consumption. Med Lav 2002; 93(3): 176-183.

CARUSO. Mercury exposure and ecosystem health in the Amazon. 2007. http://www.unites.uqam.ca/gmf/caruso/caruso.htm

Charleston, JS., Bolender, RP., Mottet, NK., Body, RL., Vahter, ME., Burbacher, TM. Increases in the number of reactive glia in the visual cortex of Macaca fascicularis following subclinical long-term methyl mercury exposure. Toxicol Appl Pharmacol. 1994; 129: 196-206.

Clarkson, TW. The Three Modern Faces of Mercury. Environ Health Persp 2002; 110(suppl 1): 11-23.

Cordier, S., Grasmick, C., Paquier-Passelaigue, M., Mandereau, L., Weber, J-P., Jouan, M. Mercury exposure in French Guiana : levels and determinants. Arch Environ Health 1998; 53(4): 299-305.

Dolbec, J., Mergler, D., Larribe, F., Roulet, M., Lebel, J., Lucotte, M. Sequential analysis of hair mercury levels in relation to fish diet of an Amazonian population, Brazil. Sci Total Environ 2001 ; 271: 87-97.

Dórea, JG., Souza, JR., Rodrigues, P., Ferrai, I., Barbosa AC. Hair mercury (signature of fish consumption) and cardiovascular risk in Mundurucu and Kayabi Indians of Amazonia. Environ Res 2005; 97:209-219.

Ebbestadt V, Gunderson and Torgrimsen TA. Nov-Dec. Simple method for the determination of inorganic mercury and methylmercury in biological samples by flameless atomic absorption. Atom Absorption Newsletter 1975; 14(6):142-143.

Ferreira, EJG., Zuanon, JAS., Santos, GM. Peixes comerciais do Médio Amazonas, Região de Santarém (PA). Brasília: IBAMA; 1998. 211 pp.

Gonçalves, A., Gonçalves, NNS. Human exposure to mercury in the Brazilian Amazon: a historical perspective. Pan Am J Public Health 2004; 16(6): 415-419.

Gregus, Z., Gyurasics, A., Csanaky, I., Pintér, Z. Effects of Methylmercury and Organic Acid Mercurials on the Disposition of Exogenous Selenium in Rats. Toxicol Appl Pharmacol 2001; 174: 177-187.

Hebert, JR., Ockene, IS., Hurley, TG., Luippold, R., Well, AD., Harmatz, MG., et al. Development and Testing of a Seven-Day Dietary Recall. J Clin Epidemiol. 1997; 50(8): 925-937.

Holsbeek, L., Das, HK., Joiris, CR. Mercury speciation and accumulation in Bangladesh freshwater and anadromous fish. Sci Total Environ 1997; 198: 201-210.

Ikingura, JR., Akagi, H. Total mercury and methylmercury levels in fish from hydroelectric reservoirs in Tanzania. Sci Total Environ 2003; 304 : 355-368.

Johnsson, C., Schutz, A., Sallsten, G. Impact of consumption of freshwater fish on mercury levels in hair, blood, urine, and alveolar air. J Toxicol Environ Health Part A 2005; 68: 129-140.

Kehrig, HA., Malm., O., Akagi, H., Guimarães, JRD., Torres, JPM. Methylmercury in Fish and Hair Samples from the Balbina Reservoir, Brazilian Amazon. Environ Res 1998; 77: 84-90.

Kelly, H., Riddell, MA., Gidding, HF., Nolan, T., Gilbert, GL. A random cluster survey and a convenience sample give comparable estimates of immunity to vaccine preventable diseases in children of school age in Victoria, Australia. Vaccine 2002; 20: 3130-3136.

Kozak, S., Forsberg, CW. Transformation of Mercuric Chloride and Methylmercury by the Rumen Microflora. Appl Environ Microbiol 1979; 38(4): 626-636.

Lebel, J., Mergler, D., Lucotte, M., Amorim, M., Dolbec, J., Miranda, D., Arantes, G., Rheaulth, I., Pichet, P. Evidence of early nervous system dysfunctions in Amazonian populations exposed to low-level of methyl mercury. Neurotoxicol 1996; 17(1): 157-168.

Lebel, J., Roulet, M., Mergler, D., Lucotte, M., Larribe, F. Fish diet and mercury exposure in a riparian Amazonian population. Water Air Soil Poll. 1997; 97: 31-44.

Lemire, M., Mergler, D., Fillion, M., Passos, CJS., Guimarães, JRD., Davidson, R., Lucotte, M. Elevated blood selenium levels in the Brazilian Amazon. Sci Total Environ 2006; 366: 101-111.

Levy, M., Schwartz, S., Dijak, M., Weber, J-P., Tardif, R., Rouah, F. Childhood urine mercury excretion: dental amalgam and fish consumption as exposure factors. Environ Res 2004; 94:283-290.

Lodenius, M., Malm, O. Mercury in the Amazon. Rev Environ Contam Toxicol. 1998 ; 157 : 25-52.

Maurice-Bourgoin, L., Quiroga, I., Chincheros, J., Courau, P. Mercury distribution in waters and fishes of the upper Madeira rivers and mercury exposure in riparian Amazonian populations. Sci Total Environ 2000; 260 : 73-86.

Passos, CJ., Mergler, D., Gaspar, E., Morais, S., Lucotte, M., Larribe, F., Grosbois, S. Characterization of the diet of a riverside population in the Brazilian Amazon. Rev Saúde Ambiente 2001; 4(1/2): 72-84.

Passos, C.J., Mergler, D., Gaspar, E., Morais, S., Lucotte, M., Larribe, F., Davidson, R., de Grosbois, S. Eating tropical fruit reduces mercury exposure from fish consumption in the Brazilian Amazon. Environ Res 2003; 93: 123-130.

Passos, C., Mergler, D., Larribe, F. Response to "Fruits, fish, and mercury: further considerations". Environ Res 2004; 96: 102-108.

Pinheiro, MCN., Nakanishi, J., Oikawa, T., Guimarães, G., Quaresma, M., Cardoso, B., Amoras, WW., Harada, M., Magno, C., Vieira, JLF., Xavier, MB., Bacelar, D. Methylmercury human exposure in riverine villages of Tapajós basin, Pará State, Brazil. Rev Soc Bras Med Trop 2000; 33(3): 265-269.

Pinheiro, MCN., Oikawa, T., Vieira, JLF., Gomes, MSV., Guimarães, GA., Crespo-Lópes, ME., Muller, RCS., Amoras, WW., Ribeiro, DRG., Rodrigues, AR., Côrtes, MIT., Silveira, LCL. Comparative study of human exposure to mercury in riverside communities in the Amazon region. Braz J Med Biol Res 2006; 39: 411-414.

Rahola, T., Hattula, T., Korolainen, A., Miettinen, JK. Elimination of free and protein-bound ionic mercury ($20Hg^{2+}$) in man. Ann Clin Res. 1973; 5 : 214-219.

Ryan, RP., Terry, CE. Toxicology Desk Reference. Third Edition 1996 ; 3 : 1075-1079.

Santos, ECO., Jesus, IM., Brabo, ES., Fayal, KF., Filho, GCS., Lima, MO., Miranda, AMM., Mascarenhas, AS., Sá, LLC., Silva, AP., Câmara, VM. Exposure to mercury and arsenic in Amazon States: a summary of studies by Evandro Chagas Institute/FUNASA. Rev. Bras. Epidemiol. 2003; 6(2): 171-185.

Stern, S., Cox, C., Cernichiari, E., Balys, M., Weiss, B. Perinatal and Lifetime Exposure to Methylmercury in the Mouse: Blood and Brain Concentrations of Mercury to 26 Months of Age. Neurotoxicol 2001; 22: 467-477.

United Nations Environment Programme (UNEP). Global Mercury Assessment. Geneva (Switzerland) 2002, 266 p.

Vahter, M., Mottet, NK., Friberg, L., Lind, B., Shen, DD., Burbucher, T. Speciation of mercury in the primate blood and brain following long-term exposure to methylmercury. Toxicol Appl Pharmacol 1994; 124: 221-229.

Vahter, ME., Mottet, NK., Friberg, LT., Lind, SB., Charleston, JS., Burbacher, TM. Demethylation of Methyl Mercury in Different Brain Sites of *Macaca fasciculares* Monkeys during Long-Term Subclinical Methyl Mercury Exposure. Toxicol Appl Pharmacol 1995; 134: 273-284.

Vahter, M., Akesson, A., Lind, B., Bjors, U., Schutz, A., Berglund, M. Longitudinal Study of Methylmercury and Inorganic Mercury in Blood and Urine of Pregnant and Lactating Women, as Well as in Umbilical Cord Blood. Environ Res 2000; 84: 186-194.

Watanabe, C. Modification of Mercury Toxicity by Selenium: Practical Importance? Tohoku J. Exp. Med. 2002; 196: 71-77.

Webb, J., Mainville, N., Mergler, D., Lucotte, M., Betancourt, O., Davidson, R., Cueva, E., Quizhpe, E. Mercury in Fish-eating Communities of the Andean Amazon, Napo River Valley, Ecuador. EcoHealth 2004; 1(suppl. 2): 59-71.

World Health Organization. Environmental Health Criteria: Mercury. Geneva WHO-IPCS, 1991.

Yokoo, EM., Valente, JG., Sichieri, R., Silva, EC. Validation and calibration of mercury intake through self-referred fish consumption in riverine populations in Pantanal Mato-Grossense, Brazil. Environ Res 2001; 86: 88-93.

Young, JF., Wosilait, WD., Luecke, RH. Analysis of methylmercury disposition in humans utilizing a PBPK model and animal pharmacokinetic data. J Toxicol Environ Health (Part A) 2001; 63: 19-52.

Zelinski, EM., Burnight, KP., Lane CJ. The Relationship between Subjective and Objective Memory in the Oldest Old: Comparisons of Findings from a Representative and a Convenience Sample. J Aging Health 2001; 13(2): 248-266.

CONCLUSION GÉNÉRALE

L'objectif général de la présente thèse était d'analyser le rôle de la consommation de fruits amazoniens sur la dynamique d'exposition humaine au mercure provenant des poissons contaminés. Elle a été réalisée dans l'optique de trouver des solutions palliatives et à court terme au problème d'exposition, sans pour autant priver ces communautés de la consommation de cet aliment à la fois très riche en nutriments et très souvent la base de l'alimentation des communautés riveraines.

La revue exhaustive de la littérature scientifique concernant la pollution environnementale et l'exposition humaine au mercure, tout comme ses effets à la santé des populations traditionnelles amazoniennes au cours de presque deux dernières décennies, montre de manière assez claire et surtout décevante que la grande et écrasante majorité des recherches n'ont pas proposé des mesures concrètes et réalistes pour l'atténuation de la charge corporelle du métal chez cette population, laquelle dépend fortement des poissons pour subvenir à ses besoins alimentaires et nutritionnels quotidiens. On peut aussi en déduire que malheureusement l'harmonie très souhaitable entre la science et les politiques publiques en matière de santé des populations est très loin d'être une réalité dans cette région.

Ce cadre plutôt décevant renforce davantage la pertinence du thème qui a été abordé dans la présente recherche. Les résultats obtenus se montrent très encourageants et très prometteurs du point de vue de leur potentiel en tant qu'outil d'intervention alimentaire permettant le maintien de la consommation de poissons tout en minimisant les risques d'atteinte à la santé dus à la bioaccumulation du métal dans les tissus biologiques. De plus, les données constituent un rappel de la nécessité d'accorder beaucoup plus d'attention et de mettre davantage en valeur la biodiversité des ressources naturelles animales et végétales retrouvées dans cette immense mosaïque d'écosystèmes, l'Amazonie. Le grand nombre d'espèces fruitiers et d'espèces de poissons de plusieurs habitudes alimentaires facilement accessibles dans les jardins potagers et dans la forêt derrière les villages, de même que dans les cours d'eau illustrent bien la viabilité de l'utilisation stratégique de l'énorme biodiversité de ces ressources naturelles pour atténuer à court terme la charge de mercure chez ces populations.

Dans le même ordre d'idées, ces recherches ont permis de confirmer qu'un certain nombre de fruits comestibles possèdent la capacité d'exercer des effets bénéfiques. La présence de ces arbres fruitiers offre des avantages tant au niveau de l'environnement qu'au niveau de la composante humaine de l'écosystème (Passos et al., 2003). Par exemple, les arbres donnant l'ingá sont de bons fixateurs d'azote et ils sont largement utilisés en milieu tropical humide (NFTA, 1993). Même si ce genre comprend environ 180 espèces en Amazonie brésilienne, seulement 4 ou 5 sont véritablement consommées. Parmi celles-ci *Inga edulis* Mart. (ingá-cipó), une importante espèce de ingá cultivé dans les jardins familiaux de la région du Tapajós, est valorisée en agroforesterie grâce à sa croissance rapide, à sa tolérance aux sols acides et également en raison de sa grande capacité de production de biomasse foliaire, laquelle aide à contrôler l'érosion des sols.

Ce type de constatation illustre bien les avantages d'une démarche écosystémique focalisant sur les problèmes de santé reliés à la dégradation de l'environnement, réunissant des chercheur-es de différentes disciplines et s'appuyant sur la participation communautaire tout comme l'analyse différenciée selon le genre. En effet, l'échange constant non seulement d'idées mais aussi de données récoltées dans une même zone d'études par des chercheur-es provenant de différents horizons disciplinaires, a été d'une importance capitale pour l'atteinte des objectifs établis au début de ce projet. Par exemple, ce n'est qu'à partir de l'intégration des données de mercure dans les poissons, obtenues par la collègue Delaine Sampaio da Silva travaillant sur les aspects ecotoxicologiques du projet, avec les informations issues des questionnaires alimentaires que nous avons pu calculer l'apport quotidien en mercure dans cette population, pour ensuite analyser le rôle protecteur des fruits contre un tel apport toxique. De la même manière, nos collègues en sciences sociales (Elizete Gaspar, Frédéric Mertens et Johanne Saint-Charles) nous ont permis de mieux comprendre la dynamique sociale au sein des communautés avec lesquelles nous effectuons des recherches, et notamment le rôle des femmes. Leurs analyses ont révélé l'importance de l'engagement de la part des femmes, lesquelles à leur tour sont des personnes clés dans des interventions alimentaires chez des populations traditionnelles amazoniennes, puisque ce sont elles les responsables de la sélection des poissons, de la préparation de la nourriture ainsi que de sa distribution aux membres du foyer familial. Dans la présente recherche, la participation des femmes à l'étude exploratoire et aux interventions a été de grande importance pour l'atteninte des objectifs établis.

Cependant, si d'une part la structure interdisciplinaire du projet CARUSO ainsi que l'implication communautaire et plus particulaièrement celle des femmes constituent des éléments caractérisant l'utilisation de l'approche écosystémique, d'un autre côté l'implication des autorités locales et d'aûtres acteurs intéressés à été moins présente. À ce titre, les ateliers de travail réalisés dans les communautés et les positions prises par les décideurs portent à croire qu'une des raisons principales (voire la principale) expliquant une telle absence est le fait que la problématique d'exposition humaine au mercure ne se place pas très haut sur la liste considérable des problèmes de santé présents dans cette region. De plus, elle n'est pas du tout prise en considération dans les aspirations de développement économique régional. Ces constats sont d'ailleurs en accord avec les difficultés et les barrières retrouvées par d'autres équipes de recherche menant des travaux d'intervention communautaires afin de diminuer les risques liés à l'exposition au mercure dans des zones minières à travers le monde (y compris dans la region du Tapajós), telles que présentées et discutées dans le chapitre I.

Du point de vue de l'évolution de la démarche écosystémique participative cherchant à trouver des solutions viables et à court terme au problème d'exposition au mercure sur le Tapajós, une des conclusions les plus importantes qu'on peut faire c'est qu'au delà des choix alimentaires visant à favoriser la consommation d'espèces de poissons herbivores au détriment d'espèces carnivores, la découverte de l'influence de la consommation de fruits régionaux sur la relation entre la consommation de poissons et l'exposition au mercure constitue un avancement majeur et prometteur qui mérite d'être exploré davantage en tant qu'effet protecteur pouvant mener à une stratégie additionnelle d'intervention à court terme visant à diminuer les niveaux d'exposition.

Toutefois, il importe de rappeler que les résultats encourageants présentés dans cette thèse ne suffiraient pas à apporter des solutions durables et à long terme au problème de contamination environnementale et humaine. Tout d'abord, il y a un besoin urgent de favoriser l'interaction entre les autorités sanitaires régionales et nationales avec les équipes de recherche en vue d'utiliser davantage les connaissances scientifiques générées au cours de ces presque vingt ans, et de stimuler la mise en œuvre de politiques publiques qui visent à diminuer les niveaux d'exposition humaine dans l'ensemble de l'Amazonie. Il serait souhaitable que les efforts d'intervention alimentaire soient couplés à des interventions à moyen et à long terme visant à réduire la lixiviation du mercure des sols vers les écosystèmes aquatiques grâce à de meilleures pratiques agricoles et à une gestion plus durable du territoire, tels que proposés par Farella et al. (2006, 2007).

Au niveau de la poursuite des recherches, plusieurs questions demeurent et un certain nombre de pistes restent à explorer. Par exemple, bien que cette thèse montre une influence de la consommation de fruits sur le niveau de mercure sanguin, et malgré que quelques études récentes aient montré des contenus élevés en plusieurs substances antioxydantes et nutraceutiques pour un nombre très restreint de fruits amazoniens (Del Pozo-Insfran et al, 2006, 2004; Lichtenthaler et al., 2005; Arabbi et al., 2004; Andrade et al., 2001), le(s) mécanisme(s) d'action des fruits ainsi que les nutriments impliqués dans ces réactions restent toujours à étudier, d'autant plus que la composition nutritionnelle et phytochimique de la plupart des fruits consommés dans la région du Tapajós est inconnue. Les métabolites végétaux secondaires présents dans ces fruits ont un grand potentiel en termes de propriétés bioactives, et ils sont à la base des principales hypothèses émises en vue d'expliquer les possibles mécanismes d'action protectrice des fruits contre la bioaccumulation du mercure chez l'humain.

À ce chapitre, la présente thèse laisse voir que la déméthylation du mercure organique en mercure inorganique ne semble pas être influencée par la consommation de fruits, du moins lorsqu'on utilise l'urine comme indicateur d'excrétion de ces formes inorganiques. Néanmoins, compte tenu de l'importance de l'excrétion fécale, il se peut que la consommation de fruits influence l'excrétion du mercure par cette voie. Nous n'avons pas pu examiner l'excrétion fécale dans cette thèse, ce qui constitue une limite de l'étude, et ceci demeure donc une hypothèse à tester par de futures recherches. La thèse a tout de même permis de mettre en évidence l'importance du mercure inorganique chez les consommateurs de poissons et les relations entre la consommation de poissons carnivores et les concentrations de mercure inorganique sanguin et urinaire.

La possible action protectrice de l'ingestion de fruits régionaux contre les effets neurotoxique, immunotoxique et/ou cardiotoxique du mercure reste également à examiner. Des études qui visent à approfondir la compréhension du lien entre la consommation de poissons et les teneurs élevées en mercure inorganique dans cette population devraient être poursuivies, puisque de telles études présentent aussi un grand potentiel pour apporter de meilleures explications non seulement aux effets toxiques du mercure chez les riverains, mais aussi à de possibles voies physiologiques impliquées dans l'effet des fruits sur la bioaccumulation du mercure, comme la possible excrétion fécale des formes inorganiques augmentée par la consommation de fruits régionaux. Finalement, nos études renforcent l'importance de considérer l'influence des facteurs alimentaires et nutritionnels lors des prochaines études épidémiologiques évaluant les risques et les effets toxiques du mercure.

RÉFÉRENCES BIBLIOGRAPHIQUES

Andrade, EH., Zoghbi, MGB., Maia, JGS., Fabricius, H., Marx, F. 2001. Chemical characterization of the fruit of *Annona squamosa* L. occurring in the Amazon. Journal of Food Composition and Analysis 14: 227-232.

Arabbi, PR., Genovese, MI., Lajolo, FM. 2004. Flavonoids in vegetable foods commonly consumed in Brazil and estimated ingestion by the Brazilian population. J. Agric. Food Chem 52: 1124-1131.

Del Pozo-Insfran, D., Percival, SS., Talcott, ST. 2006. Açai (Euterpe oleracea Mart.) polyphenolics in their glycoside and aglycone forms induce apoptosis of HL-60 leukemia cells. J. Agric. Food Chem. 54: 1222-1229.

Del Pozo-Insfran, D., Brenes, CH., Talcott, ST. 2004. Phytochemical composition and pigment stability of Açai (Euterpe oleracea Mart.). Journal of Agricultural and Food Chemistry 52: 1539-1545.

Farella, N., Lucotte, M., Davidson, R., Daigle, S., 2006. Mercury release from deforested soils triggered by base cation enrichment. Sci Total Environ 368: 19-29.

Farella, N., Davidson, R., Lucotte, M., and S. Daigle., 2007. Nutrient and mercury variations in soils from family farms of the Tapajós region (Brazilian Amazon): recommendations for better farming. Agric Ecosyst Environ 120: 449-462.

Lichtenthaler, R., Rodrigues, RB., Maia, GS., et al. 2005. Total oxidant scavenging capacities of *Euterpe oleracea* Mart. (Açai) fruits. International Journal of Food Sciences and Nutrition 56(1) : 53-64.

NFTA 93-04. 1993. Nitrogen Fixing Tree Association, *Inga edulis*: a tree for acid soils in the humid tropics.

Passos, C.J., Mergler, D., Gaspar, E., Morais, S., Lucotte, M., Larribe, F., Davidson, R., de Grosbois, S., 2003. Eating tropical fruit reduces mercury exposure from fish consumption in the Brazilian Amazon. Environ Res 93: 123-130.