UNIVERSITÉ DU QUÉBEC À MONTRÉAL

# UNE APPROCHE ÉCOSYSTÉMIQUE AUX SOURCES DE SÉLÉNIUM DANS L'ALIMENTATION ET À SES EFFETS SUR LA SANTÉ EN LIEN AVEC L'EXPOSITION AU MERCURE EN AMAZONIE BRÉSILIENNE

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« Comme le démontrent Kiriki au milieu de la forêt en flammes et tous les colibris qui s'illustrent dans les histoires et les légendes de tant de peuples, ce n'est pas l'animal le plus gros, le plus puissant ou le plus téméraire qui apporte le plus grand bien ou exerce la plus grande influence.

Ce sont plutôt ceux qui n'hésitent pas à agir, qui sont conscients des enjeux de leur combat, qui accomplissent les plus grandes choses. »

Michelle Benjamin, *Flight of the Hummingbird: A Parable for the Environment*

Traduit de l'anglais par Richard Desjardins

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# RÉSUMÉ

Le mercure (Hg) est un polluant global qui pose plusieurs risques pour la santé humaine. Le poisson, qui peut bioaccumuler des quantités importantes de Hg, renferme aussi des nutriments et assure la sécurité alimentaire de plusieurs populations humaines. Le projet CARUSO, au sein duquel les présentes recherches ont été réalisées, utilise une approche écosystémique, interdisciplinaire et participative pour étudier les sources de Hg et ses effets dans la région du Bas-Tapajós, en Amazonie brésilienne. Au début des années 2000, lors d'un atelier avec les villageois sur les résultats des études précédentes, les femmes du village ont demandé aux chercheur-es s'il existait des aliments qui pourraient influencer les effets du Hg sur leur santé. Ceci a amené l'équipe du CARUSO à initier de nouvelles études sur les sources possibles et le rôle du sélénium (Se) dans cette région.

Le Se est un oligoélément qui constitue le centre actif d'au moins 25 sélénoprotéines. Ces dernières sont indispensables pour le cerveau, notamment pour le protéger contre les dommages du stress oxydatif. Plusieurs études animales, souvent parcellaires et contradictoires, soutiennent que le Se protège contre les effets toxiques du Hg. Ces auteur-es suggèrent que le Se pourrait soit compenser les effets du stress oxydatif causé par le Hg, former des complexes inertes Hg-Se ou réduire la biodisponibilité du Hg dans les organes-cibles. Les quelques études chez les populations exposées au Hg n'ont toutefois pas montré de tels effets du Se chez les humains. Par ailleurs, quelques études suggèrent que le Se pourrait aussi réduire le stress oxydatif causé par l'exposition au plomb (Pb), une autre substance toxique récemment identifiée chez les riverains de cette région.

Les aliments sont la principale source de Se et leurs teneurs en Se varient suivant plusieurs facteurs dont les niveaux de Se présents dans les sols. Un apport alimentaire insuffisant ou excessif en Se peut avoir des effets néfastes sur la santé. La déficience de Se est associée notamment à une augmentation des maladies cardiaques et de divers types de cancer. Les effets toxiques causés par un excès de Se sont moins fréquents et ont été rapportés chez des populations chinoises vivant dans des écosystèmes naturellement très riches en Se. Les signes et les symptômes de toxicité associés à un excès de Se sont l'apparition graduelle de signes dermiques et de problèmes gastro-intestinaux suivis de troubles moteurs et sensoriels. Récemment, un statut élevé de Se a aussi été associé à une prévalence élevée de diabète, d'hypercholestérolémie et d'hypertension chez différentes populations aux États-Unis, en France et en Angleterre, qui consomment une quantité importante de suppléments alimentaires de Se.

La première étude sur le Se menée par l'équipe du CARUSO montre que, chez les riverains du Bas-Tapajós, le statut de Se varie de normal à très élevé et de façon importante entre les communautés. Selon la littérature, certains aliments présents dans la région d'étude pourraient contenir des niveaux considérablement élevés de Se. Cependant, aucune information pouvant expliquer les variations du statut de Se chez les riverains, par exemple les niveaux de Se dans les aliments ou les niveaux de Se dans les écosystèmes terrestres et aquatiques de la région du Tapajós, n'est disponible.

Le Hg constitue un problème important pour ces communautés amazoniennes. L'exposition au Pb, récemment découverte, pourrait exacerber les effets néfastes du Hg sur la santé des riverains. La présente recherche utilise une approche écosystémique pour mettre en lumière dans quelle mesure le Se peut contribuer à réduire les effets de ces substances toxiques sur la santé des riverains. Les objectifs de cette thèse sont: (i) d'évaluer les variations saisonnières du statut de Se à l'aide de différents bioindicateurs; (ii) d'identifier les sources alimentaires de Se dans plusieurs villages du Bas-Tapajós; (iv) d'examiner les relations entre les bioindicateurs de Se et les effets connus de la toxicité du Se, et; (v) d'évaluer les relations entre les bioindicateurs de Se et les effets sur les fonctions motrices et les cataractes séniles, seuls et en lien avec le Hg et le Pb.

Une première phase de cette recherche a eu lieu dans six communautés de la région du Bas-Tapajós en 2003 et 2004. Deux études transversales ont été réalisées: à la saison de la décrue des eaux (N = 259) et à la saison de la crue des eaux (N = 137), avec des mesures répétées pour un sous-groupe (N = 112). Les niveaux de Se ont été mesurés dans le sang (B-Se), les cheveux (H-Se) et l'urine (U-Se). Les résultats de cette étude montrent une importante variation interindividuelle des niveaux de B-Se (de 142 to 2447 µg/L), sans variations particulières d'une saison à l'autre (médiane 284 et 292 µg/L respectivement). Toutefois, l'analyse séquentielle de segments de cheveux montre une variation significative du statut de Se dans le temps : les niveaux de H-Se étaient significativement plus bas à la saison de la décrue des eaux comparé qu'à celle de la crue des eaux (médiane: 0.7 et 0.9 µg/g, plage d'étendue: 0.2 – 4.3 et 0.2 – 5.4 µg/g respectivement). Lors des deux saisons, les relations entre les niveaux de B-Se et de H-Se étaient linéaires et hautement significatives, alors que celle entre les niveaux de B-Se et de U-Se était mieux décrite par une courbe sigmoïde. Les variations temporelles de H-Se suggèrent qu'il y ait des variations saisonnières importantes des sources locales de Se. Chez les populations présentant un statut de Se hautement variable ou élevé, les analyses séquentielles de H-Se peuvent constituer un bioindicateur utile pour évaluer les variations temporelles et le statut mensuel de Se.

Pour la seconde phase de cette recherche, réalisée en 2006, nous avons recruté 448 personnes, âgées de 15 à 87 ans, dans 12 communautés de la région. Les niveaux de Se et de Hg ont été mesurés dans le sang, le plasma, les cheveux et l'urine et les

niveaux de Pb ont été mesurés dans le sang. Des questionnaires ont permis de collecter des données sociodémographiques, sur les fréquences de consommation des aliments potentiellement riche en Se, sur la présence des symptômes de toxicité du Se et sur l'histoire médicale des participants. Une évaluation de signes de la toxicité du Se (cheveux, ongles et peau) a été réalisée par une infirmière. Tous les participants ont également subi un examen visuel complet et réalisé une batterie de tests neuromoteurs pour évaluer la coordination motrice, la dextérité manuelle et la force de préhension. Des échantillons d'aliments et d'eau consommée par les riverains ont aussi été collectés dans quatre de ces communautés.

Les analyses du Se dans les aliments de la région ont montré que les noix du Brésil (*Bertholletia excelsa*) sont une excellente source de Se, mais avec des concentrations hautement variables, 0.4 à 158.4µg/g. D'autres aliments comme le poulet, la viande de chasse, les œufs et le bœuf avaient des niveaux élevés de Se (0.3–1.4µg/g), alors que le borécole, les patates douces, le riz et les *pupunha* avaient des niveaux moyens de Se (0.1–0.2µg/g). Selon le type d'aliment, les niveaux de Se ne suivaient pas les mêmes profils de variation entre les communautés. Les niveaux de Se dans l'eau potable étaient négligeables (< 1.4µg/L). Les niveaux de B-Se étaient positivement associés à la consommation de noix du Brésil, de poulet et de viande de chasse. Les noix du Brésil contenaient aussi des niveaux hautement variables et parfois très élevés de Ba (1.9–1437 µg/g) et de Sr (3.3–173µg/g).

Chez les participants, les niveaux de B-Se variaient entre 103.3–1500.2µg/L (médiane 228.4µg/L), de Se plasmatique (P-Se) entre 53.6–913.2µg/L (médiane 134.8µg/L), de H-Se entre 0.4–3.8µg/g (médiane 0.7µg/g) et de U-Se entre 2.3 – 1375.0µg/g creat. (médiane 33.6µg/g creat.). Les médianes de Hg sanguins (B-Hg) et Pb sanguins (B-Pb) étaient de 42.5µg/L et de 113.0µg/L respectivement. Même si les niveaux de B-Se et P-Se dépassaient les concentrations considérées toxiques, aucun signe ou symptôme de toxicité du Se n'était associé aux bioindicateurs de statut de Se. Par ailleurs, les bioindicateurs de Se (surtout le P-Se) étaient positivement reliés à de meilleures performances pour tous les tests moteurs, en tenant compte des variables sociodémographiques et des bioindicateurs de B-Hg et de B-Pb. Les coefficients de régression pour les bioindicateurs de Se étaient considérablement plus élevés lorsque les modèles étaient contrôlés pour le B-Hg. Aucune interaction entre les bioindicateurs de Se, Hg et Pb n'a été observée.

Chez les adultes de 40 ans et plus, 32.7% présentaient des cataractes séniles. Les rapports de cote pour la prévalence des cataractes séniles, ajustés pour l'âge et la consommation de cigarette, montrent un effet bénéfique du P-Se à des concentrations $\geq$ 111µg/L et des effets néfastes du B-Hg à des concentrations $\geq$ 25µg/L (0.25 [0.10–0.69] et 4.52 [1.35–15.16], respectivement). Lorsque les concentrations de P-Se étaient $\geq$ 111µg/L, aucune association entre le B-Hg et la prévalence de cataractes séniles n'a été observée.

Cette thèse est la première recherche à montrer l'absence d'effets toxiques du Se chez des populations ayant un statut élevé de Se venant des aliments et des effets bénéfiques du Se sur les fonctions motrices et les cataractes séniles. Cette recherche met aussi en lumière que des interrelations entre le Se et le Hg chez l'humain sont possibles, mais plus complexes qu'un ratio molaire Se:Hg de 1:1. Chez des populations exposées à des niveaux élevés de Hg, un statut adéquat, et même élevé de Se, pourrait jouer un rôle crucial pour atténuer les effets toxiques du Hg ou maintenir l'activité optimale des sélénoprotéines. Plus d'études sont nécessaires pour comprendre dans quelles conditions le Se dans les aliments affecte la santé chez des populations avec une exposition faible ou modérée au Hg.

Mots-clés : Sélénium, Mercure, Noix du Brésil, Fonctions motrices, Cataractes séniles, Amazonie brésilienne.

# INTRODUCTION

## *Historique*

Le mercure (Hg) est un polluant global qui pose plusieurs risques pour la santé des écosystèmes, incluant les humains (Mergler et al., 2007). Dans les années 80, il a été mis en évidence que l'orpaillage artisanal, qui utilise le Hg pour amalgamer les particules d'or dans les sols et les sédiments lacustres, cause de la contamination de plusieurs écosystèmes aquatiques en Amazonie (Martinelli et al., 1988; Pfeiffer et al., 1988). Le Dr. Fernando Branches (1948-2002), cardiologue de Santarém (État du Pará, Brésil), a été le premier à sonner l'alerte quant aux niveaux élevés de Hg non seulement chez les mineurs, mais aussi chez les habitants de Brasília Legal, une communauté riveraine du Bas-Tapajós, en Amazonie brésilienne, (Branches et al., 1993; Lebel, 2003). Brasília Legal est une communauté située à plusieurs kilomètres des sites d'orpaillage qui, à l'époque, vivait essentiellement de la pêche et de l'agriculture de subsistance. En 1994, l'équipe de recherche canado-brésilienne, composée de Donna Mergler, Fernando Branches, Marc Lucotte, Jean Lebel, Marc Roulet (1968-2006) et Marúcia Amorim, y ont fondé le projet CARUSO, ainsi nommé en l'honneur du célèbre chanteur d'opéra Enrico Caruso qui a inauguré de *Teatro Amazonas*, à Manaus en Amazonie.

Depuis ses débuts, le projet CARUSO utilise une approche écosystémique, interdisciplinaire et participative pour caractériser les voies et les conditions de transmission du Hg des sources jusqu'aux humains et pour étudier effets de l'exposition au Hg sur la santé des riverains, dans le but de promouvoir des solutions qui préserveraient l'environnement et la santé humaine (CARUSO, 2009). Par cette approche systémique, les chercheurs-es de l'équipe ont été les premiers à mettre en évidence que la déforestation et l'agriculture sur brûlis, et non seulement l'orpaillage, sont les principaux responsables de la contamination au Hg des écosystèmes du Bas-

Tapajós, qui sont fort éloignés des sites d'orpaillage du Haut-Tapajós (Roulet et al., 1998a; 1998b). Ces activités humaines contribuent de façon importante au largage du mercure inorganique (HgI), contenu naturellement dans les sols, vers les écosystèmes aquatiques où le HgI est transformé par l'action bactérienne en méthylmercure (MeHg), qui se bioaccumule et se bioamplifie dans la chaîne trophique aquatique, du phytoplancton vers les grands poissons prédateurs (Farella et al., 2001; 2006; 2007; Guimaraes et al., 2000a; 2000b; Roulet et al., 1998a; 1998b; 1999; 2001; Sampaio da Silva et al., 2005; 2009). En fait, les niveaux de Hg dans les poissons et les écosystèmes aquatiques de la région auraient commencé à augmenter il y a environ cinquante ans, suite à l'essor de la colonisation des terres amazoniennes par les habitants venant du nord-est et du sud du Brésil (Roulet et al., 1999).

Pour les riverains, les poissons constituent la principale source d'exposition au Hg (Lebel et al., 1997; Passos et al., 2003; 2007; 2008). Toutefois, ces poissons sont aussi la base de l'alimentation quotidienne et source de protéines, de sélénium (Se) et d'acides gras oméga-3 (Passos et al., 2001; Philibert et al., *soumis*; Sampaio et al., *en révision*). La pêche communautaire est l'une des principales activités de subsistance de la région et plusieurs traditions orales et alimentaires sont intimement liées à cette relation étroite qu'ont les populations amazoniennes avec les écosystèmes aquatiques (Sampaio da Silva, 2005).

Malheureusement, à l'heure actuelle, plusieurs communautés riveraines d'Amazonie présentent les niveaux les plus élevés d'exposition au Hg dans le monde (Passos et Mergler, 2008). Dans le Bas-Tapajós, l'exposition au Hg a été associée à des altérations précoces du système nerveux chez les adultes et les enfants (Dolbec et al., 2000; Grandjean et al., 1999; Lebel et al., 1996; 1998). Des études récentes suggèrent également que l'exposition au Hg peut mener à une augmentation de la pression sanguine et une diminution de la variabilité du rythme cardiaque (Fillion et al., 2006; Philibert et al., *en préparation*).

Récemment, des niveaux élevés de plomb (Pb) ont également été observés dans le sang des riverains du Bas-Tapajós, possiblement à cause des plaques de métal utilisées pour torréfier la farine de manioc, un aliment traditionnel riche en amidon qui accompagne souvent les repas (Barbosa et al., 2009). L'exposition au Pb pourrait aussi avoir des effets néfastes sur la santé cardiovasculaire et neurologique des riverains, en particulier sur celle des enfants (Bellinger, 2008; Navas-Acien et al., 2007). Par ailleurs, des études animales suggèrent que l'exposition au Hg et au Pb pourraient aussi contribuer à la formation de cataractes à l'âge adulte, qui affectent considérablement l'acuité visuelle (Head, 2001).

Au début des années 2000, lors d'un atelier communautaire à Brasilia Legal sur les résultats des études précédentes, des femmes du village ont demandé aux chercheurs-es du projet s'il existait des aliments qui pourraient influencer les effets du Hg sur leur santé. En effet, plusieurs études suggèrent que certains nutriments présents dans l'alimentation pourraient jouer un rôle important pour contrer les effets du Hg, et peut-être aussi contre les effets du Pb (en revue dans Ahamed et Siddiqui, 2007; Chapman et Chan, 2000). Le questionnement des villageoises nous a amené à étudier différents facteurs dans l'alimentation locale tels que les fruits (Passos et al., 2003; 2008), les oméga-3 (Philibert et al., *soumis*) et le sélénium (Se).

## *Problématique*

Le Se est un oligoélément essentiel au bon fonctionnement de l'organisme (Rayman, 2000). Il est requis pour l'activité d'au moins 25 sélénoprotéines, des enzymes uniques impliquées, entre autres, dans la protection de l'organisme contre le stress oxydatif, l'équilibre redox et le métabolisme des hormones thyroïdiennes (Papp et al., 2007; Reeves et Hoffmann, 2009). Plusieurs sélénoenzymes sont indispensables pour le cerveau, notamment pour le protéger contre les dommages du stress oxydatif. Toute forme de vie ayant un système nerveux exprime ou préserve de

façon prioritaire l'activité optimale des sélénoenzymes dans les tissus cérébraux et neuroendocriniens (Chen et Berry, 2003; Kohrle et al., 2005; Schweizer et al., 2004a).

Plusieurs études animales, souvent parcellaires et contradictoires, soutiennent que le Se protège contre les effets toxiques du Hg (Khan et Wang, 2009). Ces auteurs-es suggèrent que le Se pourrait compenser les effets du stress oxydatif causé par le Hg, interagir directement avec le Hg pour former un complexe inerte avec le Se ou réduire la biodisponibilité du Hg pour les organes cibles (Curvin-Aralar et Furness, 1991; Ganther et al., 1972; Ganther et Sunde, 2007; Khan et Wang, 2009; Watanabe, 2002). Quelques études ont aussi proposé que le Se pourrait réduire les effets du stress oxydatif causé par le Pb (Ahamed et Siddiqui, 2007).

Jusqu'à ce jour, peu d'études épidémiologiques se sont attardées aux effets du Se sur la santé en lien avec l'exposition au Hg ou au Pb. Chez les populations nordiques ayant des niveaux élevés de Se et de Hg venant de la consommation de poissons et de mammifères marins, aucun effet du Se sur la santé neurologique des nouveau-nés et des enfants n'a été observé (Choi et al. 2008a; Després et al., 2005; Steuerwald et al. 2000). Saint-Amour et al. (2006) ont montré que le Se avait des effets opposés aux effets du Hg sur le système neuro-visuel des enfants Inuits, mais les résultats sont difficiles à interpréter.

L'alimentation, notamment le poisson, les viandes, les œufs et certaines espèces végétales, est la principale source de Se pour les humains (OMS, 1986). Les teneurs en Se des aliments peuvent varier grandement selon les régions du monde, et les niveaux de Se chez les populations humaines reflètent généralement le niveau de Se présent dans les sols (Fordyce, 2005). Comme plusieurs éléments essentiels, un apport alimentaire insuffisant ou excessif de Se, tel qu'illustré à la Figure 1, peut conduire à des effets néfastes sur la santé (OMS, 1986; ATSDR, 2003).

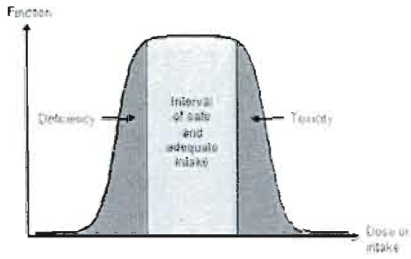


Figure 1: Relation dose-réponse des oligoéléments
(tiré de Lindh, 2007)

La déficience de Se chez des populations vivant dans des régions particulièrement pauvres en Se est associée, entre autres, à une augmentation des maladies cardiaques et de divers types de cancers (Rayman, 2008). Des effets toxiques causés par un excès de Se ont aussi été rapportés chez des populations chinoises vivant dans des écosystèmes naturellement très riches en Se (Yang et al., 1983; 1989a). Les signes et les symptômes de toxicité associés à un excès de Se sont l'apparition graduelle de signes dermiques, incluant la perte de cheveux, la déformation et la chute des ongles, des lésions de la peau, et des problèmes gastro-intestinaux suivis de troubles moteurs et sensoriels (Yang et al., 1983; 1989a). Les écosystèmes pauvres en Se sont en général beaucoup plus fréquents que ceux renfermant des niveaux élevés de Se, désignés comme sélénifères (Fordyce, 2005).

Le plasma et le sérum sont les bioindicateurs de Se les plus souvent utilisés pour comparer le statut de Se entre les pays et étudier ses effets sur la santé chez les populations ayant un statut pauvre ou normal de Se (Rayman 2000; 2008; Thomson 2004). Toutefois, puisque les niveaux de Se dans le plasma tendent à plafonner lorsque l'apport alimentaire de Se devient élevé (Hansen et al., 2004; Yang et al., 1989a,b), le sang total est le bioindicateur favorisé chez les populations ayant un statut élevé de Se. Selon la littérature, le sang, les cheveux et l'urine pourraient tous constituer des bioindicateurs appropriés pour évaluer le statut de Se. Toutefois, les études sur les relations entre ces bioindicateurs de Se et les effets du Se sur la santé sont limitées (Barceloux, 1999).

Chez les riverains du Bas-Tapajós, le statut de Se varie de normal à très élevé et de façon importante entre les communautés (Lemire et al., 2006; Pinheiro et al., 2005). Certaines espèces de poissons de la région sont des sources non négligeables de Se (Dorea et al., 1998; Sampaio et al., *en révision*). Selon les saisons, d'autres aliments de la région, comme les noix du Brésil, pourraient aussi s'avérer être d'excellentes sources de Se (Dumont et al., 2006; Fordyce, 2005). Dans les régions hautement sélénifères, les niveaux de Se dans l'eau potable peuvent atteindre des concentrations élevées dépassant les normes recommandées pour la consommation humaine (Yang et al., 1983). Cependant, aucune information pouvant expliquer les variations du statut de Se chez les riverains, par exemple les niveaux de Se dans les aliments ou les niveaux de Se dans les écosystèmes terrestres et aquatiques de la région du Tapajós, n'est disponible.

Le Hg constitue un problème important pour ces communautés amazoniennes et l'exposition au Pb, récemment découverte, peut exacerber les effets néfastes du l'Hg sur la santé des riverains. Le Se pourrait contribuer à réduire les effets néfastes de ces toxiques sur la santé. Toutefois, les niveaux de Se chez certains riverains dépassent les limites considérées nuisibles pour la santé. La présente recherche utilise une approche écosystémique à la santé pour caractériser les voies d'exposition et les effets du Se sur la santé et pour évaluer dans quelle mesure le Se peut apporter une solution viable aux problématiques du Hg et du Pb dans la région du Bas-Tapajós. Une telle approche procure un cadre d'analyse écosystémique et interdisciplinaire qui permet d'aborder la problématique du Se en lien avec celles du Hg et du Pb sous différents angles. Cette approche implique également de collaborer avec les riverains, et ce dès le début de la recherche, pour développer les questions de recherche et identifier des pistes de solutions à court, moyen et long terme afin de mieux orienter les choix alimentaires et agricoles dans la région.

## *Présentation des chapitres*

La section intitulée État des connaissances présente une revue de la littérature exhaustive sur les différents aspects de la problématique du Se en lien avec le contexte amazonien et l'exposition au Hg et au Pb. Une attention particulière a été portée au fait que le statut de Se des riverains du Bas-Tapajós varie de normal à passablement élevé (Lemire et al., 2006) afin de mettre en évidence : (i) les sources potentielles de Se dans la région, (ii) les bioindicateurs de Se les mieux adaptés à une telle situation et (iii) les effets possibles d'un statut élevé de Se sur la santé des riverains. Deux études du projet CARUSO, réalisées en parallèle à cette recherche, ont été publiées récemment et sont présentées dans cette section (Grotto et al., 2010; Jacob-Ferreira et al., 2009).

Les objectifs de cette thèse ont été formulés pour aborder de façon écosystémique la problématique du Se dans la région du Bas-Tapajós. Les différents aspects de la santé abordés sont la présence de toxicité du Se, les fonctions motrices et la santé oculaire. L'effet du Se sur les fonctions visuelles, la pression sanguine et la variabilité du rythme cardiaque sera abordé dans des études complémentaires, réalisées par Myriam Fillion et Aline Philibert, toutes deux membres de l'équipe de recherche du CARUSO. Cette section est suivie de la description des différentes populations à l'étude pour répondre aux objectifs de la thèse. Les méthodes utilisées sont décrites en détail dans chacun des chapitres, lesquels sont rédigés sous forme d'articles scientifiques. Les questionnaires conçus pour l'étude sont présentés à l'Appendice B et l'Appendice C.

Le premier chapitre de cette thèse présente les variations saisonnières du statut de Se de la saison de la décrue des eaux en 2003 à la saison de la crue des eaux en 2004 dans six communautés de la région à l'étude. Ce chapitre présente également la

validité des différents bioindicateurs utilisés pour évaluer les fluctuations temporelles du statut de Se.

Le deuxième chapitre présente les niveaux de Se dans les aliments venant de quatre communautés, les variations des niveaux de Se de ces aliments entre les communautés, en comparaison à ceux du marché public et du supermarché, et les relations entre le statut de Se et la consommation d'aliments riches en Se. Cette recherche a été réalisée en 2006, parallèlement à celles décrites aux chapitres suivants.

Le troisième chapitre évalue la présence des signes et symptômes de toxicité du Se en lien avec le statut de Se dans 12 communautés de la région. Ce chapitre présente en détail les différents aspects de la toxicité du Se en lien avec les formes de Se présentes dans l'environnement naturel et de travail, dans les aliments et les suppléments alimentaires, et en lien avec l'exposition au Hg.

Le chapitre quatre présente l'effet du Se sur les fonctions motrices telles que la coordination motrice, la force de préhension ainsi que la dextérité et la précision du mouvement des mains en tenant compte de l'effet de l'exposition au Hg et au Pb et des autres covariables sur ces fonctions. Le cinquième et dernier chapitre présente les effets combinés du Se, du Hg et du Pb sur la prévalence de cataractes séniles en tenant compte des covariables. Ces deux recherches ont été menées dans les mêmes 12 villages.

La thèse se termine avec les conclusions générales concernant l'ensemble de la problématique du Se en Amazonie et ailleurs, et propose de nouvelles études nécessaires à l'approfondissement des connaissances. Par souci d'économie de papier et pour éviter les répétitions, les références de chacune des sections sont présentées uniquement après la conclusion.

Tous les chapitres de la présente thèse sont intimement liés entre eux et aux recherches de Myriam Fillion et Aline Philibert. Dans le cadre de l'approche interdisciplinaire et participative utilisée pour le projet, l'équipe de recherche s'est assurée de l'arrimage entre les volets environnementaux, sur les réseaux sociaux et de santé du projet pour être en mesure de bâtir, en collaboration avec les riverains, une intervention communautaire à la fois scientifiquement rigoureuse et accessible à tous. L'ensemble des différentes phases de consultation des riverains et de campagnes de retour des résultats du projet CARUSO sont présentées à l'Appendice A.

# ÉTAT DES CONNAISSANCES

## *1. Le cycle du Se dans les écosystèmes*

Le Se est inégalement réparti à la surface du globe. Les concentrations de Se dans les sols varient considérablement selon l'origine de la roche mère, formant des milieux déficients en Se (< 0.06µg/g) et des régions riches en Se (≈ 8µg/g) à hautement sélénifères (> 30µg/g) (Magos et Berg, 1986). Les roches ignées et métamorphiques ont généralement des concentrations inférieures à 1µg/g alors que les roches sédimentaires telles que le grès, le calcaire, la phosphorite et le schiste argileux peuvent contenir de 1 à 100µg/g (Figure 2) (OMS, 1986). Aucune information n'est disponible quant aux niveaux de Se dans les sols au Brésil et dans la grande région Amazonienne. Le nord du Venezuela (État du Portuguesa) est souvent cité comme une région sélénifère, toutefois les niveaux de Se dans les sols ne sont pas disponibles (Jaffe et al., 1972; Bratter et al., 1991; Combs Jr., 2001).

Le Se est un métalloïde qui est reconnu pour ses propriétés photoélectriques et semi-conductrices ayant plusieurs usages industriels. Certains procédés tels que la micro-électronique, la photocopie, la métallurgie ou l'usage agricole de certains engrais et pesticides contenant du Se peuvent contribuer à déverser des concentrations importantes de Se dans les écosystèmes (Fordyce, 2005). Aucune de ces industries n'est toutefois présente dans le contexte amazonien.

TABLE III. Selenium Concentrations in Selected Natural Materials

| Material | Total Se (mg kg$^{-1}$) | Water-soluble Se (ng g$^{-1}$) | Material | Total Se (mg kg$^{-1}$) |
|---|---|---|---|---|
| Earth's crust | 0.05 | | Water (μg L$^{-1}$): | |
| | | | World freshwater | 0.02 |
| Igneous rocks (general): | 0.35 | | Brazil River Amazon | 0.21 |
| Ultramafic (general) | 0.05 | | U. S. (general) | <1 |
| Mafic (general) | 0.05 | | U. S. seleniferous | 50–300 |
| Granite (general) | 0.01–0.05 | | U. S. Kesterson | <4200 |
| | | | U. S. River Mississippi | 0.14 |
| Volcanic rocks (general). | 0.35 | | U. S. River Colorado | 10–400 |
| United States | <0.1 | | U. S. River Gunnison | 10 |
| Hawaii | <2.0 | | U. S. Lake Michigan | 0.8–10 |
| Tuffs (general) | 9.15 | | U. S. seleniferous gw | 2–1400 |
| | | | U. S. drinking water | 0.0–0.01 |
| Sedimentary rocks: | | | Spain freshwater | 0.001–0.202 |
| Limestone (general) | 0.03–0.08 | | China Se-deficient sw | 0.005–0.44 |
| Sandstone (general) | <0.05 | | China Se-adequate sw | 1.72 |
| Shale (general) | 0.05–0.06 | | China seleniferous sw | 0.46–275 |
| W. USA shales | 1–675 | | Finland stream water | 0.035–0.153 |
| Wyoming shales | 2.3–52 | | Canada stream water | 1–5 |
| South Korea shales | 0.1–41 | | Norway groundwater | 0.01–4.82 |
| China Carbon-shale | 206–280 | | Slovakia groundwater | 0.5–45 |
| Mudstones (general) | 0.1–1500 | | Bulgaria drinking water | <2 |
| Carbonates (general) | 0.08 | | Sweden drinking water | 0.06 |
| Marine carbonates | 0.17 | | Germany drinking water | 1.6–5.3 |
| Phosphates (general) | 1–300 | | Ukraine surface water | 0.09–3 |
| U. S. Coal | 0.46–10.65 | | Ukraine groundwater | 0.07–4 |
| Australia Coal | 0.21–2.5 | | Argentina surface water | 2–19 |
| China stone-coal | <6471 | | Reggio, Italy dw | 7–9 |
| Oil (general) | 0.01–1.4 | | Sri Lanka drinking water | 0.056–0.235 |
| | | | Greece drinking water | 0.05–0.700 |
| Soil: | | | Polar ice (general) | 0.02 |
| World (general) | 0.4 | | Seawater (general) | 0.09 |
| World seleniferous | 1–5000 | | | |
| U. S. (general) | <0.1–4.3 | | Plants: | |
| U. S. seleniferous | 1–10 | | U. S. grasses | 0.01–0.04 |
| England/Wales (general) | <0.01–4.7 | 50–390 | U. S. clover and alfalfa | 0.03–0.88 |
| Ireland seleniferous | 1–1200 | | Norway moss | 0.8–1.23 |
| China (general) | 0.02–3.81 | | Canada tree bark | 2–16 |
| China Se-deficient | 0.004–0.48 | 0.03–5 | Norway grain | 0.006–0.042 |
| China Se-adequate | 0.73–5.66 | | Norway forage | 0.05–0.042 |
| China seleniferous | 1.49–59.4 | 1–254 | Finland hay | 0–0.04 |
| Finland (general) | 0.005–1.241 | | Finland grain | 0.007 |
| India Se-deficient | 0.025–0.71 | 19–66 | | |
| India seleniferous | 1–19.5 | 50–620 | Algae: | |
| Sri Lanka Se-deficient | 0.112–5.24 | 4.9–43.3 | Marine (general) | 0.04–0.24 |
| Norway (general) | 3–6 | | Freshwater (general) | <2 |
| Greece Se-deficient | 0.05–0.10 | | | |
| Greece Se-adequate | >0.2 | | Fish: | |
| New Zealand (general) | 0.1–4 | | Marine (general) | 0.3–2 |
| | | | Freshwater (general) | 0.42–0.64 |
| Stream sediments: Wales | 0.4–83 | | | |
| | | | Animal tissue (general) | 0.4–4 |
| Atmospheric dust (general): | 0.05–10 | | | |
| Air (ng m$^{-3}$) (general) | 0.00006–30 | | | |

Note: gw = groundwater; sw = surface water; dw = drinking water.

From Fleming (1980); Thornton et al. (1983); Lewander (1986); WHO (1987); Jacobs (1989); Nriagu (1989); Tan (1989); Ferguson (1990); Hem (1992); Haygarth (1994); Neal (1995); Rapant et al. (1996); Fordyce et al. (1998); Reimann and Caritat (1998); Vinceti et al. (1998); Oldfield (1999); British Geological Survey (2000); Fordyce et al. (2000a).

Figure 2: Les concentrations naturelles de Se retrouvées dans l'environnement (tiré de Fordyce, 2005)

Quatre stades d'oxydation du Se sont naturellement présents dans les sols : le sélénium élémentaire ($Se^0$), le sélénide ($Se^{-2}$), le sélénite ($Se^{+4}$) et le sélénate ($Se^{+6}$). L'accumulation du Se, qui varie en fonction des espèces végétales, est dictée par plusieurs facteurs dont la forme chimique du Se, le pH, la texture et la présence d'autres ions dans les sols (Mikkelsen et al., 1989). Le sélénate est généralement la forme la plus disponible pour l'absorption par les plantes. Le sélénite l'est également, mais à moindre échelle. À des pH $\geq$ 7, le sélénate est le stade d'oxydation du Se prédominant. Dans les sols inondés ou particulièrement acides, le Se élémentaire et le sélénide sont les formes de Se thermodynamiquement stables et peu disponibles pour les végétaux terrestres ou aquatiques (Mikkelsen et al., 1989).

Les sélénates lessivés des sols de surface sont la principale source de Se qu'on retrouve dans l'eau. L'eau de mer contient généralement des concentrations allant de 0.06 à 0.12µg/L alors que l'eau de la Rivière Amazone contient 0.21µg/L (OMS, 1986; Fordyce, 2005). L'eau potable excède rarement 10µg/L, limite de l'OMS pour le Se dans l'eau potable (OMS, 1994). Dans l'eau de surface des régions sélénifères de la Chine, les concentrations atteignent 140µg/L. Toutefois, chez ces populations où la sélénose (intoxication chronique causée par un excès de Se) est endémique, l'eau potable n'est pas une source majeure d'exposition en comparaison des aliments, dont l'apport de Se a été estimé à 5000 µg/jour (Yang et al., 1983).

L'alimentation est l'apport principal de Se chez l'humain. Les niveaux Se dans les plantes, les poissons et les mammifères reflètent la biodisponibilité du Se dans les sols, les sédiments et l'eau, qui varie substantiellement d'une région à l'autre du globe (Levander, 1987). Le schéma de la Figure 3 résume le cycle du Se et son transfert de l'environnement aux humains.

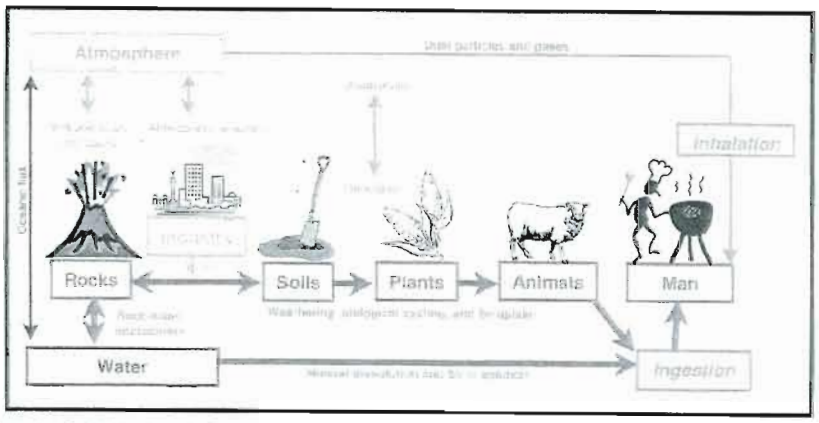


FIGURE 1 Simplified schematic diagram of the cycling of selenium from the environment to man. The main geochemistry and health pathways are shown in red.

Figure 3: Schéma du cycle du Se de l'environnement aux humains
Les flèches en rouges sont les principales voies de circulation du Se dans le cycle
(tiré de Fordyce, 2005)

Selon les espèces, les plantes peuvent accumuler des concentrations très variables de Se. Les différentes catégories de plantes sont présentées à la figure 4. Les espèces connues comme « accumulatrices primaires de Se » croissent bien dans des écosystèmes très riches en Se et peuvent accumuler plus de 1000µg/g de Se. Parmi ces espèces, on retrouve la famille des Lecythidaceae qui comprend plus de 325 espèces d'arbres des régions sub-tropicales d'Amérique (Fordyce, 2005). La noix du Brésil (*Bertholletia excelsa*) est l'espèce comestible la plus connue. Elle peut contenir de 0.1 à 512.0µg/g de Se (Chang et al., 1995). Les plantes « accumulatrices secondaires de Se », qui peuvent concentrer jusqu'à 50-100µg/g de Se, incluent la famille des *Brassica sp.*, dont le borécole (aussi appelé kale ou kole) et les choux qui sont fréquemment consommés en Amazonie (Passos et al., 2001). Par ailleurs, d'autres espèces végétales de la région pourraient aussi accumuler des niveaux non négligeables de Se (jusqu'à 1.0µg/g) : les végétaux des espèces *Allium sp* et les fruits de la famille des palmiers (*Arecaceae*) (Aleixo et al., 2000; Combs Jr., 2001; Yuyama et al., 2003).

**TABLE X.** Examples of the Three Types of Selenium Accumulating Vegetation

| *Type* | *Examples (genus, family, or species)* |
|---|---|
| Primary accumulator | G. *Astragalus* (e.g., milk vetch) |
| | G. *Machaeranthera* (woody aster; U. S.) |
| | G. *Haplopappus* (North and South American goldenweed) |
| | G. *Stanleya* (Prince's Plume) |
| | G. *Morinda* (rubiaceous trees and shrubs, Asia/Australia) |
| | F. Lecythidaceae (South American trees) |
| | Sp. *Neptunia* (Legume Asia/Australia) |
| Secondary accumulator | G. *Aster* |
| | **G. *Astragalus*** |
| | G. *Atriplex* (Saltbush) |
| | G. *Castilleja* (North and South American perennials) |
| | G. *Grindelia* (gummy herbs of western North and Central America) |
| | G. *Gutierrezia* (perennial herbs of western North and South America) |
| | G. *Machaeranthera* |
| | G. *Mentzelia* (bristly herbs of western America) |
| | Sp. *Brassica* (mustard, cabbage, broccoli, cauliflower) |
| Non-accumulator | Sp. *Pascopyrum* (wheat grass) |
| | Sp. *Poasecunda* (blue grass) |
| | **Sp. *Xylorhiza* (Woody Aster)** |
| | Sp. *Trifolium* (clover) |
| | Sp. *Buchloe* (buffalo grass) |
| | Sp. *Boutelova* (North and South American tuft grass) |
| | Sp. *Beta* (sugar beet) |
| | Sp. *Horedeum* (barley) |
| | Sp. *Triticum* (wheat) |
| | Sp. *Avena* (oats) |

Figure 4: Les catégories de plantes accumulatrices de Se (tiré de Fordyce, 2005)

Les céréales représentent une source de Se hautement variable, allant généralement de pauvre à modérée (< 0.1 à ≥ 0.8µg/g) (Fordyce, 2005; OMS, 1986). Même dans les régions hautement sélénifères, les cultures céréalières n'atteignent pas plus de 30µg/g de Se (Yang et al., 1983). Les tubercules et les bulbes accumulent généralement plus de Se que les légumes feuillus et les fruits, qui sont pauvres en Se (< 0.01µg/g) (Fordyce, 2005). Par ailleurs, les poissons, les fruits de mer, les organes des mammifères et les jaunes d'œufs sont des aliments généralement riches en Se (0.4 à 1.5µg/g), alors que les produits laitiers et la chair de mammifères peuvent contenir entre 0.1 à 0.4µg/g (OMS, 1986).

Les niveaux de Se dans les aliments peuvent aussi varier au sein d'une même région. En effet, les concentrations d'un aliment peut varier de 10 fois selon la biodisponibilité du Se dans les écosystèmes (U.S. Institute of Medicine, 2000). Dans un rayon de 10 km, il est possible de rencontrer des écosystèmes avec très peu ou un excès de Se (Fordyce et al., 2000). Par exemple, les noix du Brésil en provenance de la région centrale de l'Amazonie brésilienne contiennent beaucoup plus de Se (moyenne 36.0µg/g, variant de 1.3 à 512.0µg/g) que celles de la portion Nord-Ouest de l'Amazonie (moyenne 3.1 µg/g, variant de 0.1 à 31.7µg/g) (Chang et al., 1995).

La spéciation du Se peut varier considérablement selon les aliments. Plusieurs formes organiques de Se sont synthétisées chez les plantes, surtout de la sélénométhionine (SeMet), mais aussi la sélénocysteine (SeCys) et plusieurs autres composés intermédiaires tels la Se-methyl-SeCys, la γ-glutamyl-Se-methyl-SeCys, etc. (Whanger, 2002). Ces composés intermédiaires sont principalement synthétisés par les plantes accumulatrices de Se, afin d'éviter qu'il devienne toxique pour l'organisme. Certaines espèces produisent des composés volatiles et sont utilisées pour retirer le Se des sols contaminés au Se (Fordyce, 2005). Les plantes peuvent aussi accumuler directement le sélénate dans les sols. Les différentes voies d'accumulation du Se par les plantes sont présentées à la figure 5.

Dans les aliments d'origine animale, les principaux composés sont la SeCys et la SeMet. Cependant, différentes proportions de ces composés organiques ont été rapportées pour un même type d'aliment (Whanger, 2002; Rayman, 2008). Les voies de synthèse des sélénoprotéines chez les mammifères seront abordées plus en détail à la section suivante.

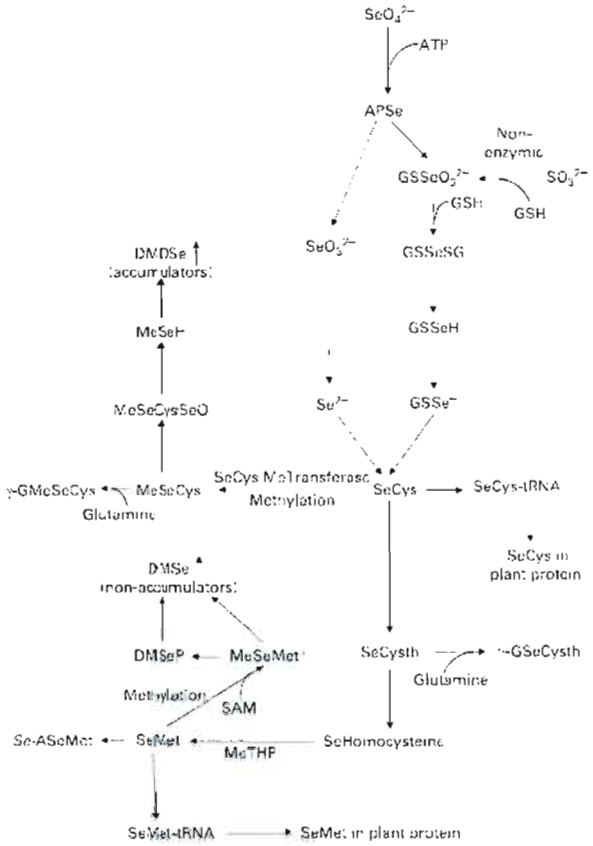


Fig. 1. Biosynthetic pathways elucidated for Se in higher plants (some by analogy with S pathways) adapted from Ellis & Salt[3], Wrobel[45], Terry et al.[6], Tagmount et al.[7] and Sors et al.[8]. It should be noted that reactions vary from species to species so that compounds formed and their relative quantities differ between species and strains. APSe, adenosine 5'-phosphoselenate; $GSSeO_3^{2-}$, glutathione *S*-selenite; GSH, glutathione; DMDSe, dimethyl diselenide (volatile); GSSeSG, selenodiglutathione; MeSeH, methyl selenol; GSSeH, glutathione-selenopersulfide; MeSeCysSeO, *Se*-methyl-selenocysteine selenoxide; $GSSe^-$, glutathione-conjugated selenide; γ-GMeSeCys, γ-glutamyl *Se*-methyl-selenocysteine; MeSeCys, *Se*-methyl-selenocysteine; SeCys MeTransferase, selenocysteine methyltransferase; SeCys, selenocysteine; DMSe, dimethyl selenide (volatile); DMSeP, dimethyl-selenonio-propionate ($(CH_3)_2Se^+(CH_2)_2CH_2COO^-$); MeSeMet, *Se*-methyl-selenomethionine; SeCysth, selenocystathionine; γ-GSeCysth, γ-glutamyl-selenocystathionine; SAM, *S*-adenosyl methionine; *Se*-ASeMet, *Se*-adenosyl-selenomethionine; SeMet, selenomethionine; MeTHF, methyl-tetrahydrofolate; SeHomocysteine, selenohomocysteine.

Figure 5: Les voies d'accumulation du Se dans les plantes
(Tiré de Rayman et al., 2008)

Les formes de Se dans les aliments pourraient grandement influencer la biodisponibilité et les effets de ces derniers sur la santé, surtout lorsqu'ils contiennent des niveaux élevés de Se (Rayman et al., 2008). Plusieurs études ont tenté d'élucider les différentes formes de Se présentes dans les aliments. Les techniques analytiques ont grandement évolué au cours des dernières années. Malgré cela, l'isolation, la caractérisation et la quantification des différentes formes de Se dans les aliments demeurent, à ce jour, un défi analytique (Pedredo and Madrid, 2009; Rayman et al., 2008).

## *2. Le métabolisme du Se et les différentes sélénoprotéines*

Le Se est ingéré sous différentes formes organiques (SeMet et SeCys) et inorganiques (sélénite, sélénate, etc.). Les composés organiques proviennent surtout des aliments alors que ceux inorganiques sont retrouvés dans l'eau potable et dans certains suppléments. Ces différentes formes de Se doivent être métabolisées par l'organisme en sélénide d'hydrogène ($H_2Se$), un précurseur inorganique, avant d'être insérées dans les sélénoprotéines sous la forme de l'acide aminé SeCys, essentielle pour la fonction catalytique des différentes sélénoenzymes qui sont constituées d'un nombre variable de SeCys (Reeves et Hoffman, 2009). La figure 6 résume l'ensemble des voies métaboliques du Se.

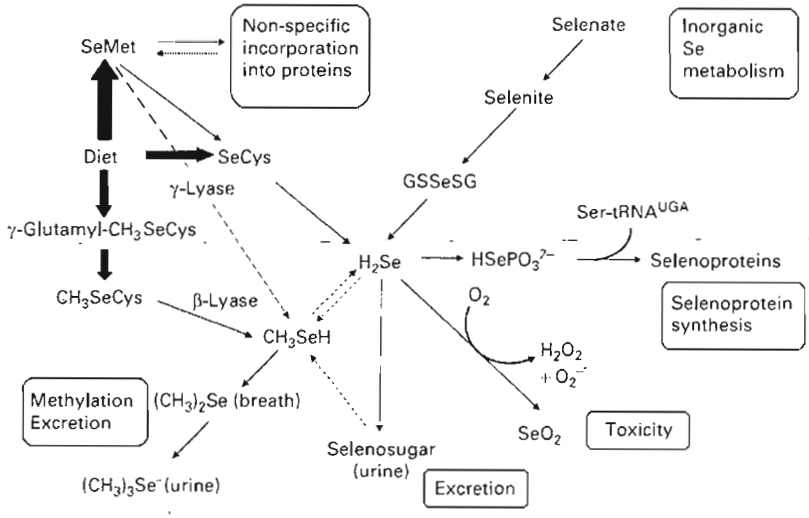


**Fig. 2.** Metabolic pathway of dietary Se in humans (adapted from Combs[11] and Rayman[12][13,14]). SeMet, selenomethionine; SeCys, selenocysteine; GSSeSG, selenodiglutathione; γ-glutamyl-$CH_3$SeCys, γ-glutamyl-*Se*-methyl-selenocysteine; $H_2Se$, hydrogen selenide; $HSePO_3^{2-}$, selenophosphate; $CH_3$SeCys, *Se*-methyl-selenocysteine; $CH_3$SeH, methyl selenol; $(CH_3)_2Se$, dimethyl selenide; $SeO_2$, selenium dioxide; $(CH_3)_3Se^+$, trimethyl selenonium ion.

Figure 6: Les voies métaboliques du Se chez l'humain
(tiré de Rayman et al., 2008)

Le $H_2Se$ en excès peut être méthylé puis excrété par les voies respiratoires (volatile) et dans l'urine. Lorsque l'apport en Se devient trop élevé et que l'organisme n'excrète plus suffisamment l'excès, le surplus de $H_2S$ peut générer du stress oxydatif, entrainant la formation de peroxydes d'hydrogènes et de radicaux libres (Rayman et al., 2008; Combs Jr., 2001).

Contrairement à la SeCys, il a été suggéré que la SeMet ingérée peut à la fois être réduite en $H_2Se$, ou bien, puisqu'elle est confondue par l'organisme avec la méthionine, s'accumuler de façon non-spécifique à la place de la méthionine dans le *pool* protéique de l'organisme, incluant les protéines des érythrocytes et l'albumine dans le plasma (U.S. Institute of Medicine, 2000). Jusqu'à ce jour, l'effet physiologique de cette accumulation de la SeMet demeure inconnu (Laclaustra et al., 2009a). Il pourrait aussi s'agir d'un "réservoir à long terme" de Se, où le Se est lentement relâché lors du *turn-over* métabolique des protéines et pourrait pallier à une éventuelle carence en Se (Xia et al., 2005). Toutefois, il n'est pas clair si la SeMet est d'abord accumulée dans le *pool* de méthionine, puis lentement relâchée, ou si l'accumulation non-spécifique se produit uniquement lorsque l'apport alimentaire en Se est élevé et que les besoins de l'organisme en sélénoprotéines ont été atteints.

Jusqu'à ce jour, des gènes codant pour au moins 25 sélénoprotéines ont été identifiées chez l'humain. La figure 7 présente l'ensemble de ces sélénoprotéines, leurs fonctions respectives, le statut de Se nécessaire pour leur activité optimale ainsi que leur localisation subcellulaire. La grande majorité est impliquée dans la protection de l'organisme contre le stress oxydatif, l'équilibre redox, notamment lors de la cascade signalétique intracellulaire, et le métabolisme des hormones thyroïdiennes (figure 8) (Papp et al., 2007; Reeves et Hoffmann, 2009). La figure 9 présente quelques exemples des réactions antioxydantes, impliquant notamment la glutathion (GSH), et des réactions redox catalysées par les sélénoprotéines. Un statut de Se supérieur à 70-90µg/L dans le plasma est essentiel pour assurer le fonctionnement optimal de la glutathionne peroxydase (GPx) (Nève, 1995). Récemment, il a été suggéré qu'un apport en Se supérieur ($\approx$ 125µg/L dans le plasma) soit nécessaire pour maximiser l'activité de l'ensemble des sélénoprotéines, incluant celle de la sélénoprotéine P (SelP) (Rayman, 2009). Les fonctions de certaines sélénoprotéines dans le cerveau et dans le cristallin de l'œil, hautement vulnérables aux stress oxydatifs (Schweizer et al., 2004; Head, 2001), sont discutées en détail aux chapitres IV et V.

Table 1 Summary of functions of selenoproteins and regulation of their expression by Se status

| Selenoprotein | Abbreviation | Important insights into function and significance | Dietary selenium effects | Subcellular localization |
|---|---|---|---|---|
| Cytosolic glutathione peroxidase | GPX1 | GPX1 knockout is more susceptible to oxidative challenge. Overexpression of GPX1 increases risk of diabetes | Very sensitive to Se status, following insufficient Se, or oxidative stress rapid GPx1 recovery. Se deficiency leads to nonsense mediated decay of GPx1 mRNA | Cytoplasmic |
| Gastrointestinal glutathione peroxidase | GPX2 | GPX1/GPX2 double knockout mice progressively develop intestinal cancer, one allele of GPX2 added back confers protection | Relatively resistant to dietary Se changes | Cytoplasmic |
| Plasma glutathione peroxidase | GPX3 | Important for cardiovascular protection, perhaps through modulation of Nitroge Oxide levels; antioxidant in thyroid gland | Sensitive to Se status | Secreted |
| Phospholipid hydroperoxide glutathione peroxidase | GPX4 | Genetic deletion is embryonic lethal; GPX4 acts as crucial antioxidant, structural protein in sperm, and sensor of oxidative stress and pro-apoptotic signals | Relatively resistant to dietary Se changes | Cytoplasmic |
| Olfactory glutathione peroxidase | GPX6 | Importance unknown | | |
| Thioredoxin reductase Type 1 | TrxR1, TR1 | Localized to cytoplasm and nucleus. Genetic deletion is embryonic lethal | Increased Se increases activity, Se deficiency decreases activity, but does not change mRNA levels | Cytoplasmic, nuclear |
| Thioredoxin reductase Type II | TrxR2, TR3 | Localized to mitochondria. Genetic deletion is embryonic lethal | Subject to dietary Se changes (i.e., increased Se increases expression) | Mitochondria |
| Thioredoxin reductase Type III | TR3/TR2, TR2, TGR | Testes specific expression | | |
| Deiodinase Type I | D1, DIO1 | Important for systemic active tyroid hormone levels | | Membrane associated |
| Deiodinase Type II | D2, DIO2 | Important for local active tyroid hormone levels | Expression levels are stable under low Se conditions | Membrane associated |
| Deiodinase Type III | D3, DIO3 | Inactivates thyroid hormone | | Membrane associated |
| Selenoprotein H | Sel H | Nuclear localization, involved in transcription. Essential for viability and antioxidant defense in *Drosophila* | Highly dependent on adequate dietary Se levels | Nuclear |
| Selenoprotein I | Sel I, EPT1 | Possibly involved in phospholipid biosynthesis | | Transmembrane |
| Selenoprotein K | Sel K | Transmembrane protein localized to endoplasmic reticulum | | ER, plasma membrane |
| Selenoprotein M, Selenoprotein 15 | Sel M, Sep 15 | Thiol-disulfide oxidoreductases localized to endoplasmic reticulum | | ER |
| Selenoprotein N | Sel N, SEPN1, SepN | Potential role in early muscle formation; involved in RyR related calcium mobilization from ER; mutations lead to multiminicore disease and other myopathies | | ER |
| Selenoprotein O | Sel O | Contains a Cys-X-X-X See motif suggestive of redox function, but importance remains unknown | | |

Figure 7: Fonctions et régulation des sélénoprotéines chez l'humain
(tiré de Reeves et Hoffmann, 2009)

Table 1 continued

| Selenoprotein | Abbreviation | Important aspects into function and significance | Dietary selenium effects | Subcellular localization |
|---|---|---|---|---|
| Selenoprotein P | Sel P | Selenium transport to brain and testes; Sel P knockout leads to neurological problems and male sterility. Sel P also functions as extracellular antioxidant in plasma/ECM | Serves as a biomarker for Se status, and is moderately sensitive to Se status | Secreted, cytoplasmic |
| Selenoprotein R | Sel R, MsrB1 | Functions as a methionine sulfoxide reductase and Sel R knockouts show mild damage to oxidative insult | | Cytoplasmic |
| Selenoprotein S | Sel S, SEPS1, SELENOS, VIMP | Transmembrane protein found in plasma membrane and endoplasmic reticulum. May be involved in ER stress | | ER |
| Selenoprotein T | Sel T | Endoplasmic reticulum protein involved in calcium mobilization | | ER |
| Selenoprotein V | Sel V | Testes specific expression | | |
| Selenoprotein W | Sel W, SEPW1 | Putative antioxidant role, perhaps important in muscle growth | Highly dependent on adequate dietary Se levels as well as levels of Sel P | Cytoplasmic |
| Selenophosphate synthetase | SPS2 | Involved in synthesis of all selenoproteins, including itself | | Cytoplasmic |

Figure 7 (suite): Fonctions et régulation des sélénoprotéines chez l'humain (tiré de Reeves et Hoffmann, 2009)

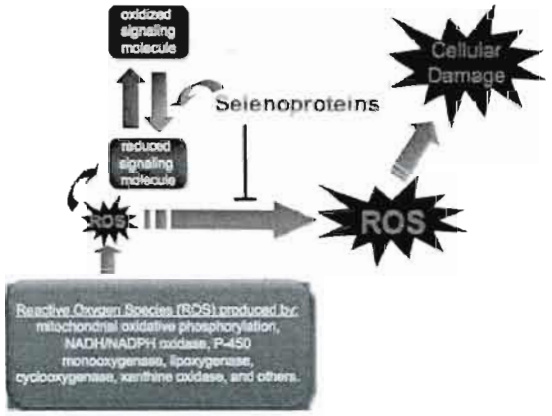


**Fig. 2** Roles for selenoproteins in regulating oxidative stress and redox status of signaling molecules. Through antioxidant selenoenzymes such as glutathione peroxidases, thioredoxin reductases, or methionine sulfoxide reductase, cellular damage caused by reactive oxygen species (ROS) is mitigated. In addition, selenoproteins may directly or indirectly modulate redox-regulated signaling

Figure 8: Rôles des sélénoprotéines dans la régulation du stress oxydatif et l'équilibre redox de la cascade signalétique
(tiré de Reeves et Hoffmann, 2009)

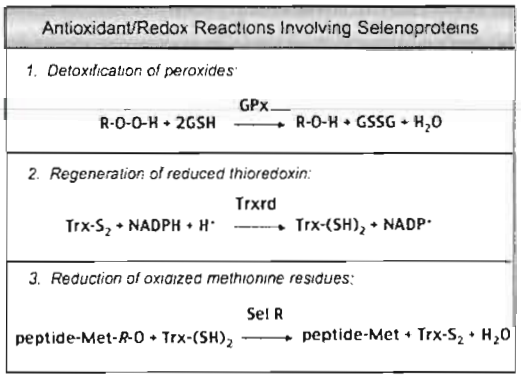


**Fig. 1** Three examples of antioxidant or redox reactions catalyzed by selenoproteins. In the first reaction, different forms of peroxide (R–O–O–H) such as hydrogen peroxide or lipid peroxide are reduced by the GPx selenoenzymes using glutathione (GSH). In the second, oxidized thioredoxin ($Trx\text{-}S_2$) is converted to reduced thioredoxin ($Trx\text{-}(SH)_2$) by thioredoxin reductase (Trxrd) using nicotinamide adenine dinucleotide phosphate (NADPH). The third reaction involves reduction of the *R*-stereoisomer of methionine-sulfoxide (Met-*R*-O) within peptides to methionine (Met) by selenoprotein R (Sel R, also called MSRB1) using Trx

Figure 9: Exemples de réactions antioxydantes et redox catalysées par les sélénoprotéines
(tiré de Reeves et Hoffmann, 2009)

## *3. Les effets sur la santé de la déficience et de l'excès de Se*

Un apport alimentaire insuffisant de Se est associé à une augmentation de la mortalité, des maladies cardiaques, des problèmes de fertilité et de reproduction, de la formation de cancer et à un déclin des fonctions cognitives, immunitaires et thyroïdiennes (en revue dans Rayman, 2008). D'autre part, dans certaines régions hautement sélénifères de la Chine, l'exposition à des niveaux très élevés de Se aurait causé l'apparition graduelle de signes dermiques, incluant la perte de cheveux, la déformation et la chute des ongles (voir Figure 10), des lésions de la peau (i.e. érythème, œdème, éruptions et démangeaisons) et des problèmes gastro-intestinaux, suivis de troubles moteurs et sensoriels (i.e. anesthésie périphérique, acroparaesthésie, douleur dans les extrémités, paralysie partielle, dysfonctions motrices et hémiplégie) (Yang et al., 1983). Récemment, un statut élevé de Se, mais à des niveaux inférieurs que ceux rapportés en Chine, a aussi été associé à une prévalence plus élevée de diabète, d'hypercholestérolémie et d'hypertension chez différentes populations aux États-Unis, en France et en Angleterre, qui consomment une quantité importante de suppléments alimentaires de Se (en revue dans Stranges et al., 2010). Le Se, sous forme inorganique, est également utilisé en laboratoire pour induire la formation de cataractes chez les souris (Flohé, 2005). Les études ayant observé des effets toxiques du Se sont abordées en détail au chapitre III.

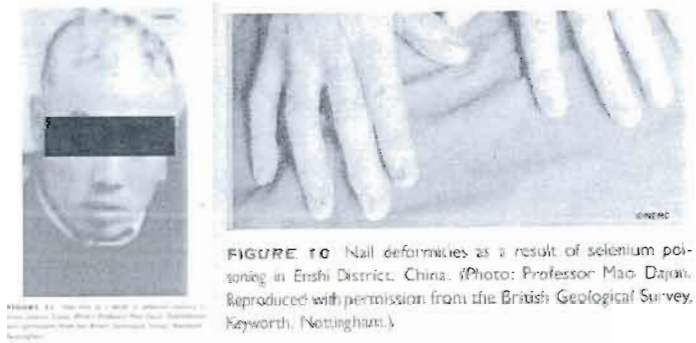

FIGURE 10 Nail deformities as a result of selenium poisoning in Enshi District, China. (Photo: Professor Mao Dajun. Reproduced with permission from the British Geological Survey, Keyworth, Nottingham.)

Figure 10 : Premiers signes cliniques de la toxicité du Se
(tiré de Fordyce, 2005)

## *4. Le statut et les bioindicateurs de Se*

Le statut de Se chez les populations humaines peut varier considérablement d'une région à l'autre. Il dépend de plusieurs facteurs, incluant les niveaux et la biodisponibilité du Se dans les écosystèmes, les préférences alimentaires, les pratiques agricoles, la disponibilité des aliments cultivés dans la région, les importations d'aliments, la consommation de suppléments alimentaires et la biodisponibilité des formes de Se présentes dans les aliments (Combs Jr., 2001). Il est donc difficile d'évaluer l'apport alimentaire de Se en croisant les données des questionnaires alimentaires et celles des niveaux de Se dans les aliments publiés par les agences gouvernementales. Ce calcul pourrait sur- ou sous-évaluer le statut de Se chez certaines populations. En effet, pour une mesure valide et reproductible du statut de Se, plusieurs auteurs favorisent les mesures des niveaux de Se dans différents bioindicateurs aux estimés alimentaires (Mayne, 2003; Mozaffarian, 2009). Le terme bioindicateur est généralement utilisé pour faire référence aux mesures de Se dans différents tissus biologiques.

La validité des différents bioindicateurs varie aussi selon les populations. Lorsque l'apport en Se est limité, les concentrations et l'activité des sélénoprotéines dans le sang et les tissus sont directement fonction de l'apport alimentaire total de Se. Toutefois, si l'apport alimentaire est suffisant, la forme chimique de Se ingérée a davantage d'influence sur la répartition du Se dans les fractions du sang et le statut de Se (U.S. Institute of Medicine, 2000). Par exemple, la SeMet ingérée, qui n'est pas sous régulation homéostatique, peut s'accumuler dans les protéines de l'organisme, particulièrement dans les érythrocytes, alors que les autres formes de Se en surplus sont plus susceptibles d'être excrétées (en revue dans Rayman, 2008). En effet, deux études ont mis en évidence que, lorsque l'apport en Se est élevé, les niveaux de Se dans le plasma tendent à saturer et atteindre un plateau alors que le Se dans le sang total, incluant les érythrocytes, continue d'augmenter (Hansen et al., 2004; Yang et

al., 1989a). Au-delà du seuil de Se requis, les concentrations de sélénoprotéines seront aussi influencées par des facteurs génétiques et environnementaux (U.S. Institute of Medicine, 2000; Rayman, 2009).

Le plasma et le sérum sont les bioindicateurs les plus utilisés pour comparer le statut de Se chez des populations ayant un statut pauvre ou normal de Se (Thomson, 2004). Toutefois, le sang total est un bioindicateur plus approprié lorsque le statut de Se tend à être élevé, ce qui n'est pas très fréquent (Combs Jr., 2001; Barceloux, 1999). Certains auteurs ont aussi proposés d'autres bioindicateurs moins invasifs et plus faciles à collecter tels que l'urine, les cheveux et les ongles, qui pourraient s'avérer utiles dans certaines conditions (Yang et al, 1983, 1989a, b; Fordyce, 2005; Barceloux, 1999). La figure 11 présente les niveaux de Se pour différents bioindicateurs dans plusieurs régions du monde.

L'urine est la principale route d'excrétion du Se et pourrait s'avérer un bon bioindicateur du statut de Se (Robberecht et Deelsta, 1984) puisqu'il reflète la capacité de l'organisme à réguler et excréter l'excès de Se. Par ailleurs, chez les populations chinoises ayant un statut élevé de Se, il a été rapporté que les niveaux de Se dans les cheveux étaient plus sensibles aux variations saisonnières de l'apport alimentaire de Se que le sang total. Les cheveux pourraient aussi agir comme une voie d'excrétion du Se dans l'éventualité où l'apport alimentaire devient trop élevé (Yang et al, 1989b). Puisque les cheveux poussent au rythme d'environ un centimètre par mois (Robbins, 2002), l'analyse séquentielle des segments de cheveux en centimètre permet d'établir un profil rétrospectif d'exposition au fil des mois, selon la longueur des cheveux (Cernichiari et al., 1995; Dolbec et al., 2001; Lebel et al., 1997). Les ongles et les ongles d'orteils, qui représentent une exposition rétrospective de 6 à 12 mois, sont aussi utilisés comme bioindicateur de Se. Toutefois, les risques de contamination des ongles au Se sont élevés et la validité de ces deux bioindicateurs reste à prouver (Slotnick et Nriagu, 2006).

TABLE XVI. Examples of Selenium Concentrations in Human Tissues From Around the World [a]

| Country | Selenium ($mg\,L^{-1}$) Whole blood | Year | Selenium ($mg\,L^{-1}$) Serum | Year |
|---|---|---|---|---|
| Average (humans) | 0.2 | | | |
| Normal (humans) | | | 0.06–0.105 | |
| Canada, Ontario | 0.182 | 1967 | | |
| China, high Se | 1.3–7.5 | 1983 | | |
| China, high Se, no disease | 0.44 | 1983 | | |
| China, mod Se | 0.095 | 1983 | | |
| China, low Se, no disease | 0.027 | 1983 | | |
| China, low Se, disease | 0.021 | 1983 | | |
| Tibet, low Se | | | <0.005 | 1998 |
| Egypt | 0.068 | 1972 | | |
| Finland | 0.056–0.081 | 1977 | | |
| Guatemala | 0.23 | 1967 | | |
| New Zealand | 0.083–0.059 | 1979 | | |
| Sweden | | 1987 | 0.86 | |
| UK | 0.32 | 1963 | | |
| U.S. | 0.256–0.157 | 1968 | | |
| Russia | 0.11–0.442 | 1976 | | |
| Venezuela seleniferous | 0.355–0.813 | 1972 | | |
| Bulgaria | | | 0.0548 | 1998 |
| Hungary | | | 0.0558 | 1998 |
| Slovenia | | | 0.0570 | 1998 |
| Croatia | | | 0.0642 | 1998 |
| Russia | | | 0.0718 | 1999 |
| Italy, Lombardy | 0.04–0.19 | 1986 | 0.033–0.121 | 1986 |
| Spain, Barcelona | | | 0.060–0.106 | 1995 |
| Canary Islands | | | 0.008–0.182 | 2001 |

| | Selenium ($mg\,kg^{-1}$) Hair | Year | Selenium ($\mu g\,L^{-1}$) Urine |
|---|---|---|---|
| China, Se deficient | 0.074 | 1983 | 0.007 |
| China, Se deficient | 0.170–0.853 | 1998 | |
| China, Se deficient | 0.094–0.359 | 1996 | |
| China, low Se | 0.16 | 1983 | |
| China, Se adequate | 0.343 | 1983 | 0.026 |
| China, high Se | 1.9–100 | 1983 | 0.04–6.63 |
| China, high Se | 0.566–141 | 1998 | |
| Italy, Lombardy | | 1986 | 0.0002–0.068 |
| Sri Lanka | 0.104–2.551 | 1998 | |

Yang et al. (1983); Levander (1986); Akesson and Steen (1987); WHO (1987, 1996); Oldfield (1999); Fordyce et al. (2000a); Vinceti et al. (2000); Romero et al. (2001); Fordyce et al. (2000b)

Figure 11 : Niveaux de Se dans des tissus humains de différentes régions du monde (tiré de Fordyce, 2005)

La majorité des études épidémiologiques ayant évalué les liens entre le statut de Se et les effets du Se sur la santé utilisent le plasma ou le sérum (Rayman, 2000, Rayman et al., 2008; Thomson, 2004). Puisque le sang total est le meilleur

bioindicateur de Se lorsque l'apport alimentaire en Se est élevé, les quelques études qui ont évalué les effets du Se sur la santé neurologique et cardiovasculaire ont favorisé le sang total ou le sang de cordon ombilical au plasma (Choi et al., 2008a; Després et al., 2005; Saint-Amour et al., 2006; Steuwerwald et al., 2000; Valera et al., 2009). Toutefois, à ce jour, les données sont limitées quant aux relations entre le Se dans le sang et les effets sur la santé lorsque le statut est pauvre ou normal ou entre le Se dans le plasma et la santé lorsque le statut est élevé (Barceloux, 1999). Par ailleurs, peu d'études ont évalué les liens entre les bioindicateurs capillaires et l'urine, et les effets sur la santé.

Plusieurs valeurs de références ont été proposées par différentes agences gouvernementales. Lorsque le Se est inférieur à 70-90µg/L dans le plasma, l'apport est insuffisant pour l'activité optimale de la GPx (Nève, 1995). Cette valeur pourrait cependant être revue à la hausse pour l'ensemble des sélénoprotéines (Rayman, 2009). La RDA (*Recommended Dietary Allowance*) est de 55µg/jour chez les femmes et les hommes adultes (U.S. Institute of Medicine, 2000). À partir des études sur les épisodes de toxicité du Se dans les régions sélénifères de Chine (Yang et al., 1983, 1989a), des valeurs maximales de Se dans le sang total ont été établies. La NOAEL (*No Observable Adverse Effect Level*) est de 1000µg/L et la LOAEL (*Lowest Observable Adverse Effect Level*) de 1350µg/L (U.S.EPA, 2002). Le UL (*Upper Tolerable Intake Level*) est estimé à 560µg/L (400µg/jour) (U.S.Institute of Medicine, 2000). Selon le Centre de Toxicologie du Québec, la plage de variation normale pour le Se est entre 100 et 340µg/L dans le sang total et entre 7 et 79µg/L dans l'urine, alors que le Se est considéré toxique au-delà de 400µg/L dans l'urine (Ryan et Terry, 1996). Selon l'OMS, un niveau inférieur à 0,1µg/g de Se dans les cheveux est équivalent à une carence de Se alors que des niveaux supérieurs à 5µg/g sont associés à des niveaux toxiques (OMS, 1994).

## *5. Les effets toxiques du Hg et les effets bénéfiques du Se – mécanismes enzymatiques compensatoires ou interactions moléculaires?*

Le Hg est un polluant global qui peut être retrouvé sous plusieurs formes dans les écosystèmes et les milieux industriels (Clarkson et al., 2003). La toxicité des différentes formes inorganiques et organiques de Hg est principalement causée par leur grande affinité pour les groupements thiols (-SH) des protéines et des enzymes, et les groupements sulfuryl de la cystéine (Cys) dans les protéines (Clarkson et Magos, 2006). Puisque ces formes de Hg ont des profils d'absorption et de distribution différents dans les organes, leurs effets toxiques sont sensiblement distincts (Gallagher et Lee, 1980). Les informations sur le mercure inorganique et organique présentées aux deux paragraphes suivants sont tirées des revues de la littérature du National Research Council (NRC, 2000), Clarkson et al. (2003), Clarkson et Magos (2006) et de Khan et Wang (2009).

Le mercure inorganique ($Hg^{2+}$ ou HgI) est surtout néphrotoxique et sa toxicité est principalement causée par l'inactivation des enzymes responsables de la dégradation des peroxydes d'hydrogènes, causant des dommages aux microtubules rénaux. Le mercure élémentaire ($Hg^0$), qui est gazeux à température ambiante, est rapidement oxydé en HgI dans le sang. Les vapeurs de $Hg^0$ qui n'auront pas été oxydées peuvent diffuser à travers la barrière hémato encéphalique et causer du dommage oxydatif dans le cerveau. Le Hg inorganique a longtemps été utilisé dans plusieurs processus industriels et à des fins médicinales. Par exemple, le nitrate de Hg utilisé dans le moulage du feutre des chapeaux au 17$^{e}$ siècle a été reconnu pour entraîner de graves lésions cognitives ou bien rendre « fous » plusieurs chapeliers, dont le célèbre *Chapelier fou* dans Alice aux pays des merveilles.

Le MeHg, la forme organique de Hg retrouvée dans le poisson et les mammifères marins, est toutefois la forme la plus neurotoxique de Hg, tant chez l'adulte que chez le fœtus en développement. En se liant au groupement -SH de la cystéine, il est activement transporté à travers les membranes cellulaires et les barrières hémato encéphalique et placentaire par les transporteurs protéiques qui le confondent à la méthionine. Plusieurs mécanismes de neurotoxicité du MeHg ont été proposés. Le MeHg pourrait générer du stress oxydatif, comme la peroxydation des lipides, et perturber plusieurs processus métaboliques incluant le fonctionnement des microtubules et des mitochondries, la balance homéostatique des ions et la synthèse des protéines. Le MeHg est reconnu pour lier la GSH (sous sa forme réduite), inactivant ainsi l'une des principales molécules antioxydantes de l'organisme, et sortir des cellules par le transporteur de la GSH. Il est aussi possible qu'une grande partie du MeHg absorbé dans le cerveau soit déméthylé *in vivo* et que les dommages cérébraux soient aussi causés par le HgI, qui, autrement, ne peut pas traverser la barrière hémato encéphalique.

Les mécanismes causant la toxicité du MeHg chez le fœtus en développement sont moins connus et ne sont pas nécessairement les mêmes que ceux causant la neurotoxicité chez les adultes. Par exemple, il a été proposé que le MeHg puisse aussi altérer les mécanismes de reconnaissance des surfaces cellulaires chez le fœtus. Tant chez l'adulte que le fœtus, il n'est pas encore clair quels sont les mécanismes prédominants causant la neurotoxicité du MeHg. L'exposition chronique au MeHg a également été associée à des effets néfastes sur le système cardiovasculaire et reproducteur.

Plusieurs études animales et *in vitro* suggèrent que le Se a des effets protecteurs contre la toxicité du HgI et du MeHg (Khan et Wang, 2009). En fait, lorsque l'on « décortique » la littérature, on remarque que les mécanismes sous-jacents à un tel effet protecteur demeurent, pour la plupart, spéculatifs. D'une part, les

sélénoprotéines pourraient jouer un rôle important en tant qu'enzymes antioxydantes impliquées dans l'inactivation et la compensation des effets des radicaux libres (ROS) générés par le HgI et le MeHg (Watanabe, 2001). Le Se pourrait même favoriser la déméthylation du MeHg *in vivo* (Ganther et al., 1972; Ganther et Sunde, 2007). D'autre part, le Se ou les sélénols (les centres actifs composés de SeCys des sélénoenzymes) pourraient contribuer à la formation d'un complexe inerte $H_2Se$-HgI, $H_2Se$-$(MeHg)_2$ ou SeCys-MeHg en circulation ou lié à des sélénoprotéines (surtout la SelP, qui contient entre six et dix molécules de SeCys) dans le plasma, le cerveau ou les reins (Khan et Wang, 2009). Plus récemment, des cristaux-granules insolubles de $HgSe_xS_{1-x}$ ont aussi été observés à l'aide de différentes techniques analytiques dans le foie de mammifères et d'oiseaux marins (Ikemoto et al., 2004). Ces différents complexes pourraient favoriser la déméthylation du MeHg, la redistribution du Hg dans l'organisme ou réduire sa biodisponibilité pour les organes-cibles (Ganther et al., 1972; Curvin-Aralar et Furness al, 1991; Yoneda et Suzuki, 1997; Watanabe, 2001; Ganther et Sunde, 2007; Khan et Wang, 2009). La figure ci-dessous résume l'ensemble des interactions moléculaires possibles entre le Se et le Hg.

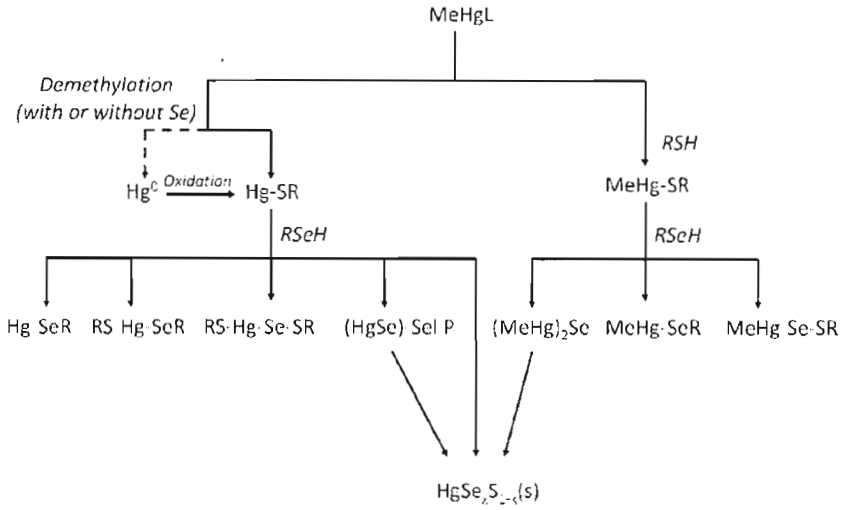


Fig. 2. Possible Hg–Se compounds involved in the metabolism of methylmercury (MeHgL), inorganic $Hg^{II}L_2$, and $Hg^0$ in biota in the presence of Se. RSH = thiols and thiol-containing proteins. RSeH = selenols and selenol-containing proteins; Sel P = selenoproteins; L = simple inorganic ligands (e.g., $H_2O$, $Cl^-$, or $OH^-$); $0 \leq x \leq 1$.

Figure 12 : Interactions moléculaires possibles entre le Se et le Hg
(tiré de Kang et Wang, 2009)

Plusieurs auteurs suggèrent que l'exposition au Hg pourrait induire une déficience de Se (Khan et Wang, 2009). Selon Ralston et al. (2008a), le Hg présente une très forte affinité pour les groupements sélénols, davantage que pour les groupements –SH. La formation de complexes Se-HgI hautement stables pourrait trapper le $H_2Se$ dans un complexe irréversible qui devient indisponible pour des cycles de synthèse futurs de SeCys et de sélénoprotéines. Par le même fait, ce complexe Se-Hg pourrait inactiver les sélénoprotéines et induire une déficience fonctionnelle de Se (Ralston et al, 2008a; Ganther et Sunde, 2007; Khan et Wang, 2009).

Des études animales ont montré que le HgI et le MeHg peuvent tous deux diminuer l'activité de la GPx1 (qui contient quatre molécules de SeCys) dans le foie, les muscles et le cerveau (Bem et al., 1985; Ganther et Sunde, 2007; Hirota, 1986; Splittgerber et Tappel, 1979). Toutefois, une étude *in vitro* montre que le HgI, mais non le MeHg, est un inhibiteur actif de la GPx1, ce qui suggère que le MeHg doit perdre son groupement méthyle avant de lier la GPx (Seppanen et al., 2004). Le MeHg pourrait aussi perturber l'activité des déiodinases, des sélénoprotéines essentielles à la synthèse et la régulation des hormones thyroïdiennes (Soldin et al., 2008). En fait, selon Ralston et al. (2008a), les sélénoprotéines pourraient être la principale cible du MeHg dans le cerveau. La figure ci-dessous résume les mécanismes d'interactions entre le Se et le Hg proposés par ces auteurs-es.

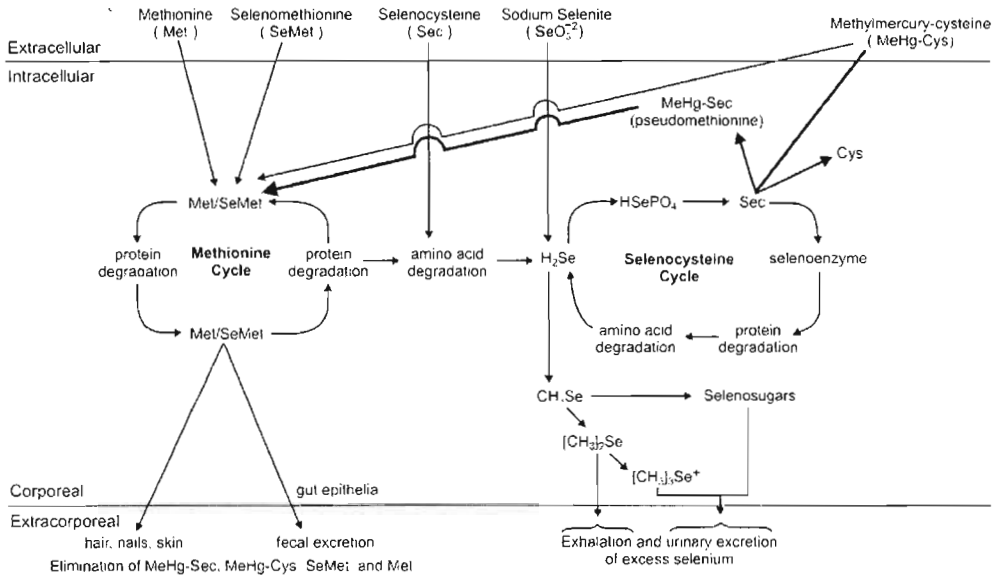


Figure 1. Metabolic cycles of selenomethionine, selenocysteine, inorganic selenium, and the proposed mechanism of MeHg dose-dependent disruption of the Se biosynthetic pathway.

Figure 13: Mécanismes d'interactions proposés par Ralston (2008b)

Chez le rat, plusieurs études ont mis en évidence qu'une diète riche en Se peut pallier aux effets neurotoxiques du MeHg, causant une diminution du Se et de l'activité des sélénoprotéines dans le cerveau, chez le fœtus et l'adulte (Folven et al., 2009; Ralston et al, 2008a; Watanabe et al, 1999a, b). Kaur et al (2009) ont aussi montré que la SeMet prévient les cellules nerveuses contre le ROS généré par le MeHg *in vitro*. Ralston et al (2008b) suggèrent ainsi qu'avec une diète riche en Se (dans un ratio moléculaire Hg:Se $\geq$ 1), les organismes pourraient accumuler des niveaux supérieurs de MeHg dans le cerveau, sans effets toxiques. L'étude de Ganther et Sunde (2007) montre que, contrairement au foie, le Se dans le cerveau des chats ne complexe pas tout le Hg présent dans le cerveau (ratio Hg:Se < 1), suggérant que l'absence d'effets toxiques ne peut être uniquement expliquée par l'interaction moléculaire directe entre le Se et le Hg dans le cerveau. Des études animales ont aussi suggéré que la vitamine E pourrait jouer un rôle important contre le Hg, agissant de pair avec le Se comme molécule antioxydante ou aidant l'organisme à protéger les sélénoenzymes contre le stress oxydatif et le Hg (présenté en revue dans Ganther et Sunde, 2007).

Certains auteurs suggèrent que l'interaction entre le Se et le Hg dans les poissons ou les mammifères marins pourrait diminuer la biodisponibilité du Se et du Hg pour les organismes qui consomment ces poissons (Rayman et al, 2008; Ralston et al, 2008b). Toutefois, aucune étude épidémiologique n'a démontré de tels effets.

Plusieurs études épidémiologiques ont rapporté des corrélations positives entre les niveaux sanguins de Se ou de Hg et les fréquences de consommation de poissons ou de mammifères marins ainsi que des corrélations entres les différents bioindicateurs de Se et de Hg chez les participants aux études (Hagmar et al., 1998; Hansen et al., 2004; Karita et Suzuki, 2002; Muckle et al., 2001; Svensson et al., 1992). Les auteurs de ces études expliquent généralement la corrélation entre le Se et le Hg par le fait que ces deux éléments proviennent de la même source; le poisson ou les mammifères marins. Par ailleurs, d'autres études ont montré une corrélation positive entre le Se et le Hg dans le sang alors que celle entre la consommation de poissons et le Se était particulièrement faible (Bensryd, et al., 1994, Grandjean et al, 1992; Lemire et al., 2006). Des corrélations positives ont aussi été démontrées chez des populations qui consomment peu de poissons (Bárány et al., 2003; Osman et al., 1998) et chez une population qui n'en consomme pas (Lindberg et al., 2004), suggérant qu'il pourrait y avoir une interaction Se-Hg dans le sang qui soit indépendante de la consommation de poissons. Il est à noter que la majorité des études considèrent les fréquences de consommation totale de poissons alors que certaines espèces peuvent contenir plus de Se ou de MeHg (Burger et Campbell, 2004). D'autres aliments riches en Se sont aussi consommés par plusieurs populations.

Une étude chez des mineurs habitant une zone contaminée au HgI, et consommant aussi des aliments possiblement contaminés au Hg (contenant diverses formes de Hg), suggère que l'exposition au Hg pourrait affecter les niveaux de Se et la distribution des sélénoprotéines dans le sérum (Chen et al., 2006). L'expression de

la GPx et de la SelP était significativement plus élevée chez les mineurs que chez la population de référence, et associée à une augmentation du Se dans le sérum. L'activité de la GPx, les concentrations de malondialdéhyde (MDA), un marqueur de peroxidation des lipides, et la proportion de Hg lié à la SelP étaient significativement plus élevés chez le groupe exposé au Hg. Les sélénoprotéines pourraient donc jouer deux rôles importants contre la toxicité du Hg : séquestrer le Hg par leurs groupements sélénols hautement réactifs et contribuer à éliminer les ROS générés par l'exposition au Hg (Chen et al., 2006). Chez cette population, les niveaux de Se et de Hg dans le sérum n'étaient pas corrélés, mais l'étaient dans l'urine.

Une étude de suivi a été réalisée avant et après la saison de pêche chez les travailleurs de la Baie James (Bélanger et al., 2008). Cette étude montre que l'augmentation de la consommation de poissons pêchés dans des lacs d'eau douce du Nord du Québec est associée à une augmentation de l'exposition au Hg, mais aussi à une augmentation des défenses antioxydantes (telles que l'activité de la GPx et de la glutathione réductase ainsi que les concentrations de GSH et d'autres molécules anti-oxydantes) et à une réduction des marqueurs de risques de maladies cardiovasculaires. Les niveaux sanguins de Se, d'acides gras oméga-3 et de vitamine E sont toutefois demeurés similaires avant et après la saison de pêche. Ces résultats suggèrent que les poissons d'eau douce de cette région renferment du Hg et plusieurs éléments bénéfiques pour la santé mais ne sont pas une source importante de Se, d'oméga-3 et de vitamine E.

Des études récentes dans la région du Bas-Tapajós en Amazonie suggèrent que les sélénoprotéines et le Se sont intimement liés aux processus métaboliques causant la toxicité du MeHg dans la région. Des membres de notre équipe de recherche ont montré que l'activité de la GPx et les concentrations de molécules antioxydantes (GSH et catalase) diminuent avec l'augmentation des niveaux de Hg dans le sang, le plasma et les cheveux (Grotto et al, 2010). Ces résultats vont dans la

direction opposée à ceux obtenus chez les mineurs (Chen et al., 2006) et les travailleurs de la Baie James (Bélanger et al, 2008) et suggèrent que lorsque l'exposition au MeHg est élevée et prolongée, l'organisme n'arrive peut être plus à compenser les effets néfastes du Hg sur les sélénoprotéines. Chez cette population, des résultats préliminaires montrent aussi que l'activité de la GPx augmente positivement avec le Se dans le plasma lorsque l'on tient compte de l'effet néfaste du Hg sur la GPx. Lorsque l'on ne tient pas compte du Hg, l'association entre le Se plasmatique et la GPx n'est pas significative. Pourtant, l'interaction entre les bioindicateurs de Se et de Hg n'a pas d'effet significatif sur l'activité de la GPx. Ceci suggère que l'augmentation du statut de Se peut aider à pallier les effets néfastes du Hg sur la GPx, et possiblement sur les autres sélénoprotéines (Barbosa Jr., *communication personnelle*). D'autres chercheurs-es ayant travaillé sur les données du projet CARUSO ont aussi montré que l'augmentation du Hg dans le plasma est corrélée à une hausse des métalloprotéinases matricielles (MMT-2 et MMT-9), associées à une augmentation de la vulnérabilité des maladies cardiovasculaires, alors que l'augmentation du Se dans le plasma est associé à une réduction de la MMT-9 (Jacob-Ferreira et al, 2009).

Les quelques études qui ont évalué les effets du Se sur la santé en lien avec les effets du Hg ont été réalisées chez les populations nordiques ayant des niveaux élevés de Se et de Hg venant de la consommation de poissons et de mammifères marins.

Aux Îles Féroé, l'augmentation du Hg dans le sang de cordon était significativement associée à une diminution des fonctions neurologiques des nouveau-nés (Steuerwald et al., 2000) et des fonctions neurocomportementales chez les enfants de 7 ans (Choi et al., 2008a). Dans ces deux études, les niveaux de Se dans le sang de cordon n'avaient pas d'effet sur la relation entre le Hg et les mesures de santé. L'information concernant l'effet direct du Se sur les fonctions neurologiques n'était toutefois pas donnée.

Chez les enfants inuit du Nunavik, une augmentation de l'amplitude du tremblement des mains était associée à l'augmentation des niveaux sanguins de Hg et aucune relation entre les niveaux de Se dans le sang et les fonctions motrices n'a été montrée (Després et al., 2005). Chez cette même cohorte, Saint-Amour et al. (2006) ont observé une plus grande latence des potentiels visuels évoqués en lien avec les niveaux sanguins de Se et ce, alors que le Hg raccourcit le temps de latence. Les auteurs évoquent que l'effet du Se sur le système neuro-visuel pourrait être un signe précoce de toxicité du Se. Ces résultats sont tout de même étonnants; le temps de latence diminue avec l'exposition au Hg au lieu d'augmenter, et l'effet du Se sur le temps de latence agit dans la direction opposée à l'effet du Hg. Plus récemment, une étude chez une population adulte du Nunavik a montré un effet bénéfique du Se sur la pression sanguine, un effet statistiquement indépendant des effets néfastes du Hg et des effets bénéfiques des oméga-3 (Valera et al., 2009).

De plus en plus d'études épidémiologiques suggèrent qu'en contrôlant les modèles d'analyses statistiques pour les effets bénéfiques du Se et des oméga-3 provenant du poisson, tout comme pour les effets de l'environnement social et des autres contaminants environnementaux, on est mieux en mesure d'évaluer les effets néfastes du MeHg sur la santé (Rice, 2008; Choi et al, 2008b; Valera et al., 2009). Le contraire est également possible, en contrôlant pour les effets des substances toxiques comme le Hg et le Pb, on évalue avec plus de précision les effets du Se sur la santé. Selon Choi et al. (2008b), les nutriments dans l'alimentation ont plus de chances d'avoir des effets sur les mêmes compartiments que le MeHg, mais dans une direction opposée, que d'être impliqués dans des interactions toxico-cinétiques directes avec le MeHg.

## *6. Les effets toxiques du Pb et les effets bénéfiques du Se*

L'exposition environnementale au Pb a des effets néfastes sur plusieurs systèmes de l'organisme, et ce, même à très faible dose (Bellinger, 2008; Needleman, 2009). Il est reconnu pour causer des troubles hématologiques, gastro-intestinaux et neurologiques chez les adultes et les enfants. Il peut aussi s'avérer néphrotoxique et causer de l'hypertension et des problèmes lors de la grossesse (Bellinger, 2005; Bellinger et Bellinger, 2006; Ekong et al., 2006; Navas-Acien et al., 2007). Les mécanismes moléculaires de la toxicité du Pb impliquent, entre autres, l'inhibition de plusieurs enzymes, l'altération des métabolismes cellulaires impliquant le calcium, un ralentissement de la propagation des influx nerveux et la génération de stress oxydatif (en revue dans Lockitch et al., 1993).

Très peu d'information est disponible quant aux effets possibles du Se sur la toxicité du Pb. Quelques études animales suggèrent que le Se pourrait contribuer à réduire les effets neurotoxiques et la néphrotoxiques du Pb, possiblement en agissant contre les dommages oxydatifs causés par le Pb (en revue dans Ahamed et Siddiqui, 2007). Chez le rat, le Se pourrait réduire la peroxydation des lipides des membranes du foie et des reins générée par le Pb (Othman et El Missiry, 1998). Chez des travailleurs exposés à des métaux, l'exposition au Pb a été associée à une augmentation de la tension artérielle, à une augmentation de la MDA et une réduction de l'activité de la GPx dans les érythrocytes. Les auteurs suggèrent que le Pb pourrait aussi lier les sélénoprotéines et induire une déficience fonctionnelle de Se (Kasperczyk et al., 2009). Pourtant, selon Whanger (1992) et Levander (1979), les effets du Se sur la toxicité du Pb sont mitigés et, contrairement au Hg, ne semblent pas impliquer une interaction directe entre le Se et le Pb.

Dans les études épidémiologiques réalisées au Nunavik, l'exposition au Pb a également été évaluée et étudiée en lien avec le statut de Se. Després et al. (2005) ont

montré une association positive entre les niveaux de Pb dans le sang et le temps de réaction simple, l'oscillation de la posture, la diadochokinésie et le tremblement d'intension des mains. Comme pour le Hg, ces effets étaient indépendants des niveaux sanguins de Se. Saint-Amour et al. (2006) n'ont pas observé d'effets du Pb sur les potentiels visuels évoqués. Valera et al. (2009) ont observé une augmentation de la pression sanguine en lien avec les niveaux sanguins de Pb, indépendants des effets bénéfiques du Se.

# OBJECTIFS

La présente recherche utilise une approche écosystémique pour mettre en lumière dans quelle mesure le Se peut contribuer à réduire les effets néfastes du Hg et du Pb sur la santé des riverains du Bas-Tapajós. Les objectifs spécifiques de cette recherche ont été réalisés lors de deux phases différentes du projet CARUSO :

## Phase 1 (2003-2005): Les variations saisonnières du statut de Se

1. Évaluer les variations saisonnières des niveaux de Se dans le sang et les segments séquentiels de cheveux;
2. Examiner les relations entre les niveaux de Se dans le sang et le premier centimètre de cheveux, et entre le sang et l'urine.

## Phase 2 (2006-2008): Les sources et les effets du Se sur la santé

1. Étudier les sources de Se dans l'alimentation et les relations avec le statut de Se :
    - 1.1. Mesurer les concentrations de Se dans les aliments potentiellement riches en Se et dans l'eau potable;
    - 1.2. Évaluer les variations des niveaux de Se dans ces aliments entre les communautés et en comparaison à ceux des marchés locaux;
    - 1.2. Examiner les relations entre les niveaux de Se sanguin chez les riverains et les fréquences de consommation des aliments riches en Se.
2. Étudier les effets du Se sur la santé des riverains:
    - 2.1. Évaluer la présence des signes et des symptômes de la toxicité du Se en lien avec les bioindicateurs de Se;
    - 2.2. Évaluer les fonctions motrices et examiner la performance en lien avec les bioindicateurs de Se, en tenant compte de l'exposition au Hg et au Pb et des autres covariables;
    - 2.2. Évaluer les paramètres de l'examen oculaire en relation avec les bioindicateurs de Se, l'exposition au Hg et au Pb et les autres covariables.

# POPULATIONS À L'ÉTUDE

La région d'étude est le Bassin du Bas-Tapajós, qui comprend les municipalités d'Itaituba, de Aveiro et de Rurópolis (Figure 14). Dans cette région, on dénombre près de 50 communautés dont la taille et l'origine des populations varient considérablement. Lors d'un voyage exploratoire de l'équipe de recherche du CARUSO en janvier 2003, les communautés riveraines de la région ont été caractérisées selon l'importance des activités traditionnelles de subsistance telles que la pêche, l'agriculture et l'extractivisme (fruits, plantes et animaux), les pratiques religieuses, leur statut socio-économique et l'accès aux soins de santé, à l'éducation et aux villes locales (Aveiro ou Itaituba).

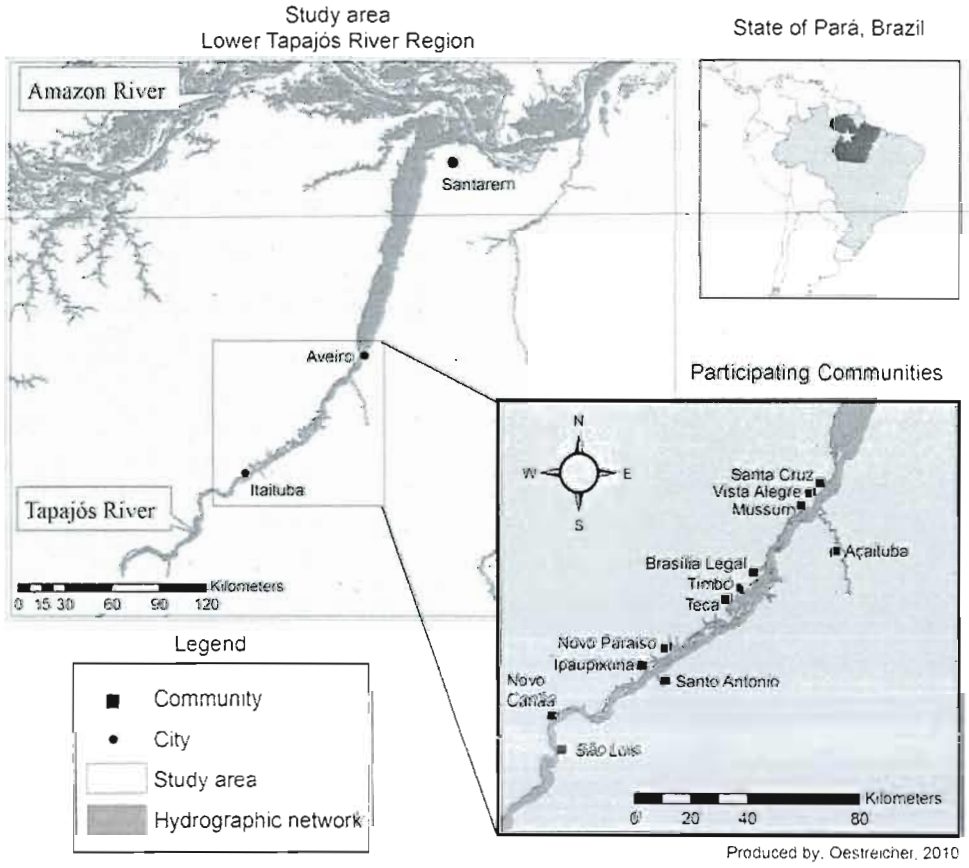


Figure 14 : Région Bas-Tapajós, Pará, Brésil

Treize de ces communautés riveraines ont ensuite été sélectionnées pour des études plus approfondies (voir les noms sur la Figure 14, excluant Brasília Legal). Ces communautés ont été choisies afin de refléter la diversité des écosystèmes aquatiques, des différents types de sols, des principales activités de subsistance, des pratiques religieuses, des conditions sanitaires, de l'accès à la route Transamazonienne, de l'année de la fondation de la communauté et de l'importance de sa composition par des habitants originaires de l'État du Pará, en comparaison de ceux provenant d'autres états brésiliens (Fillion et al., 2009; Passos et al., 2007). Six des treize communautés ont aussi été sélectionnées pour une étude préliminaire sur le statut de Se (Lemire, 2005).

Le design de la présente étude est mixte avec des composantes transversales et prospectives (Figure 15). La **Phase 1** a été réalisée en deux étapes, en 2003 et en 2004. Lors de la saison de la décrue des eaux (juin à août 2003), 236 riverains, âgés de 15 ans et plus, provenant des villages de São Luis do Tapajós (SLT), Nova Canãa (NC), Santo Antônio (SA), Vista Alegre (VA), Mussum (MU) et Açaituba (AC) ont participé à l'étude sur une base volontaire (Lemire et al., 2006). De ce nombre, 123 riverains ont de nouveau participé à l'étude lors de la saison de la crue des eaux (janvier à février 2004).

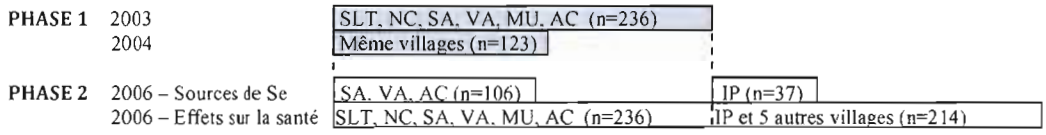


Figure 15: Schéma du design de l'étude

L'étude de la **Phase 2** a eu lieu à la saison des hautes eaux (mai à juillet 2006). L'étude des sources de Se dans l'alimentation a été réalisée auprès des villages de SA, VA et AC ainsi que celui d'Ipaupixuna (IP). Les communautés SA et IP ont

été sélectionnées parce qu'elles sont voisines, que la présence d'arbres à noix du Brésil y est importante et que les niveaux de Se sanguin chez les membres d'une famille élargie de SA étaient très élevés en 2003. Les communautés de VA et AC ont été choisies parce qu'elles sont situées dans la portion Nord du bassin du Bas-Tapajós, où les niveaux de Se sanguin étaient moyens-élevés en 2003. Les villages de VA et IP sont situés sur les berges de la rivière Tapajós alors que ceux de SA et AC sont sur des tributaires du Tapajós. L'échantillon est de 155 participants, composé de 74 femmes et 81 hommes, âgés de 15 ans et plus. L'étude des effets du Se sur la santé a été réalisée dans le cadre d'une étude plus large portant sur les facteurs pouvant influencer l'exposition sur le Hg et ses effets sur la santé. Cette étude implique 12 communautés du Bas-Tapajós (incluant Brasília Legal, mais excluant Campo Alegre et Sumaúma), dont plusieurs des habitants ont déjà été recrutés lors d'une étude précédente (Fillion et al., 2009; Passos et al., 2008). Un total de 448 personnes, composé de 216 hommes et 232 femmes, ont participé à cette étude, incluant ceux des villages SA, IP, VA et AC.

# CHAPITRE I

## Le statut de sélénium en Amazonie : les bioindicateurs de sang, d'urine et de segments séquentiels de cheveux

## *Biomarkers of selenium status in the Amazonian context: blood, urine and sequential hair segments*

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## *Résumé*

Le sélénium (Se) est un oligoélément et une déficience ou un excès de l'apport alimentaire de Se peut avoir des effets néfastes sur la santé. Des niveaux relativement élevés de Se ont été rapportés chez les riverains d'Amazonie Brésilienne. Dans cette région, l'alimentation varie de façon importante au fil des mois suivant les aliments qui sont en saison. La présente étude a été réalisée dans six communautés riveraines du Bas-Tapajós pour évaluer les variations saisonnières du statut de Se dans le sang et les segments séquentiels de cheveux (cm) et pour examiner les relations entre les niveaux de Se dans le sang et les cheveux, et entre le sang et l'urine.

Deux études transversales ont été réalisées: à la saison de la décrue des eaux (N = 259) et à la saison de la crue des eaux (N = 137), avec des mesures répétées pour un sous-groupe (N = 112). Les niveaux de Se ont été mesurées dans le sang (B-Se), les cheveux (H-Se) et l'urine (U-Se). Des analyses pairées ont été utilisées pour comparer les niveaux de Se entre les saisons et la méthode *best fit*, pour décrire les relations entre les bioindicateurs.

Les résultats de cette étude montrent une importante variation interindividuelle des niveaux de B-Se (de 142 to 2447 µg/L), sans variations particulières d'une saison à l'autre (médiane 284 et 292 µg/L respectivement). Toutefois, l'analyse séquentielle de 13 cm de segments de cheveux montre une variation significative du statut de Se dans le temps: les niveaux de H-Se étaient significativement plus bas à la saison de la décrue des eaux comparé à celle de la crue des eaux (médiane: 0.7 et 0.9 µg/g, plage d'étendue: 0.2–4.3 et 0.2–5.4 µg/g respectivement). Lors des deux saisons, les relations entre les niveaux de B-Se et de H-Se étaient linéaires et hautement significatives ($R^2$ = 67.9 et 63.6 respectivement), alors que celle entre les niveaux de B-Se et de U-Se étaient mieux décrits par une courbe sigmoïde. Le genre, l'âge, l'éducation et la consommation de cigarette n'avait pas d'influence sur les bioindicateurs de Se ou les relations entre les bioindicateurs.

Les variations temporelles de H-Se suggèrent qu'il y ait des variations saisonnières importantes des sources locales de Se. Chez les populations présentant un statut de Se hautement variable ou élevé, les analyses séquentielles de H-Se peuvent constituer un bioindicateur utile pour évaluer les variations temporelles et le statut mensuel de Se.

## *Abstract*

Selenium (Se) is an essential element and deficit or excess of dietary Se are associated with health disorders. Relatively elevated selenium levels have been reported in the Brazilian Amazon, where there are also important annual variations in the availability of different foods. The present study was conducted among six riparian communities of the Tapajós River to evaluate seasonal variations in blood and sequential hair cm Se concentrations, and to examine the relations between Se in blood and hair, and blood and urine.

Two cross-sectional studies were conducted, at the descending water ($N$ = 259) and the rising water ($N$ = 137) seasons, with repeated measures for a subgroup ($N$ = 112). Blood Se (B-Se), hair Se (H-Se) and urine Se (U-Se) were determined. Match-paired analyses were used for seasonal comparisons and the method of best fit was used to describe the relations between biomarkers.

Blood Se (B-Se) levels presented a very large range (from 142 to 2447μg/L) with no overall seasonal variation (median 284 and 292μg/L respectively). Sequential analysis of 13 cm hair strands showed significant variations over time: Se concentrations at the descending water season were significantly lower compared to the rising water season (medians: 0.7 and 0.9μg/g, ranges: 0.2–4.3 and 0.2–5.4μg/g respectively). At both seasons, the relations between B-Se and H-Se were linear and highly significant ($R^2$ = 67.9 and 63.6 respectively), while the relation between B-Se and U-Se was best described by a sigmoid curve. Gender, age, education and smoking did not influence Se status or biomarker relations.

Variations in H-Se suggest that there may be seasonal availability of Se sources in local food. For populations presenting a large range and/or elevated Se status, sequential analyses of H-Se may provide a good reflection of temporal variations and monthly Se status.

## *1. Introduction*

Selenium (Se) is an essential element, yet deficit and excess in Se status are associated with health disorders (Rayman, 2000). Se is present in a number of foods and the Se content in the food chain is highly dependent on local soil Se levels, which vary all over the world (WHO, 1986). The most important sources are fish and marine mammals, organ meat, eggs, meat, mushrooms and some comestible plants such as cereals, *Brassica sp.* and *Allium sp.* vegetables, and Brazil nuts (*Bethollethia excelsa* Humb. & Bonpl.) (reviewed in Dumont et al., 2006). However, the bioavailability of Se in a particular food varies with the digestibility of the Se-containing food proteins and the pattern of Se-amino acids, such as selenocysteine and selenomethionine (Combs Jr., 2001). Thus, Se status in humans reflects soil composition (Fordyce et al., 2000), agricultural practices, preferences and availability of foods grown in the area, food imports and the bioavailability of Se forms in the diet (Combs Jr., 2001).

Because of underlying geological variations, Se content of the same food item can have more than a 10-fold difference (U.S. Institute of Medicine, 2000). For example, in the Enshi District of China, Se-deficient and Se-toxic environments occur within 20km of each other (Fordyce et al., 2000). Consequently, estimates of the Se exposure through dietary assessment may under- or over- evaluate Se status for some populations. For this reason, epidemiologic studies rely on biomarkers rather than dietary estimates (Mayne, 2003).

Plasma and serum are the favored biomarkers for comparison of Se status among countries (Thomson, 2004). However, most studies of Se status have focused on Se-deficient or Se-adequate populations. There are few reports on the validity of biomarkers in populations with high Se. Plasma or serum biomarkers may be

inadequate for populations with high Se since plasma Se tends to saturate at whole blood Se levels between 300 and 900µg/L, while erythrocytes continue to accumulate Se (Yang et al., 1989a; Hansen et al., 2004). Urinary Se may also constitute a relevant biomarker since urine is the most important route for Se excretion (Robberecht and Deelsta, 1984) and reflects the organism's capacity to regulate and eliminate excess Se. Several authors have reported high correlations between Se in urine and Se intake, in whole blood, plasma or serum (Alaejos and Romero, 1993; Longnecker et al., 1991; 1996; Valentine et al., 1978; Yang et al., 1989b).

Recent studies in the Brazilian Amazon show highly variable and relatively elevated whole blood Se levels (Lemire et al., 2006) and hair Se levels in the upper normal range (Pinheiro et al., 2005; Soares de Campos et al., 2002). In this region, there are important annual variations in the availability of different foods (Passos et al., 2001), which may result in seasonal differences in Se-intake. In China, Yang at al. (1989b) reported that significant seasonal fluctuations in measured Se intake were not detected in whole blood Se levels while hair Se biomarker was more sensitive and may act as an excretory organ at higher intakes. Since hair grows approximately 1 centimeter (cm) a month (Robbins, 2002), segmental hair analysis can provide a retrospective profile over several months of exposure depending of the length of the hair (Cernichiari et al., 1995; Dolbec et al., 2001; Lebel et al., 1997).

The present study sought to better understand variations in biomarkers of Se in a population with a wide range of Se levels. It was conducted in the Brazilian Amazon at two different seasons with a view to: (i) evaluate seasonal variations of Se concentrations in whole blood and hair; and (ii) examine the relations between Se concentrations in hair and urine with respect to blood.

## 2. Methods

### Study design

This study is part of a larger project that uses an integrated approach to examine factors that modulate mercury transmission through aquatic ecosystems, human uptake and toxicity in the Lower Tapajós River Valley (CARUSO, 2009). In order to evaluate seasonal variations in biomarkers of exposure, the study took place over two different periods of time: (i) June and July 2003, during the descending water season (DWS); and (ii) January and February 2004, during the rising water season (RWS). A schema of the hydrologic cycle of the Tapajós River region is presented in Figure 16. Two cross-sectional studies were carried out and repeated measures were obtained for a subgroup of persons.

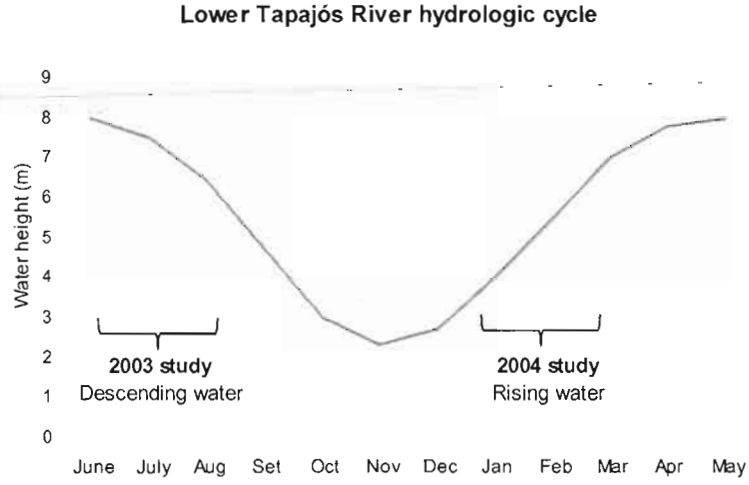


Figure 16 : Schema of the annual Lower Tapajós River hydrologic cycle and the traditionally associated seasons

Six riparian communities of the Tapajós River, one of the major tributaries of the Amazon (Figure 17) were targeted. The communities were selected so as to reflect the mosaic of the local ecosystems and diversity of riverside human

populations. The valley of the Lower Tapajós is composed of villages established on the riverbanks for more than a century, and immigrants, mostly from northeast states of Brazil, who began arriving in Amazon region in the early 1960's. The communities of São Luis do Tapajós (SLT) and Nova Canaã (NC) are located on the south of the municipality of Itaituba, on the east and west shores of the Tapajós River, respectively. The community of Santo Antônio (SA) is located on the shores of the Itapacurazinho River, a small tributary of the main stream. The communities of Vista Alegre (VA) and Mussum (MU) are neighbour and located on the west shore of the Tapajós River, close to the small municipality of Aveiro. The community of Açaítuba (AC) is located on the south shore of the Cuparí River, another tributary of the Tapajós.

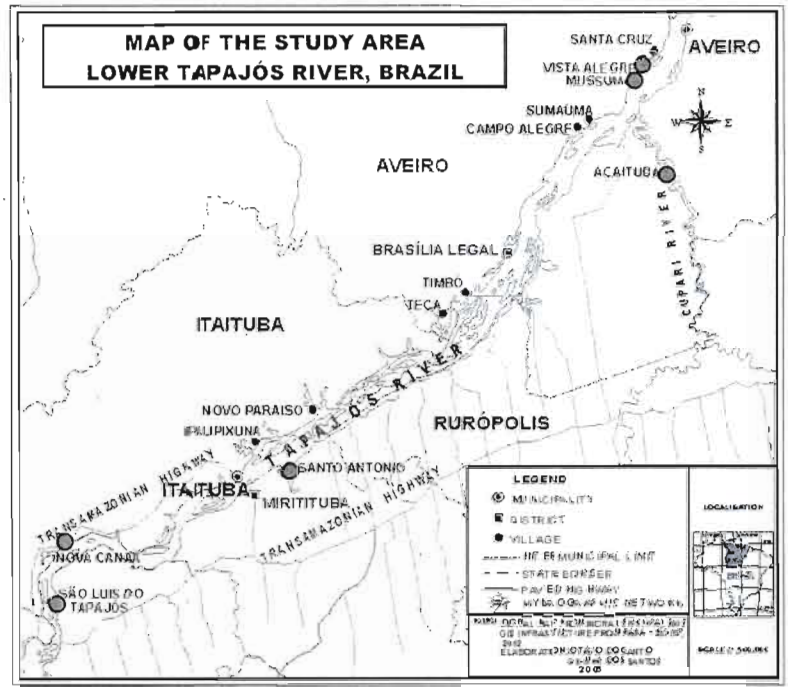


Figure 17 : Map of the study area, the Lower Tapajós River Valley
Participating communities are identified by a large dot (●)

## Recruitment

At DWS, a house-to-house socio-demographic survey was undertaken, during which the study was explained to each household and villagers were invited to participate. Meetings were also held in each community to further explain the study. Inclusion criteria for the present study were: fish-eating, age ≥ 15 years, not pregnant or breastfeeding. In all, 259 persons (39% of the total adult population of these villages) accepted to participate. Reasons provided for non-participation included time constraints, lack of interest and religious beliefs.

Six months later, during RWS, the results were returned to the communities. Village meetings were held to explain the aggregate results and house-to-house visits served to provide the confidential individual results. Villagers were likewise invited to provide further samples and respond to a questionnaire. A total of 12% of the participants had moved away from their community and because of flooding it was difficult to reach many of the houses. As RWS sampling was carried out during a period of high precipitation, we were only able to contact and re-sample 43% of the original participants. Twenty-five (25) persons asked to be included since they had not been available at DWS. At RWS there were 137 participants, with 112 persons who participated in both periods. The age and sex distribution of participants was similar to the underlying population (Lemire et al., 2006; Passos et al., 2007).

The study was approved by the Ethics Review Boards of the University of Quebec at Montreal (Canada) and the Federal University of Rio de Janeiro (Brazil). For both studies, all participants signed an informed consent form, which was read to them.

## Socio-demographic data

Socio-demographic characteristics including age, sex, smoking habits, alcohol consumption, years of education and subsistence practices (e.g. fishing, farming, etc.) were evaluated by an interview-administered questionnaire (Appendice B).

## Sampling and analyses of biomarkers of Se status

For each phase of the study, participants provided at least one of the biomarkers: (i) DWS: hair and blood; and (ii) RWS: hair, blood and urine. A nurse collected whole blood samples by venipuncture into 6mL heparinized Becton Dickinson Vacutainer® (BD7863). Spot-urine samples were collected in polypropylene bottles (Nalgene 125mL # 2104-0004) and then transferred to screw cap tubes with conical base (RPK PPGWB 15mL, SARSTEDT™) for transport purposes. All blood and urine samples were kept frozen at –20°C on the research boat and were later sent to the laboratory of the Quebec Toxicology Centre of the Quebec Public Health Institute (CTQ-INSPQ), Canada, for analysis of total Se. Collection materials were pre-screened for internal (plastic bottles and tubes) and external Se contamination. Whole blood and urine samples were analysed by inductively coupled plasma mass spectrometry (ICP–MS) on a Perkin-Elmer instrument (Elan 6000), according to the methods described by Labat et al. (2003) and Baskett et al. (1994) respectively. The detection limit for Se analysis was 7.9µg/L in solution. Urine gravity was analysed by refractrometry (Cambridge Instruments Inc. # 3461) to normalize samples for inter-individual dilutions caused by random miction sampling. Spot-urine Se results were then adjusted for specific urine gravity (SG) to the overall average gravity of the study population of 1.018g/mL [Spot-urine Se*(1.018–1)/(SG–1)]. This method was preferred to creatinine adjustment since it is less sensitive to age, gender, body size and nutritional status (Miller et al., 2004; Suwazono et al., 2005). Fifteen individuals (12%) had urine gravity under 1.010g/mL, which is

considered low, but as no individual presented highly-diluted urine samples (≤ 1.001g/mL), no one was excluded (Vahter et al., 2006). Analytical quality control was ensured by routine checks of accuracy and precision, using reference materials from CTQ-INSPQ Inter-Laboratory Comparison Programs and participation in the periodic evaluations of the same programs.

Selenium deficiency was set at whole blood selenium levels (B-Se) < 44µg/L and upper cut-offs were set 500µg/L, which corresponds to the Tolerable Upper Intake Level (UL) (U.S. Institute of Medicine, 2000) and 1000µg/L, which is the No Observable Adverse Effect Level (NOAEL) (U.S. EPA, 2002), based on the study of Yang et al. (1989a). For urine there are fewer guidelines, and the absence of symptoms of toxicity is usually associated with urine selenium levels (U-Se) < 100µg/L, while > 400 - 700µg/L are considered excessive (WHO, 2001). Here we used > 400µg/L as a high level and > 1200µg/L as a very high level.

Hair strands from the occipital region were cut next to the scalp with stainless steel scissors and then placed in plastic bags, with the root end stapled. The samples were analysed at the Geochemical Laboratories of the Earth and Planetary Sciences Department of McGill University (Canada), by hydride generation atomic absorption spectrometry (HG-AAS) on a Perkin-Elmer instrument (Analyst 100, FIAS-400 flow injection system) using sodium borohydrate solution (NaBH4 0.2% w/v and NaOH 0.05% w/v) as the hydrogen source and HCl solution (HCl 10% v/v) as the carrier stream. Hair strands were placed on a stainless steel module, standardized for 1cm length, and cut with a stainless steel scalpel. Mineralization and reduction of the samples was done using a technique adapted from Soares de Campos et al. (2002). From 5 to 23mg of hair were weighed in a 20mL beaker. Then, 0.5mL of $HClO_4$, 0.5mL of $HNO_3$, 0.5mL of $H_2SO_4$ and 0.5mL of high-purity water (Ω18 M) were added and heated at 90°C on a hotplate for 30 min with beaker covers. After cooling,

the digested sample was made up to 10 ml in a volumetric flask with high-purity water (Ω18 M). Two mL of HCl and 1mL of sulfamic acid (15% w/v) were added and left overnight. The sample was then carefully boiled for 10min to reduce Se (VI) to Se (IV). All glassware was washed with neutral detergent (Micro, model 8790-00, Cole-Parmer), rinsed twice in bi-distilled water, left in 10% HCl for 12h, rinsed twice with bi-distilled water and dried at 300°C. The detection limit for analysis was 0.2μg/L in solution. Precision and accuracy of the analytical quality control of Se determination was ensured by the use of reference material (Human Hair 086) provided by the International Atomic Energy Agency (IAEA). The accuracy of the results was checked daily by running three replicates of the reference material and variation was observed in the recovery of the reference material. Overall, mean results (± SD) for the reference material triplicates was 0.70 ± 0.13μg/g ($N$ = 65) of Se, while the reference material contained 1.00 ± 0.20μg/g. Since there were significant differences between the daily sets of analyses (Wilcoxon Rank Sums test $\chi^2$, $P$ = 0.04), segmental cm hair Se results were corrected on the basis of the mean results of the IAEA three daily replicates. The normal range for hair selenium (H-Se) concentration was considered to be between 0.1 and 5.0μg/g (WHO, 1994).

## Statistical analyses

Descriptive statistical analyses were used to characterize the study population and biomarkers of Se status at the two seasons. Gender, smoking and alcohol consumption status, subsistence practices and villages were included as categorical variables.

Biomarker differences with respect to socio-demographic variables and community groups were tested by non-parametric Wilcoxon / Kruskall-Wallis tests ($\chi^2$, $\alpha_{error}$ = 0.05). Two-by-two comparisons were used to examine differences between multiple categories, such as communities. Student's match-paired $t$-test analyses were

performed to test inter-seasonal differences for those who participated in both seasons. As most of variables did not display a normal distribution, the correlations between biomarkers of Se were examined using non-parametric correlational statistics (Spearman's $\rho$).

For the simple and multiple regression models that were used to examine the relations between Se biomarkers, and factors influencing biomarkers' variability, logarithmic transformations ($\log_{10}$) were performed for variables with non-normally distributed residuals. The general linear model (GLM) univariate procedure provided linear regression analyses and Student's *t*-test analyses were used to obtain the regression parameters.

When the relationship between biomarkers was not linear, the best-fit for a non-linear regression model was estimated using the methods of least mean square and Student's *t*-test analyses for the parameters of the regression. We assessed threshold levels below and above which the increase of both biomarkers was no longer proportional (linear). For the relation between B-Se and U-Se, three linear models appeared to be reasonable choices in the absence of information on toxicokinetic mechanisms (Wyzga, 1990). Thus, we used a sub-application of the change point problem in two-phase regression ($\chi^2$ test of one degree of freedom), considering one phase as a constant line. In accordance with this model, the maximum-likelihood technique was used. This technique was previously described in Campagna et al. (1996). Results were defined as statistically significant for a value of $P < 0.05$. Analyses were performed using JMP software, Sigma Plot and SPSS (version 5.0.1a, 6.00 and 8.02 respectively; SAS Institute Inc., Cary, NC).

## *3. Results*

Socio-demographic characteristics of the DWS study population are presented in Table 1. A large proportion (83.4%) was originally from the Tapajós region, and those who were not from the State of Pará were mostly immigrants from the northeastern states of Brazil, essentially from Maranhão (10.1%) and Ceará (2.0%). Socio-demographic data at RWS and the repeated measures subgroup had similar distributions.

Tableau 1: Socio-demographic characteristics of the study population

| | Descending water season (DWS) June – July 2003 | | |
|---|---|---|---|
| | X ± SD | *N* | % |
| Women | | 124 | 48.1 |
| Men | | 135 | 51.9 |
| Age (years) | 35.7 ± 15.9 (15.0 – 89.0) | | |
| Education (years) | 3.6 ± 2.6 (0 – 11) | | |
| Born in the region (State of Pará) | | 216 | 83.4 |
| Current alcohol consumer | | 99 | 38.2 |
| Current smoker | | 77 | 29.8 |
| Fisher [a] | | 182 | 70.2 |
| Farmer | | 159 | 61.4 |

[a]124 participants (47.9 %) were both fisher and farmer.

The distribution of whole B-Se, Se in the first cm of hair ($H_1$-Se) and U-Se are presented in Table 2. For both seasons, no individuals showed B-Se or $H_1$-Se deficiency and both biomarkers presented a large inter-individual variation. At DWS, 24 participants (10.2%) had B-Se levels above 500µg/L and 10 participants (4.2%) had B-Se levels over 1000µg/L. No one had $H_1$-Se levels above 5µg/g. At RWS, 15 participants (12.7%) presented B-Se levels above 500µg/L and 5 persons (4.2%) above 1000µg/L. Two participants (1.6%) had $H_1$-Se levels higher than 5µg/g. For both seasons, all of those who presented the highest Se levels (B-Se levels >

1000µg/L and $H_1$-Se levels > 5µg/g) were part of an extended family from SA. Their mean hair and blood Se levels were significantly higher (Wilcoxon / Kruskall-Wallis test $\chi^2$, $P < 0.0001$) than those of the rest of the population (DWS B-Se: 1156 ± 617µg/L vs. 305 ± 121µg/L and $H_1$-Se: 3.1 ± 1.3µg/g vs. 0.9 ± 0.4µg/g ; RWS phase B-Se: 1183 ± 466µg/L vs. 313 ± 125µg/L and $H_1$-Se: 2.6 ± 1.0µg/g vs. 0.8 ± 0.3µg/g). We thus considered the extended family separately from the others of this village: SA-1 and SA-2 (extended family).

Tableau 2: Selenium biomarkers levels for both phases of the study

| | Biomarkers [a] | | |
|---|---|---|---|
| | B-Se (µg/L) | $H_1$-Se (µg/g) | U-Se (µg/L) |
| Descending water season (DWS) June – July 2003 | | | |
| X ± SD | 361 ± 259 | 0.9 ± 0.5 | |
| Median | 284 | 0.8 | |
| Range | 142 – 2029 | 0.3 – 4.1 | |
| *N* | 236 | 235 | |
| Rising water season (RWS) January – February 2004 | | | |
| X ± SD | 394 ± 340 | 1.1 ± 0.8 | 220 ± 355 |
| Median | 292 | 0.9 | 85 |
| Range | 142 – 2447 | 0.3 – 5.4 | 29 – 2579 |
| *N* | 118 | 127 | 125 |

[a] Whole blood Se (B-Se), first hair cm Se ($H_1$-Se) and urine Se (U-Se) concentrations

U-Se levels presented a larger inter-individual variation, up to 100-fold, compared to B-Se and $H_1$-Se. Sixty-nine participants (55.2%) had U-Se levels < 100µg/L. However, 14 individuals (11.2%) had high U-Se levels, varying between 436 – 1184µg/L, which included not only persons from SA-2 but also participants from SA-1 and AC. Two individuals had extremely high U-Se levels (1954 and 2579µg/L respectively) and came from the community of AC and MU.

Blood, hair and urine biomarkers did not vary with gender, age, years of education and smoking habits (Wilcoxon / Kruskall-Wallis test $\chi^2$ and Spearman's ρ, $P > 0.05$). At DWS, alcohol consumers had significantly higher B-Se (Wilcoxon / Kruskall-Wallis test $\chi^2$, $P = 0.01$), but no relation was observed for $H_1$-Se. At RWS, no relation was observed between alcohol intake and B-Se, $H_1$-Se or U-Se. For both seasons, farmers had consistently higher Se biomarker levels (Wilcoxon / Kruskall-Wallis test $\chi^2$, $P < 0.007$), while no difference was observed for fishing practices.

All Se biomarkers presented significant inter-community differences (Wilcoxon / Kruskall-Wallis test $\chi^2$, $P < 0.0001$). Interestingly, except for SA-2 whose Se biomarkers remained consistently higher than other communities, two-by-two community comparisons showed that certain communities had higher Se levels compared to others. These differences were observed for all biomarkers (Wilcoxon / Kruskall-Wallis test $\chi^2$, $P < 0.05$). At DWS, the communities AC, VA, MU, and SA-1 had higher Se status than SLT, whose Se levels were lower but above NC. At RWS, the community AC had Se levels above VA, MU, SA-1 and NC, and SLT presented the lowest Se status (data not shown).

Inter-seasonal comparisons showed that, although total population $H_1$-Se levels were significantly higher at RWS (Mean difference 0.15µg/g, CI $_{95\%}$ [0.04 – 0.25]; Student's paired *t*-test, $P = 0.004$), B-Se levels did not change significantly (Mean difference 14.0µg/L, CI $_{95\%}$ [-15.0 – 43.1]; Student's paired *t*-test, $P = 0.341$) (Table 3). Within communities, B-Se and $H_1$-Se seasonal variations were in the same direction but $H_1$-Se seasonal differences were more pronounced for most communities compared to those of B-Se. Mean hair to whole blood ratio (µg/g vs. mg/L) were 2.67 ± 0.77 at the DWS and 2.88 ± 0.96 at RWS, and did not differ between seasons (Student's paired *t*-test, $P = 0.216$). At RWS, mean urine to whole blood Se ratio (µg/L vs. µg/L) was 0.47 ± 0.57.

Tableau 3 : *T*-test results of match-pair analysis for DWS (June and July 2003) to RWS (January to February 2004) variations of selenium biomarkers

| Village | B-Se [a] | | $H_1$-Se [a] | |
|---|---|---|---|---|
| | Results [b] | *N* | Results [b] | *N* |
| SLT | $\downarrow^{**}$ | 27 | $\downarrow^{**}$ | 23 |
| NC | $\uparrow^{*}$ | 10 | $\uparrow^{*}$ | 12 |
| SA-1 | $\uparrow^{\dagger}$ | 13 | $\uparrow^{**}$ | 13 |
| SA-2 | ns | 10 | $\uparrow^{\dagger}$ | 8 |
| VA | ns | 10 | $\uparrow^{**}$ | 12 |
| MU | ns | 9 | ns | 14 |
| AC | $\uparrow^{**}$ | 14 | $\uparrow^{*}$ | 18 |
| Subgroup population | ns | 93 | $\uparrow^{**}$ | 100 |

[a] Whole blood Se (B-Se) and first hair cm Se ($H_1$-Se) concentrations
[b] $^{**}$: $P < 0.01$; $^{*}$: $P < 0.05$; $^{\dagger}$: $P < 0.10$; ns : non-significant results.

For 56 women, we were able to analyse a length of 13 cm of hair, cut into centimeters. Segmental H-Se monthly profiles of the women's hair by community are presented in Figure 3. H-Se levels of the SA-2 family were higher and significantly different from the rest of the study population over the whole year, from January 2003 to January 2004 (Wilcoxon / Kruskall-Wallis test $\chi^2$, $P < 0.0001$). For some communities there were too few women to statistically examine inter-community differences, but visual observation suggest that H-Se levels were higher at the beginning of the rainy season (from January to March 2003) and were lower and presented less inter-community variations during the dry season (from June to December 2003); there was a slight increase again in January 2004. Two individuals from the SA-2 family showed H-Se levels above 5.0µg/g, one from January to June 2003 and the other during January and February 2003, reaching 7.1µg/g.

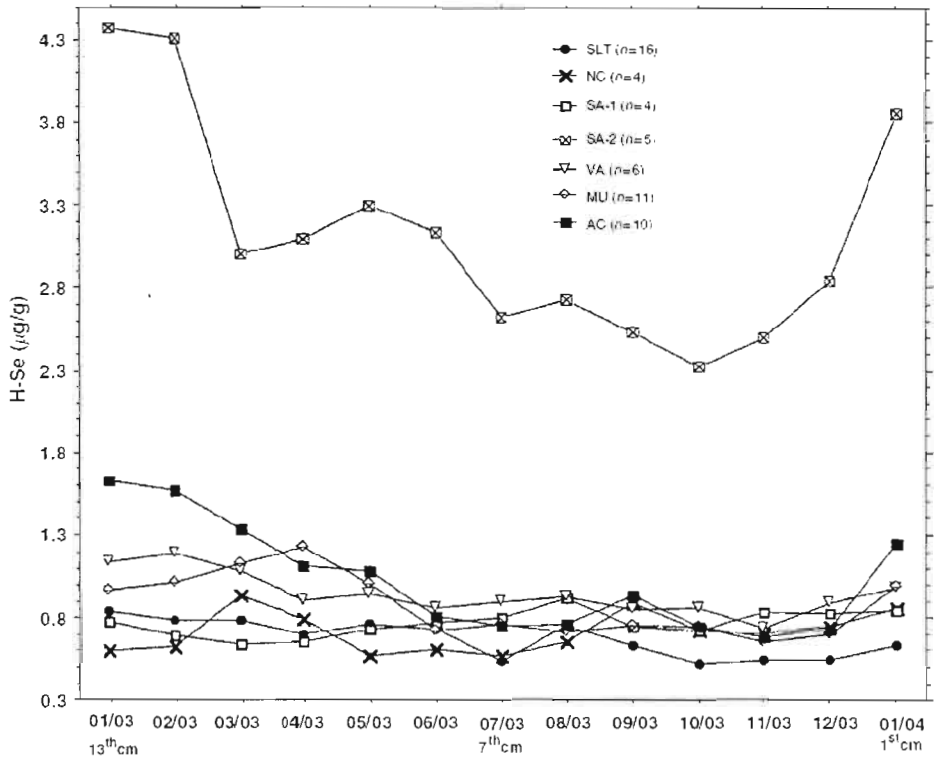


Figure 18 : Sequential H-Se analysis for 13 cm of 56 women hair strands. Dots represents the community mean results for each month of analyse.

Paired comparisons (Student's match-pair *t*-test) of $H_I$-Se from DWS, carried out in June and July 2003, to the corresponding months of the sequential H-Se results of the women's hair (mean of June and July 2003 results), showed no difference ($P = 0.43$, $N = 42$). However, two by two pair analyses of the sequential hair concentrations, showed that June 2003 results were significantly lower compared to January 2004 (Student's match-paired *t*-test $P = 0.001$), which is in the same direction as the results of $H_I$-Se seasonal variations presented in Table 5. The H-Se concentrations from June 2003 were also significantly lower than January 2003 (Student's paired *t*-test, $P < 0.0001$), and the same trend was observed for January 2004, where concentrations tended to be slightly lower than January 2003 results (Student's paired *t*-test, $P = 0.075$).

Figure 19a and 19b presents the seasonal relations between whole blood and the previous month's hair Se biomarkers. The slopes of the linear regression model, for $\log_{10}$ transformed B-Se and $H_1$-Se variables, were similar for both seasons ([f = ax+b]; DWS: a = 0.799, $P < 0.0001$; and RWS: a = 0.800, $P < 0.0001$), even when the SA-2 family is excluded (DWS: a = 0.748, $P < 0.0001$; and RWS: a = 0.624, $P < 0.0001$). At DWS, B-Se levels explained 67.9% of the $H_1$-Se variation and at RWS, 63.6% of the $H_1$-Se variation. Spearman's correlations between B-Se and $H_1$-Se were also highly significant for both seasons ($\rho = 0.737$, $P < 0.0001$ and $\rho = 0.663$, $P < 0.0001$ respectively). The relation between whole blood and urine Se levels at the RWS is shown in Figure 20. The best non-linear relation between B-Se and U-Se was described by a three parameter sigmoid curve ($[f = a/(1+\exp(-(x-x_0)/b))]$; a = 7.435, $P < 0.0001$; b = 1.100, $P = 0.002$; $x_0 = 5.205$, $P < 0.0001$; $R^2 = 0.500$). The assessment of a threshold B-Se value at the lower part of the curve, for which no U-Se increase (plateau), was statistically detectable and estimated at 212µg/L of B-Se (corresponding to 49µg/L of U-Se). This estimate was highly significant ($\chi^2 = 11.2$, $P < 0.0001$; CI $_{95\%}$ [< 142-295µg/L]). At the upper part of the curve, a "pseudo-plateau" was estimated at 601µg/L of B-Se (corresponding to 377µg/L of U-Se). This estimate was also highly significant ($\chi^2 = 30.8$, $P < 0.0001$; CI $_{95\%}$ [< 426-988µg/L]). Between 212 and 601µg/L of B-Se (49 and 377µg/L of U-Se), U-Se levels increased linearly with B-Se levels. Nineteen participants from SLT, NC, MU and VA (18%) had B-Se levels ≥ 142 and < 212µg/L, 74 participants (69%) had ≥ 212 and < 601µg/L (from all communities) and 14 participants (13%) from SA-2, SA-1, AC had ≥ 601µg/L. Participants' gender, age and education, as well as alcohol and smoking status, and farming and fishing practices did not influence blood-hair and blood-urine relations.

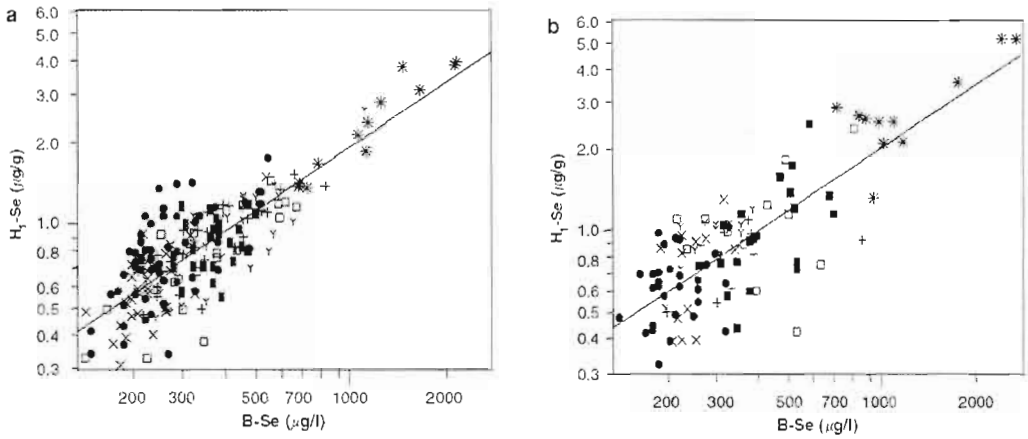


Figure 19 : (a) Relation between $\log_{10}$ B-Se and $\log_{10}$ $H_1$-Se biomarkers at the descending water season ($N = 212$); (b) Relation between $\log_{10}$ B-Se and $\log_{10}$ $H_1$-Se biomarkers at the rising water season ($N = 110$)

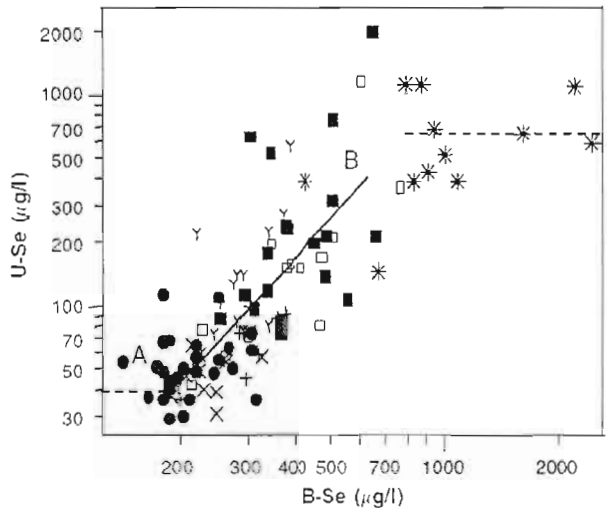


Figure 20 : Relation between $\log_{10}$ B-Se and $\log_{10}$ U-Se biomarkers at the RWS. The strait line between the cut-offs A (212µg/L, 49µg/L) and B (601µg/L, 377µg/L) represents the segment of the regression where the relation between B-Se and U-Se is linear. Pointed lines represent the two 'pseudo-plateau' evaluated by the sigmoid curve model ($N = 107$).

Legends of the Figures 4 and 5: Different dots represented the studied villages: STL: São Luis do Tapajós ( ), NC: Nova Canaã ( ), SA-1: Santo Antônio ( ), SA-2: Extended family of Santo Antônio ( ), VA: Vista Alegre ( ), MU: Mussum (+), AC: Açaítuba ( ).

## *4. Discussion*

In this region of the Amazon, all biomarkers of Se are relatively high with important seasonal and inter-community variations. High Se status is not common in human populations and is generally associated to staple food consumption from seleniferous regions of the world like the Enshi District in China (Yang et al., 1989b), Nawan Shanhar District in India (Hira et al., 2004), North Dakota and Wyoming States in U.S.A. (Longnecker et al., 1991) and Portuguesa province in Venezuela (Bratter et al., 1991). For Inuit populations from Nunavik in Canada (Muckle et al., 2001) and Greenland (Hansen et al., 2004), high Se status has been related to consumption of marine mammals. Most cases of selenosis have been reported from the Enshi District of China (Yang et al., 1983; 1989a), where nail deformations were observed at B-Se concentrations ≥1054µg/L, which are similar to the upper levels observed in the present study. In the present study, over 10% of participants presented B-Se levels above 500µg/L, which corresponds to the Tolerable Upper Intake Level (U.S. Institute of Medicine, 2000). However, a recent study on long-term Se supplementation and the incidence of Type 2 Diabetes suggests that this level may be too high (Stranges et al., 2007).

Overall H-Se results from the present study are in the same range as the H-Se levels of non-pregnant women from 3 villages of the Tapajós River (mean 2.5µg/g, range 0.9 – 5.7µg/g) reported by Pinheiro et al. (2005). This latter study did not specify the months of data collection, which could be important since our results indicate important monthly fluctuations of Se status in the region. No previous data were available for U-Se levels in the Brazilian Amazon.

In the present study, the relations for both seasons between whole blood Se and the 1st centimeter of hair Se were highly similar; the slope of the regressions were comparable and hair to blood ratios remained constant. Yang et al. (1989b) observed a similar whole blood – hair Se correlation for individuals with low to extremely high Se status. However, mean hair to blood ratios measured in the present population (2.67 and 2.88) were lower compared to results from Se-adequate and Se-high environments in China, which we estimated from their published data to be between 3.79 and 10.75. In the China studies, B-Se ranged from 95 to 3200µg/L (Yang et al., 1983; 1989b). There are several possible explanations for these differences: in the present study, the 1st centimeter of hair was used to calculate the ratio while for the China studies, no information was provided about the number of centimeters that were considered. Other factors such as lower protein status and external exposure through Se-rich coal smoke in China (Yang et al., 1983), different bioavailability of Se in food sources, individual requirements and concomitant exposure to other metals such as mercury may explain the lower excretion ratio measured in the Amazon Tapajós riverside population.

According to several authors, urine excretion (24h) is highly and linearly related to Se-intake and plasma Se concentration (Alaejos and Romero, 1993; Yang et al., 1989b). In the present study, the range of Se exposure was large, and the relation between whole blood and urine Se was best described by a sigmoid curve with two thresholds. There was a significant linear correlation between the lower and upper cut-offs at B-Se levels of 212µg/L and 601µg/L. Since Se is an essential element, it is probable that at lower levels, homeostatic mechanisms retain Se in blood, while at normal levels, Se is excreted proportionally to its concentration in blood, and as blood levels further increase, excretory mechanisms may saturate so that excess Se remains in the blood. This upper level may be a threshold for possible Se toxicity, but this requires further study. On the other hand, the relation between blood Se and hair

Se remained linear; suggesting that hair Se would constitute a better biomarker for Se body burden.

Inter-seasonal differences suggest that sequential analyses for H-Se may be more sensitive to seasonal fluctuations in Se status than B-Se. Indeed, although B-Se and H-Se were strongly correlated, H-Se was more sensitive to seasonal differences. This may be due to higher variability in B-Se compare to H-Se, which reflects a longer time period. Yang et al. (1989b), who showed that the consumption of unusually high Se food can occasionally and strongly influence B-Se content (bolus dose), suggest that as global Se-intake gets higher, whole blood is in general a less sensitive biomarker than hair, nails and urine. Considering the seasonal variations of Se food sources, even if intra-hair growth is variable and hair growth varies between individuals (Harkins and Susten, 2003), sequential hair analysis can provide relevant information on overall monthly Se status.

The Environmental Health Criteria of the International Program on Chemical Safety (WHO, 1986) has not set reference values for hair and urine Se. U-Se may be a useful biomarker to assess very recent intake, but there are several limitations: incomplete urine collection (< 24h) may not provide valid information about the Se status (WHO, 1986); the expression of U-Se per unit of volume of urine or adjustment techniques are not consistent between studies; and urine Se excretion is highly susceptible to variation (Yang et al., 1983) mostly because of current individual Se status, recent consumption of food with high Se content and bioavailability and/or interfering compounds in the diet (Robberecht and Deelstra, 1984).

It has been suggested that in circumstances where external Se contamination can be excluded, such as medicated shampoos and cosmetics (Senofonte et al., 2000; Leblanc et al., 1999), H-Se content could be a useful biomarker to assess Se status (WHO, 1986). Indeed, as a biomarker, hair has notable advantages compared to other media: sampling is less invasive; hair can be easily stored for long periods and transported; it can provide information over an extended period of time; it contains one order of magnitude more Se (μg/L vs. μg/g); and it can provide an integrated measure, reflecting total body intake better than the more common biomarkers such as plasma and urine (Senofonte et al., 2000; Perreira et al., 2004). In this study, we saw no benefit in washing samples in a pre-treatment step before digestion because of inconsistent data on washing methods, which can extract endogenous hair Se or fail to remove external contamination (Leblanc et al, 1999; Morton et al., 2002). None of the participants used medicated shampoo containing Se disulfide, one of the most common sources of external Se contamination, whose long-term use can increase individual H-Se levels by more than 100-fold (Leblanc et al, 1999).

For the population studied here, in general, H-Se status was consistently and significantly higher at the rising water season. However, within communities, seasonal variations were not necessarily in the same direction, which is probably related to local food availability and consumption patterns. In the Tapajós region, there are more than a hundred fish species, whose predominance varies over short distances and with seasons. Furthermore, Se content varies between Amazonian fish species and ecosystems (Dorea et al., 1998; Lima et al. 2005). There are also important seasonal variations in the availability of fruits and vegetables (Passos et al., 2001). Farmers present consistently higher Se status for both seasons, suggesting that there may be multiple local Se sources in the Tapajós ecosystems. Brazil nuts (*Bertholletia excelsa)* constitute an important potential source of dietary Se (Chang et al., 1995), but are not available throughout the year; the mature nut capsules usually

fall from the trees from December to April, which corresponds to the RWS. In addition, the distribution of Brazil nut trees is not uniform throughout the Tapajós Valley. This nut is an excellent source of protein and fat (Chunhieng et al., 2004) and nuts can be stored for further consumption through the year. Most of those with the highest levels of Se live in an area surrounded by over 300 Brazil nut trees. We are currently examining Se content in local foods.

The findings of this study suggest that for populations with high Se status, $H_1$-Se may provide a more integrated measure of Se status than B-Se since it is less sensitive than plasma, whole blood or urine Se to recent consumption of food with high Se content. Segmental H-Se may be a good biomarker of Se status for public health surveys and epidemiologic studies, particularly in remote areas where blood sampling and storage is difficult and in areas where there are important seasonal variations in diet. In addition, segmental H-Se can be a useful biomarker to provide a retrospective profile of the past Se status and its monthly or seasonal variations. Further studies should examine the relations between Se intake and all of the Se biomarkers, as well as between biomarkers and health outputs.

# CHAPITRE II

## Niveaux élevés de sélénium dans l'alimentation quotidienne des populations riveraines d'Amazonie

## *Elevated levels of selenium in the typical diet of Amazonian riparian populations*

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## *Résumé*

Dans les écosystèmes ruraux, l'apport de sélénium (Se) vient surtout des aliments, notamment les poissons, les abats et la viande de boeuf, le poulet, les œufs et certaines plantes commestibles comme les *Brassica sp.* et les *Allium sp.*, et les noix du Brésil (*Bethollethia excelsa*). Les niveaux de Se dans ces aliments varient selon les niveaux de Se présents dans les sols et la capacité des plantes à accumuler le Se. Pour les humains, un apport adéquat de Se est essentiel à l'activité optimale des sélénoenzymes, impliquées entre autres dans la protection contre le stress oxydatif. Dans la région du Bas-Tapajós en Amazonie brésilienne, le statut de Se est élevé et varie de façon importante entre les communautés et les saisons. La présente étude a été réalisée dans quatre communautés du Bas-Tapajós pour mesurer les concentrations de Se dans les aliments et dans l'eau potable, pour évaluer les variations des niveaux de Se dans ces aliments selon la localisation géographique et pour examiner les relations entre les niveaux de Se sanguin chez les riverains et les fréquences de consommation des aliments riches en Se.

Un total de 155 personnes a participé à l'étude. Ces personnes ont fourni plus de 400 échantillons d'aliments locaux et 40 échantillons d'eau potable. Elles ont répondu à un questionnaire sur les fréquences de consommation alimentaire et fourni un échantillon de sang. Les aliments du marché consommés par les riverains ont aussi été échantillonés. Les niveaux de Se dans les aliments, dans l'eau potable et dans le sang ont été mesurés par ICP-MS. Puisque les noix du Brésil peuvent également contenir des niveaux élevés de Ba et Sr, ces deux éléments ont aussi été analysés par ICP-MS dans les noix échantillonés.

Les concentrations les plus élevées de Se ont été mesurées dans les noix du Brésil (*Bertholletia excelsa*). Leur teneur en Se était aussi hautement variable, 0.4 à 158.4μg/g. D'autres aliments comme le poulet, la viande de chasse, les œufs et le bœuf avaient des niveaux élevés de Se (0.3–1.4μg/g), alors que le borécole, les patates douces, le riz et les *pupunha* avaient des niveaux plutôt moyens de Se (0.1–0.2μg/g). De façon générale, les aliments riches en Se qui proviennent des communautés renfermaient plus de Se que ceux des marchés locaux. Toutefois, selon le type d'aliment, les niveaux de Se ne suivaient pas les mêmes profils de variation entre les communautés. Les niveaux de Se dans l'eau potable étaient négligeables (< 1.4μg/L). Les niveaux de Se dans le sang étaient hautement variables (103–1500μg/L), et positivement associés à la consommation de certains aliments locaux tels que les noix du Brésil, le poulet domestique et la viande de chasse. Les noix du Brésil de cette région contenaient aussi des niveaux hautement variables, et dans certains cas, très élevés de Ba (1.9–1437 μg/g) et de Sr (3.3–173μg/g).

Chez cette population fortement exposée au Hg, un apport alimentaire élevé de Se pourrait jouer un rôle important pour atténuer certains effets toxiques du Hg. Plus d'études dans cette région sont nécessaires afin de mieux connaître les relations entre les bioindicateurs de Se, de Hg et les effets sur la santé.

## *Abstract*

In rural ecosystems, selenium (Se) intake is generally from foods, notably fish, organ meat and meat, chicken, eggs, and some edible plants such as *Brassica sp.* and *Allium sp.*, and Brazil nuts (*Bethollethia excelsa*). Se content depends on soil Se concentration and plant accumulation. For humans, adequate Se intake is essential for selenoenzymes, which are involved in many functions and processes, including protection against oxidative stress. In the Lower Tapajós region of the Brazilian Amazon, Se status is elevated with large variability between communities and seasons. The present study was conducted in four communities of the Lower Tapajós region to evaluate Se content in their typical diet and drinking water, and to examine relations between food Se concentrations and geographic location, whole blood Se status and consumption frequencies of Se-rich food.

A total of 155 persons participated in the study. They provided more than 400 local foods and 40 drinking water sources samples, and responded to an interview-administered food frequency questionnaire. Market foods consumed by the participants were also sampled. Blood samples were taken. Food Se content, drinking water Se levels and blood Se levels were assessed by ICP-MS. Since Brazil nuts may also contain significant levels of barium (Ba) and strontium (Sr), these elements were likewise analyzed by ICP-MS analyses in nuts.

The highest Se concentrations in foods were observed for Brazil nuts (*Bertholletia excelsa*), whose levels were highly variable, from 0.4 to 158.4μg/g. Foods such as chicken, game meat, eggs and beef contained considerable levels of Se (0.3–1.4μg/g), while kale, sweet potato, rice and *pupunha* had somewhat lower levels (0.1–0.2μg/g). There were important geographic variations of food Se concentrations and those with higher Se content were not necessarily from the same community. In general, Se-rich food from the communities had higher Se content than market food. Se levels in drinking water were very low (< 1.4μg/L). Blood Se levels presented a very large range (103–1500μg/L), and were positively related to regular consumption of some local foods: Brazil nuts, domestic chicken and game meat. Brazil nuts from this region likewise contain highly variable and, in some cases, very high concentrations of Ba (1.9–1437 μg/g) and Sr (3.3–173μg/g).

In this population, highly exposed to Hg, Se intake may possibly play a role in offsetting some of the deleterious effects of Hg. Further studies should examine the relations between biomarkers of Se, Hg and health outcomes in this region.

## 1. Introduction

In rural ecosystems, Se intake is generally from foods such as fish, organ meat and meat, chicken, eggs and some edible plants such as *Brassica sp.* and *Allium sp.*, and nuts from the Lecitidaceae family, including Brazil nuts (*Bethollethia excelsa* H & B) and *sapucaia* nuts (*Lecythis usitata* Miers). Se content of these foods depends on soil Se concentration and bioavailability as well as plants' ability to accumulate Se from soils (Andrade et al., 1999; Combs Jr., 2001; Fordyce, 2005). Adequate Se intake is essential for several selenoenzymes involved in protection against oxidative stress, redox status, immune and thyroid regulation (Reeves and Hoffmann, 2009).

Along the Tapajós River, in the State of Pará of the Brazilian Amazon, the Se status of riverside populations ranges from normal to very high and presents significant variations between villages and seasons (Lemire et al., 2006; 2009). The highest Se levels were observed among persons who consume large amounts of Brazil nuts (up to 2447µg/L in whole blood), particularly during the Brazil nuts season, from December to April, when the mature nut capsules fall from the trees (Lemire et al., 2009). When Brazil nuts are not in season, the Se status of the villagers remains in the upper normal range, suggesting that other local sources of Se are present in their diet (Lemire et al., 2006, 2009). Some authors have suggested that soil Se levels in the Eastern Amazon (State of Pará and Amazonas) may be high compared to the other areas of the Amazon (Chang et al., 1995), however, no information is available regarding the soil Se content in the Lower Tapajós region.

Subsistence activities along the Tapajós include fishing, farming, cattle raising, hunting, and gardening, as well as plant, fruit and nut extrativism (Farella et al., 2007). For inhabitants of this region, fish is an important dietary mainstay (Lebel et al., 1996; Passos et al., 2001). Manioc, rice, beans and fruits are staple foods, and,

at times, meat, chicken, eggs, game meat and vegetables complete the daily food intake, depending on the local availability (Passos et al., 2001). While Se content in fish may be high, it varies markedly between species and fish habitat (Dorea et al., 1998; Sampaio da Silva, *personal communication*). In fruit and vegetables, Se concentration is generally low, although some native palm species such as coconut (*Cocos nucifera* L.) and *pupunha* (*Bactris gasipaes*), and root vegetables may contain substantial amounts (Aleixo et al., 2000; Yuyama et al., 2003, Fordyce, 2005; Paiva Oliveira et al., 2005). In most areas of the globe, drinking water Se concentrations rarely exceed 10µg/L, the drinking water standard for Se of the World Health Organization (WHO, 1996). However, in Se rich-areas, water Se may be as high as 160µg/L (Yang et al., 1983).

Brazil nut Se content is extremely variable depending of the region of origin, from 0.03 to 512.0µg/g, and can likewise contain highly variable levels of barium (Ba) and strontium (Sr), as high as 4000µg/g and 115µg/g respectively (Lisk et al., 1988; Chang et al., 1995; Pacheco and Scussel, 2007; Parekh et al., 2008; Welna et al., 2008).

The present study was conducted in four communities of the Lower Tapajós region. The objectives were to evaluate Se content in their typical diet and drinking water, to determine the geographic distribution, and to examine the relations between consumption frequencies of Se-rich food and whole blood Se status. Ba and Sr content in Brazil nuts were also assessed.

## 2. Methods

### Study population

This cross-sectional study is part of a larger project on factors that affect human mercury exposure and its health effects. The study targeted villagers aged 15 years and older from 4 communities along the Lower Tapajós River Basin (State of Pará, Brazil), illustrated in Figure 21. Based on our previous studies (Lemire et al., 2006; 2009), the targeted communities were selected to represent a wide range of Se status and Brazil nut availability and consumption. Ipaupixuna (Ip) and Santo Antônio (SA) are located on the southern part of the Lower Tapajós Basin, while Vista Alegre (VA) and Açaituba (Ac) are located on the more northerly portion. VA and Ip are located directly the shore of the Tapajós River, while SA and Ac are located on the shore of Itapacurazinho and Cupari affluent rivers. Recruitment was carried out in each village through house-to-house visits and at village meetings, during which the research project was explained, and villagers were invited to participate on a voluntary basis. The persons who accepted to participate (N = 155) provided food and drinking water samples.

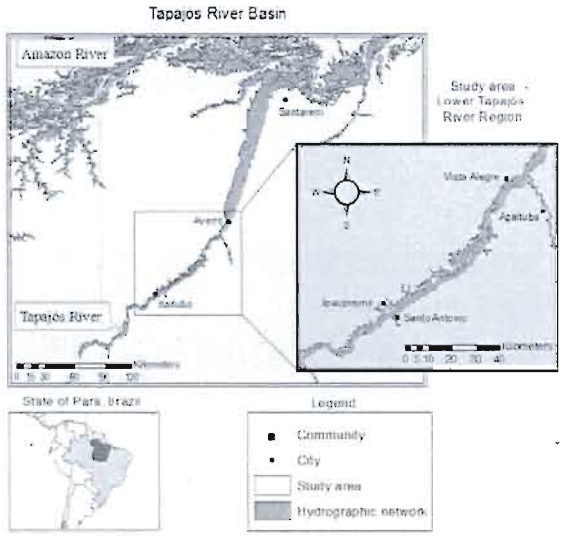


Figure 21 : Map of the study area, the Lower Tapajós River Valley.

Blood sampling was carried out at a technical college in the nearby city of Itaituba. General exclusions for the present study were pregnant (N = 1) and breastfeeding women (N = 8), and persons reporting cerebrovascular accidents (N = 3). A total of 143 participants (65 women and 78 men), were included in the present analyses.

The study was approved by the Ethics Review Boards of the University of Quebec at Montreal and the Faculty of Pharmaceutical Sciences of the University of São Paulo – Ribeirão Preto. All participants signed an informed consent form, which was read to them. There was no remuneration for study participation.

## Food samples and drinking water collection and analysis

All foods produced by the villagers that could potentially be a good source of Se were sampled from a maximum of households in each village. Food that villagers bought at local food markets and grocery stores were also sampled; when applicable, different brands of these foods were purchased in Itaituba, the nearby city. Food samples were collected before cooking, with decontaminated stainless steel scalpels, and stored in sterilized Sarstedt® 15 ml tubes or freezer-type Ziplock® bags.. In each community, a maximum number of water drinking sources were sampled (open well, artesian well, lake and river, two replicas and two blanks) and stored in 250mL Nalgene 1-Chem® (N319-0250) bottles, with 5% of HCl (12M). All food and water samples were kept frozen at –20°C on the research boat and were later sent to the *Laboratório de Toxicologia e Essencialidade de Metais*, University of São Paulo, Ribeirão Preto (Brazil), for analysis of total Se. Brazil nuts were kept in their shell until analysis and were also analyzed for Ba and Sr content.

Food samples (0.10–0.25g) were weighed, and then 3mL of nitric acid were added and heated overnight at 80°C. On the next day, 1mL of nitric acid, 1mL of

concentrated hydrogen peroxide and 1mL of magnesium chloride (10%) were added and heated for 2h at 150°C. Following this procedure, the samples were cooled and their volume was made up to 50mL with Milli-Q water in sterilized 50mL Falcon® tubes. Then, rhodium was added as internal standard to a final concentration of 10μg/L. For total Se, Ba and Sr determination in food or water samples, an ICP-MS (Elan DRC II, PerkinElmer, Norwalk, CT) was used.

All reagents used were of analytical-reagent grade, except $HNO_3$ which was purified in a quartz sub-boiling stills (Kürner) before use. A clean laboratory and laminar-flow hood capable of producing class 100 air were used for preparing solutions. High purity de-ionized water (resistivity 18.2 mΩ cm) obtained using a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used throughout. Plastic bottles, auto-sampler cups and glassware materials were cleaned by soaking in 10% (v/v) $HNO_3$ for 24 h, rinsing five times with Milli-Q water and dried in a class 100 laminar flow hood before use. Multi-element Stock solutions containing 1000mg/L of each element were obtained from Perkin-Elmer (PerkinElmer, Norwalk, CT). Analytical calibration standards were prepared daily over the range of 0–20μg/L for all elements by suitable serial dilutions of multi-element stock solution in 2% (v/v) $HNO_3$. Rhodium stock solution, 1000mg/L, was obtained by Perkin-Elmer (PerkinElmer, Norwalk, CT, USA). Two Standard Reference Materials from the National Institute of Standards and Technology (NIST) (Whole Egg Powder RM 8415, Rice Flour SRM 1568a) were analyzed before and after ten ordinary samples. A Standard Reference Material from the National Institute of Standards and Technology (NIST) SRM 1640 Trace Elements in water was analyzed for the validation of Se levels in water samples. Measured values were always within the provided reference or certified values. The value of 0.0025μg/g was given to food samples with Se content under the detection limit (0.005μg/g). All drinking water Se values under the detection limit (0.1μg/L) were set at 0.05μg/L.

## Blood sample collection and analysis

For each participant, an experienced Brazilian phlebotomist collected a 6mL blood sample in "trace metal free" evacuated tubes (BD Vacutainer®). Blood Se (B-Se) was determined by inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer DRC II) according to the method proposed by Batista et al. (2009), at the *Laboratório de Toxicologia e Essencialidade de Metais*, University of São Paulo, Ribeirão Preto, SP, Brazil. Quality control was guaranteed by analyzing various secondary reference materials provided by the New York State Department of Health's Proficiency Testing program for trace elements in whole blood, and by the external quality assessment scheme (EQAS) for trace elements operated by the *Institut national de santé publique du Québec*, Canada. Reference materials were analyzed before and after ten ordinary samples. Measured values were always within the provided reference or certified values.

## Questionnaires

Interview-administered questionnaires were used to survey (i) socio-demographic characteristics including age, sex, smoking habits, alcohol consumption and medical history, (ii) drinking water sources; (iii) food consumption of potential sources of Se over the last three months. Participants were asked if they had eaten the particular food 'every day', 'a few times a week', 'a few times a month' and 'rarely or never'. For the present analyses, the responses 'every day' and 'a few times a week' were grouped together (eat regularly) and those that reported eating the food a few times a month, rarely or never were grouped (do not eat regularly) (Appendice C).

## Statistical analysis

Descriptive statistics were used to examine the distribution of Se in food, drinking water and blood, and Ba and Sr content in Brazil nuts. For most food categories, drinking water and blood, Se concentrations were not normally distributed and non-parametric tests were used for comparisons. Blood Se levels were $\log_{10}$ transformed. The Wilcoxon Rank Sum test ($\chi^2$) was used to assess differences in food and drinking water Se content with respect to food type and community of origin, and to examine blood Se status variations between communities and with respect to food intake frequencies and socio-demographic data. Spearman correlations ($\rho$) were used to evaluate the relations between Se, Ba and Sr content in Brazil nuts and between blood Se and socio-demographic data. Contingency analysis (Likelihood ratio $\chi^2$) were used to evaluate the relations between food frequencies and communities, and when 20% of cells had an expected count less than 5, the Likelihood $\chi^2$ test was considered invalid. Multiple regression models were used to evaluate the relations between blood Se levels and food intake frequencies, taking into account co-variables relevant co-variables. Results were defined as statistically significant at $P \leq 0.05$. Analyses were performed using JMP 8.0.1 software (SAS Institute Inc.).

## *3. Results*

Se content of foods produced in the four communities is presented in Table 4. Se content in Brazil nuts, Brazil nut milk and sapucaia nuts was very elevated and highly variable, ranging from 0.4 to 158.4µg/g. Chicken (meat and organ meat), eggs (yolk and white), dry beef and game meat (paca and armadillo) presented high Se content, varying between 0.3 and 1.4µg/g. As shown in Table 1, egg yolks contained more Se than egg whites (Wilcoxon $\chi^2 = 19.8$, $p < 0.0001$) and chicken organ meat tended to have more Se than the meat itself (Wilcoxon $\chi^2 = 2.9$, $p = 0.10$). Medium Se

concentrations were measured in kale, sweet potato, *pupunha* and rice (with median Se levels between 0.1 and 0.2µg/g), while Se content was low in *tucumã*, coconut pulp and water, cow's milk, manioc flour, beans, yucca, green onions, *cará* and yam (with median Se levels below 0.09µg/g or 0.08µg/mL).

Tableau 4 : Se content (µg/g) in food samples collected in the communities

| | N | Mean | Median | Min. | Max. |
|---|---|---|---|---|---|
| LECITIDACEAE NUTS AND PALM FRUITS | | | | | |
| Brazil nut (*Bertholletia excelsa*) | | | | | |
| nut | 103 | 27.75 ± 33.54 | 13.93 | 0.40 | 158.42 |
| milk[1] | 3 | 6.63 ± 2.65 | 7.14 | 3.76 | 8.98 |
| *Sapucaia* nut (*Lecythis usitata*) | 2 | 9.69 ± 6.61 | 9.7 | 5.01 | 14.36 |
| *Tucumã* (*Astrocaryum aculeatum*) | 18 | 0.09 ± 0.10 | 0.06 | 0.003 | 0.28 |
| *Pupunha* (*Bactris gasipaes*) | 5 | 0.12 ± 0.07 | 0.14 | 0.003 | 0.17 |
| Coconut (*Cocos nucifera*) | | | | | |
| pulp | 16 | 0.10 ± 0.12 | 0.05 | 0.003 | 0.36 |
| water[1] | 14 | 0.08 ± 0.11 | 0.04 | 0.003 | 0.32 |
| MEAT, EGGS AND DAIRY | | | | | |
| Chicken (*Gallus domesticus*) | | | | | |
| meat | 17 | 0.50 ± 0.05 | 0.51 | 0.22 | 0.89 |
| organ meat (tripe, heart, liver, gizzard) | 25 | 0.63 ± 0.05 | 0.60 | 0.28 | 1.21 |
| Egg (*Gallus domesticus*) | | | | | |
| yolks | 27 | 0.66 ± 0.26 | 0.64 | 0.12 | 1.08 |
| whites | 24 | 0.29 ± 0.20 | 0.27 | 0.04 | 0.83 |
| Dry beef (*Bos Taurus*) | 2 | 0.39 ± 0.05 | 0.39 | 0.36 | 0.43 |
| Cow's milk (boiled)[1] | 5 | 0.06 ± 0.04 | 0.09 | 0.003 | 0.10 |
| Paca (*Cuniculus paca*) | 2 | 1.06 ± 0.45 | 1.06 | 0.73 | 1.38 |
| Armadillo (*Dasypodidae*) | 3 | 0.52 ± 0.47 | 0.58 | 0.03 | 0.97 |
| STAPLE FOODS | | | | | |
| Bitter manioc flour (*Manihot esculenta*) | 28 | 0.11 ± 0.12 | 0.06 | 0.003 | 0.45 |
| Rice (*Oryza sp.*) | 12 | 0.10 ± 0.08 | 0.11 | 0.003 | 0.23 |
| Beans (*Phaseolus vulgaris*) | 6 | 0.06 ± 0.05 | 0.05 | 0.01 | 0.11 |
| BRASSICA, ALLIUM AND OTHER ROOT VEGETABLES | | | | | |
| Kale (*Brassica oleracea*) | 8 | 0.20 ± 0.19 | 0.15 | 0.003 | 0.61 |
| Green onion (*Allium schoenoprasum*) | 15 | 0.10 ± 0.09 | 0.06 | 0.003 | 0.35 |
| Yucca (*Manihot esculenta*) | 20 | 0.11 ± 0.13 | 0.08 | 0.003 | 0.51 |
| Sweet potato (*Ipomoea batatas*) | 7 | 0.17 ± 0.13 | 0.17 | 0.02 | 0.36 |
| *Cará* (*Dioscorea alata* L.) | 25 | 0.08 ± 0.09 | 0.06 | 0.003 | 0.28 |
| Yam (Dioscorea sp.) | 5 | 0.02 ± 0.03 | 0.003 | 0.003 | 0.07 |

[1] µg/mL

Important inter-community variations of Se content were observed for Brazil nuts (Figure 2a). The highest Se concentrations were measured in a Brazil nut natural plantation of approximately 300 trees in SA (median = 40.6μg/g, range: 5.2 – 158.4μg/g). These nuts contained more Se compared to nuts from other trees of SA and those from VA (median = 18.0μg/g, range: 3.2 – 70.0μg/g; Wilcoxon $\chi^2 = 3.5$, $p = 0.06$), and nuts from Ip and Ac (median = 4.9μg/g, range: 0.4 – 28.3μg/g; Wilcoxon $\chi^2 = 38.8$, $p < 0.0001$). The nuts from other trees in SA and VA had similar Se content and contained significantly more Se than those from Ip and Ac (Wilcoxon $\chi^2 = 22.1$, $p < 0.0001$). Within the communities, Se content of Brazil nuts also varied significantly ($p < 0.05$) between the different households where they were grown and even between nuts from a same tree. For further analyses, participants (and all the foods they produced) living in proximity to the Brazil nut tree plantation with very high Se were categorized as SA-2, to distinguish them from participants and foods from the other households of SA (SA-1).

Brazil nuts concentrations of Ba and Sr are presented in Figure 2b and 2c. Median Ba content was 88.0μg/g (range: 1.9 – 1436.6μg/g) and median Sr content was 38.7μg/g (range: 3.3 – 172.6μg/g). Nuts from VA contained significantly more Ba compared to other villages (median = 710.4 μg/g, range: 17.4 – 1436.6μg/g versus median = 63.8μg/g, range: 1.9 – 446.5μg/g; Wilcoxon $\chi^2 = 29.8$, $p < 0.0001$), while nuts from SA-1 and SA-2 contained significantly more Sr compared to VA, Ip and Ac (median = 56.3 μg/g, range: 4.8 – 172.6μg/g versus median = 27.5μg/g, range: 3.3 – 104.1μg/g; Wilcoxon $\chi^2 = 22.5$, $p < 0.0001$). Significant differences in Ba and Sr content were also observed between different households and trees in VA, SA-1, SA-2 and Ip.

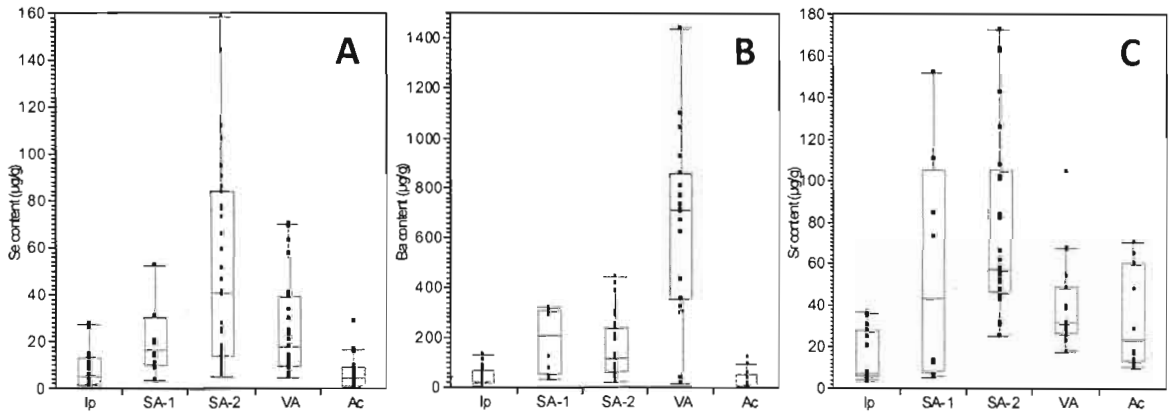


Figure 22 : Brazil nuts Se, Ba and Sr content and distribution by community
Legend: Ip (N = 19), SA-1 (N = 10), SA-2 (N = 34), VA (N = 23), Ac (N = 17)

Se, Ba and Sr concentrations in Brazil nuts were highly positively correlated (Se and Ba: Spearman $\rho = 0.58$, $p < 0.0001$; Se and Sr: Spearman $\rho = 0.68$, $p < 0.0001$; Ba and Sr: Spearman $\rho = 0.39$, $p = 0.0002$). Within each community, similar correlations were observed; the nuts with higher Se likewise tended to have higher Ba and Sr content. However, theses associations between Se, Ba and Sr were not similar in all four communities: no significant correlation was found between Ba and Se in nuts from SA-1, between Ba and Se, and Sr and Ba in nuts from VA, and between Sr and Se, and Ba and Sr in nuts from Ac.

Se content was likewise different between villages for other foods. Coconut water (N = 6) and egg whites (N = 7) from Ac had higher Se content compared to other villages (N = 8 and 17 respectively) (median = 0.14 vs 0.003µg/L; Wilcoxon $\chi^2 = 4.5$, $p = 0.03$, and median = 0.43 vs 0.22µg/g; Wilcoxon $\chi^2 = 6.0$, $p = 0.01$ respectively). Coconut pulp (N = 4) Se content was higher in VA compared to other villages (N = 12) (median = 0.20 vs median = 0.02µg/g; Wilcoxon $\chi^2 = 4.9$, $p = 0.03$), while egg yolks (N = 7) and chicken organ meat (N = 7) Se concentrations in VA were lower compared to other villages (N = 20 and 17 respectively) (median = 0.42 vs 0.79µg/g; Wilcoxon $\chi^2 = 5.4$, $p = 0.02$, and median = 0.55 vs 0.90µg/g;

Wilcoxon $\chi^2$ = 7.3, p = 0.007 respectively). *Tucumãs* were collected only in two villages; those from SA-1 and SA-2 (N = 9) had higher Se content compared to those from Ip (N = 9) (median = 0.18 vs 0.02μg/g; Wilcoxon $\chi^2$ = 9.5, p = 0.002).

Table 2 presents the Se content of foods from markets and groceries stores. Beef in the markets, which are from local cattle producers, had high Se content; Se concentrations were in the same range as the dry beef samples collected in the communities. Kidney samples had very high Se compared to meat and other organs. When comparing Se content between local food (Table 5) and commercial food (Table 2), we observed that commercial chicken meat, chicken organs, egg yolks and egg whites had a substantial Se content, but lower than those produced in the communities (Wilcoxon $\chi^2$ = 9.9, p = 0.002; Wilcoxon $\chi^2$ =6.4, p = 0.01; Wilcoxon $\chi^2$ = 2.8, p = 0.10 and Wilcoxon $\chi^2$ = 4.3, p = 0.04 respectively), while rice, yucca, potatoes, sweet potatoes and corn flour had medium Se content and no significant difference was observed between samples from markets, grocery stores and communities.

Tableau 5: Se content (μg/g) in food samples from markets and grocery stores

| | N | Mean | Median | Min. | Max. |
|---|---|---|---|---|---|
| MARKET FOOD | | | | | |
| Beef | | | | | |
| meat | 4 | 0. 37 ± 0.12 | 0.36 | 0.24 | 0.51 |
| organ meat (liver, stomach, tripe, heart) | 8 | 0.25 ± 0.09 | 0.24 | 0.16 | 0.42 |
| kidney meat | 2 | 1.32 ± 0.52 | 1.32 | 0.95 | 1.69 |
| Rice | 5 | 0.16 ± 0.11 | 0.21 | 0.03 | 0.25 |
| Beans | 7 | 0.04 ± 0.09 | 0.03 | 0.003 | 0.14 |
| Onion (*Allium cepa*) | 4 | 0.03 ± 0.12 | 0.02 | 0.003 | 0.06 |
| Garlic (*Allium sativum*) | 3 | 0.06 ± 0.14 | 0.06 | 0.03 | 0.08 |
| Yucca | 3 | 0.16 ± 0.14 | 0.11 | 0.05 | 0.32 |
| Potato | 3 | 0.11 ± 0.14 | 0.11 | 0.01 | 0.23 |
| Sweet potato | 6 | 0.11 ± 0.10 | 0.10 | 0.003 | 0.26 |
| *Cará* | 3 | 0.04 ± 0.14 | 0.003 | 0.003 | 0.10 |
| GROCERY STORES | | | | | |
| Chicken | | | | | |
| meat | 6 | 0.16 ± 0.08 | 0.13 | 0.07 | 0.27 |
| organ meat (liver and gizzard) | 5 | 0.32 ± 0.21 | 0.25 | 0.16 | 0.67 |
| Egg | | | | | |
| yolks | 5 | 0.46 ± 0.12 | 0.48 | 0.26 | 0.57 |
| whites | 6 | 0.13 ± 0.07 | 0.14 | 0.03 | 0.22 |
| Rice | 3 | 0.14 ± 0.08 | 0.16 | 0.06 | 0.21 |
| Beans | 4 | 0.05 ± 0.04 | 0.04 | 0.02 | 0.10 |
| Corn flour | 2 | 0.14 ± 0.02 | 0.14 | 0.12 | 0.16 |
| Garlic | 4 | 0.10 ± 0.05 | 0.09 | 0.03 | 0.14 |
| Pasta | 4 | 0.06 ± 0.11 | 0.003 | 0.003 | 0.22 |
| Milk powder | 2 | 0.09 ± 0.01 | 0.09 | 0.08 | 0.09 |

Drinking water Se levels are shown in Table 6. Overall, median Se levels were 0.05μg/L (range 0.05 – 1.39μg/L) and Se levels in VA were higher compared to other communities (Wilcoxon $\chi^2$ = 26.3, $p < 0.0001$). Almost all drinking water samples in VA were from open wells (N = 7/8) and their Se levels were higher than those from open wells in other communities (N = 7) (median = 0.67μg/L, range 0.12 – 1.39μg/L versus median = 0.05μg/L, range 0.05 – 1.23μg/L; Wilcoxon $\chi^2$ = 6.1, p = 0.01). The other drinking water sources had very low Se concentrations (median = 0.05μg/L, range 0.05 – 0.28μg/L). These included artesian wells (N = 7), the river (N = 12) and lakes (N = 4).

Tableau 6: Drinking water Se content (µg/L) between studied communities

| | Ip | SA-1 | SA-2 | VA | Ac |
|---|---|---|---|---|---|
| Mean ± SD | 0.05 ± 0.00 | 0.18 ± 0.35 | 0.05 ± 0.00 | 0.59 ± 0.10 | 0.05 ± 0.00 |
| Median | 0.05 | 0.05 | 0.05 | 0.54 | 0.05 |
| Range | 0.05 | 0.05 – 1.23 | 0.05 | 0.12 – 1.39 | 0.05 |
| N | 11 | 11 | 2 | 8 | 5 |

Bitter manioc flour, rice, fish (mostly *aracu* and *pescada* species), beef and domestic eggs are regularly eaten by a large proportion of this study population, but the distribution of regular consumption of Se-rich foods varied between communities (Table 7). For example, significantly more people from SA-2 regularly ate more Brazil nuts and derivates (milk, juice and in meat ragout cooked with Brazil nut milk) than any of the others. In VA more persons were regular consumers of nuts and juices compared to SA-1 and Ac, who consumed more nuts and cooked meat with Brazil nut milk than persons in Ip (Likelihood ratios, $p < 0.05$). Proportionally fewer persons in VA and Ac regularly consume chicken, eggs and beef from markets (Likelihood ratios, $p < 0.05$). However, proportionally more persons from these villages regularly consume game meat and domestic eggs compared to SA-1 and Ip. A higher percentage of persons in SA-2 also regularly consumed domestic chicken, game meat in comparison to SA-1 and Ip (Likelihood ratios, $p < 0.05$). In general, proportionally more persons consumed armadillo and paca game meats, compared to the consumption of other game meat including capybara, agouti, terrestrial and aquatic turtle, deer, caiman, wild pork and several bird species.

Tableau 7: Percentage of persons who regularly consumed Se-rich food for all the study population and by village

| | All villages (N = 143) % | Ip (N = 37) % | SA-1 (N = 34) % | SA-2 (N = 14) % | VA (N = 34) % | Ac (N = 24) % |
|---|---|---|---|---|---|---|
| IMPORTANT SOURCES | | | | | | |
| Brazil nut | | | | | | |
| nut | 35.0 | 10.8 | 20.6 | 92.9 | 52.9 | 33.3 |
| milk or juice | 13.3 | 0.0 | 8.8 | 71.4 | 11.8 | 8.3 |
| in meat plate | 7.8 | 0.0 | 3.0 | 35.7 | 5.9 | 12.5 |
| Sapucaia nut | 0.7 | 0.0 | 0.0 | 0.0 | 0.0 | 4.7 |
| Domestic chicken | | | | | | |
| meat | 35.7 | 10.8 | 38.2 | 42.8 | 38.2 | 62.5 |
| organ meat | 11.9 | 18.9 | 20.6 | 7.1 | 3.0 | 4.2 |
| eggs | 50.3 | 43.2 | 41.2 | 43.9 | 52.9 | 75.0 |
| Commercial chicken | | | | | | |
| meat | 16.8 | 24.3 | 29.4 | 14.3 | 3.0 | 8.3 |
| organ meat | 11.9 | 18.9 | 20.6 | 7.1 | 3.0 | 4.2 |
| eggs | 9.8 | 10.8 | 14.7 | 0.0 | 14.7 | 0.0 |
| Beef [1] | | | | | | |
| meat | 65.0 | 81.1 | 70.6 | 78.6 | 55.9 | 37.5 |
| organ meat | 9.0 | 10.8 | 11.8 | 14.3 | 5.9 | 4.2 |
| Game meat (all species) | 23.8 | 0.0 | 8.8 | 35.7 | 29.4 | 66.7 |
| paca | 8.4 | 0.0 | 0.0 | 0.0 | 20.6 | 20.8 |
| armadillo | 18.2 | 0.0 | 3.0 | 35.7 | 29.4 | 41.7 |
| Fish meat (all species) | 95.8 | 97.3 | 97.1 | 92.9 | 100.0 | 87.5 |
| *aracu* (*A. laticeps*) | 54.5 | 56.8 | 61.8 | 57.1 | 50.0 | 47.1 |
| *pescada* (*P. squamosissimus*) | 83.9 | 89.2 | 79.4 | 71.4 | 94.1 | 75.0 |
| OTHER SOURCES | | | | | | |
| Kale | 16.8 | 24.3 | 5.9 | 7.1 | 20.6 | 20.8 |
| Sweet potato [1] | 9.1 | 2.7 | 0.0 | 0.0 | 11.8 | 33.3 |
| Potato | 11.8 | 8.1 | 20.6 | 0.0 | 8.8 | 12.5 |
| *Pupunha* | 4.9 | 5.4 | 3.0 | 0.0 | 3.0 | 12.5 |
| Rice [1] | 97.9 | 100.0 | 97.1 | 92.9 | 97.1 | 100.0 |
| Commercial corn flour | 41.3 | 62.2 | 29.4 | 14.3 | 44.1 | 37.5 |
| Yucca [1] | 28.7 | 10.8 | 23.5 | 21.4 | 55.9 | 29.2 |
| Bitter manioc flour | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |

[1] No significant difference of Se content between communities, food markets and grocery stores

Blood Se levels were highly variable (median 291.8µg/L, range: 132.1 – 1500.2µg/L), and blood Se levels paralleled Brazil nut Se content distribution between communities (Figure 23 and Figure 22a respectively). Persons from SA-2 had median blood Se levels of 1078.1µg/L (range: 558.3 – 1500.2µg/L), which was

much higher than the other communities (Wilcoxon $\chi^2 = 76.8$, $p < 0.0001$). Blood Se levels in VA (median = 365.4µg/L, range: 173.3 – 635.1µg/L) were significantly higher than those from SA-1 and Ac (median = 286.16µg/L, range: 132.1 – 900µg/L; Wilcoxon $\chi^2 = 10.7$, $p = 0.001$). Persons from Ip presented the lowest blood Se levels (median = 208.7µg/L, range: 135.3 – 356.8µg/L; Wilcoxon $\chi^2 = 40.0$, $p < 0.0001$).

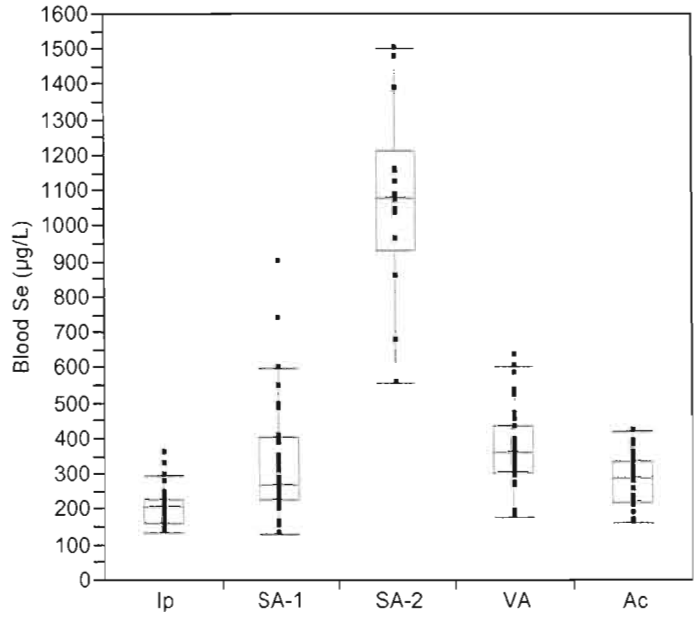


Figure 23 : Blood Se distribution of the study participants by community
Legend: Ip (N = 37), SA-1 (N = 34), SA-2 (N = 14), VA (N = 34), Ac (N = 24)

Overall, persons who consumed Brazil nuts regularly (N = 50) had significantly higher B-Se concentrations compared to the others (N = 93) (median = 430.7 vs 243.7µg/L; Wilcoxon $\chi^2 = 33.3$, $p < 0.0001$). The same was true for those who ate Brazil nut derivatives regularly (N = 21) or not (N = 122) (median = 583.0 vs 269.6µg/L; Wilcoxon $\chi^2 = 26.0$, $p < 0.0001$). Persons frequently consuming domestic chicken (N = 51) and game meat (N = 34) also had higher blood Se levels compared to the others (median = 340.3 vs 257.8µg/L; Wilcoxon $\chi^2 = 6.5$, $p = 0.01$ and median = 368.6 vs 263.1µg/L; and Wilcoxon $\chi^2 = 12.3$, $p = 0.0005$, respectively). Fish meals,

manioc flour and rice are consumed almost every day and statistical analyses were not possible. No other food was related to blood Se levels.

Certain socio-demographic variables were also related to Se status. Univariate analyses showed that blood Se was lower for those who reported smoking (N = 52) compared to those who did not (N = 91) (median = 235.1 vs 309.8µg/L; Wilcoxon $\chi^2$ = 4.4, p = 0.04), but was unrelated to age and was similar between women and men, and was not influenced by drinking status.

The multiple regression model for log blood Se, which included age, smoking, regular consumption of Brazil nuts, game meat and domestic chicken showed that significant contributions were from regular consumption of Brazil nuts (Beta estimate = 0.11; p < 0.0001), game meat (Beta estimate = 0.04; p = 0.05) and domestic chicken (Beta estimate = 0.03; p = 0.08). A total of 31% of the variance of log blood Se was explained by this model. Neither smoking status nor age entered significantly into the model.

Since Brazil nuts were the most important source of Se, and also contain Sr and Br, we calculated possible intake of these elements from these nuts. The average weight of a Brazil nut is approximately 4g, but can vary widely depending of sub-varieties (between 2g and 9g). Table 5 presents estimates of the minimum, median and maximum intakes of Se, Sr and Ba per Brazil nut of 4g and 9g.

Tableau 8 : Estimates of Se, Ba and Sr intakes per Brazil nut

| | Nuts' content (µg/g) | | Estimated intake per nut (µg) | | | |
|---|---|---|---|---|---|---|
| | Median | Range | Minimal intake per nut of 4g | Median intake per nut of 4g | Maximal intake per nut of 4g | Maximal intake per nut of 9g |
| Se | 14.0 | 0.4 – 158.4 | 1.6 | 56.0 | 233.6 | 1425.6 |
| Ba | 88.0 | 1.9 – 1436.6 | 7.6 | 352.0 | 5746.4 | 12 929.4 |
| Sr | 38.7 | 3.3 – 172.6 | 13.2 | 154.9 | 690.4 | 1553.4 |

## *4. Discussion*

Brazil nuts (*B. excelsa*) and derivates are the most important source of Se for riverside populations of the Tapajós River, and probably for the entire Amazonian Basin. Brazil nut is a Se-accumulator plant species, part of the Lecitidaceae family (Fordyce, 2005). Results from the present study show that Se content in Brazil nuts is highly variable and at least one order of magnitude higher than other rich-Se foods, such as fish, chicken, game meat, eggs and beef. When consumed on a regular basis, these nuts are the major contributor to Se intake and variations of blood Se status reflect inter-community fluctuations of Se content in Brazil nuts.

*B. excelsa* is a long-lived and large tree (up to 60m tall), and a native dominant species of the upper canopy of the Amazon and the Guianas non-flooded forests. The period of Brazil nut tree productivity is during the rainy season, between December and April, when the ripe fruits fall from the trees. The fruit is a woody capsule containing 10-25 nuts; extraction relies on the capacity of breaking through the hard pericarp. Large rodents (such as agoutis) and humans are the major consumers of Brazil nuts (reviewed by Wadt et al., 2005). Brazil nut, Brazil nut milk and their multiple derivates are part of the Amazonian culinary tradition; they are often collected and stored for further use throughout the following months. The present study was carried out from June to August, and the consumption of Brazil nuts was probably lower during this period compared to the rainy season. Indeed, in a previous study, we showed that blood Se levels can be as high as 2447 µg/L during the rainy season in SA-2 (Lemire et al., 2009), where almost all participants reported regularly consuming Brazil nuts and derivates.

The study region, here, covers approximately 95 km along the Tapajós and there are important variations of Se content in Brazil nuts, not only between communities, but also within communities, between trees in a plantation and even in nuts from a same tree. These results are consistent with the findings of other authors about Brazil nuts. Their Se content can vary up to 1000 times, depending on several factors including soil characteristics (geology, pH and moisture content) as well as the plant species sub-variety, the maturity and extent of the root system of the tree, and the position of the nut on the tree, which would cause a variation of the efficiency of the vascular system (Secor and Lisk, 1989; Chang et al., 1995). Two previous studies showed that Brazil nuts from the Central region of the Amazon (State of Pará and East of the State of Amazonas) contained significantly more Se (varying between 1.3 and 512.0µg/g) than nuts from the Western part of the Amazon (varying between 0.03 and 31.7µg/g) (Chang et al., 1995; Pacheco and Scussel, 2007). In the present study, Brazil nut Se content in the Lower Tapajós region (State of Pará) ranged between 0.4 and 158.4µg/g, confirming that important fluctuations of Se content can be observed within a much smaller geographic scale.

Brazil nuts from the Lower Tapajós likewise contain variable and, in some cases, very high concentrations of Ba and Sr. Soils of the Amazon basin are high in Hollandite, a Ba-rich mineral ($Ba_2Mn_6O_{16}$), and Ba concentrations up to 4000µg/g have been reported in Brazil nuts (Bowen, 1966 cited in Chang et al., 1995). Parekh et al. (2008) reported highly variable Ba concentrations (96 to 1990µg/g) in Brazil nuts from different regions of South America, with an average content of 3.6 ± 0.4µg/g in nuts from Brazil; however the nuts' origin within the country was unspecified. The median Ba concentration in the present study was considerably higher (88µg/g, range 1.9 – 1436.6µg/g) than those reported by Parekh et al. (2008), and suggests high Hollandite content in some parts of the Lower Tapajós Basin, particularly in VA. In humans, Ba accumulates in muscles and bones (Browning, 1969). Although there are still uncertainties about health effects of Ba from foods,

high concentrations of Ba in drinking water have been mostly associated with cardiovascular and neurologic diseases (WHO, 2004). The World Health Organization (2004) guidelines value for Ba in drinking water is 700µg/L. No guideline is available for Ba in foods. We did not assess Ba in blood and do not know how much is absorbed from Brazil nuts. These relations should be examined if Brazil nuts are to be proposed as an important source of Se.

A recent study likewise reported high and variable Sr content in Brazil nuts from unknown origin (115 ± 12µg/g) (Welna et al., 2008), which is slightly higher than the median Sr concentrations observed in the present study (38.7µg/g, range 3.3 – 172.6µg/g). Sr is a molecular surrogate for calcium and, like Ba, accumulates in the skeleton. There is no evidence that Sr is toxic for adults, although in children, it may impair mineralization of the developing bones. The U.S. drinking water safety limit for Sr is 4000µg/L (ATSDR, 2004). No permissible daily intake for Sr in food has been established yet.

Estimates of Se, Ba and Sr intakes per Brazil nut from the Lower Tapajós region are almost unpredictable. For a nut of standard weight (4g), Se, Ba and Sr intakes can vary up to 150 fold, 750 fold and 50 fold respectively. These intakes increase by one order of magnitude for a big nut of 9g. In the present study, Se, Ba and Sr contents in Brazil nuts were positively correlated. However, within communities, inconsistent relations between these elements in nuts suggest much complex soil-tree interactions. Parekh et al. (2008) reported inverse correlation between Se and Ba concentrations in nut samples from different regions of South America. In depth botanical studies which take into account soil geology and the element' availability as well as plant sub-varieties and intra-tree variability are required to better understand the variations of these elements within and between ecosystems.

Brazil nuts can also contain small amounts of radioactive radium (Ra), an alpha-emitter and potent carcinogen that accumulates in bones due to its similarity with calcium (WHO and IARC, 2001). Penna-Franca et al. (1968) reported from 5.9 to 133mBq/g of $^{228}$Ra in Brazil nuts. In this study, nuts with high Ba content likewise had the highest Ra content. More recently, Parekh et al. (2008) reported relatively constant Ra isotopes concentrations in Brazil nuts from different South American countries, with an average content of 31 ± 1 mBq/g of $^{226}$Ra and 31 ± 3 mBq/g of $^{228}$Ra in nuts from Brazil. The ingestion of one 4g nut would yield a radiation dose of 0.21μSv for a 25 years old adult (ICRP, 1996), which is negligible considering that the worldwide average radiation exposure from all sources is 2800μSv per year (UNSCEAR, 2000). High $^{228}$Th fecal excretion was reported in a worker consuming ≤ 25g of Brazil nuts per day. The authors suggest that this unexpectedly high $^{228}$Th activity would result from *in vivo* decay of high $^{228}$Ra intake from Brazil nuts (Bull et al., 2006).

Depending on moisture preservation conditions, high concentrations of aflatoxins, which are potent carcinogens synthetised by fungi, have also been reported in Brazil nuts (Pacheco and Scussel 2007). Since consumers are able to visually sort out fungi-contaminated Brazil nuts, they should be informed of the benefits of avoiding nuts with such defects (Marklinder et al., 2005). Brazil nuts can also contain high levels of vitamin E and unsaturated fatty acids (Chunhieng et al., 2008). Further studies need to address the possible combined health effects of all these potentially toxic compounds and beneficial nutrients present in these nuts.

*Sapucaia* nuts (*L. usitata*), another native nut from the Lecitidaceae family, also contained very high levels of Se. However, they were not easily found and were only consumed by a few persons in these communities. At maturity, the operculum of *sapucaia* fruits opens directly on the tree, the seeds fall to the ground and are readily eaten and/or carried away by rodents and small animals (Andrade et al., 1999).

Participants of the present study often mentioned that *sapucaia* nut is easily found on their land. High Se content in *sapucaia* nuts may account for Se content in the meat of small animals that were consumed by participants in this study. Other nuts of Lecitidaceae plant family such as monkey nuts (*L. ollaria*) can contain up to 5800μg/g, about 500 times more Se than Brazil and *sapucaia* nuts (Andrade et al., 1999). Monkey nuts are usually considered toxic by local populations (Kerdel-Vegas, 1966), possibly due to their very high Se content and/or their particular dominant chemical Se form, selenocystathionine, a Se compound that has not been identified in Brazil nuts (Rayman et al., 2008).

High Se concentrations were found in domestic chicken and game meat, and their regular consumption contributed substantially to Se intake. Eggs yolks from domestic chickens also had considerably high Se content, but since whites and yolks are usually eaten simultaneously, the resulting Se intake per gram is probably lower. In this region, free-range chicken are fed with dry corn seeds, grown by the inhabitants, and anything else that they may pick up when pecking, including soil and nut shells. Domestic chicken meat and eggs may therefore represent a good indicator of Se content and bioavailability in soils of the local ecosystem. This is probably likewise the case for wild animals, who consume nuts and other plants they find in the forest.

Some studies have suggested that cooking processes (boiling, baking or grilling) can reduce Se content of foods by volatilization, although the results are inconsistent (Higgs et al., 1972; Zhang et al., 1993; Dumont et al., 2006). Brazil nuts are mostly consumed as such. In the present study, where samples were uncooked, regular consumption of chicken and game meat was well correlated to blood Se levels. This suggests that even after cooking, there is sufficient Se content in these foods to influence blood Se concentration.

Se content of rich-Se and moderate-Se foods from villages of the Lower Tapajós is proportionally higher compared to Se content of foods from Southern States of Brazil (Ferreira et al., 2002; Paiva Oliveira et al., 2004), U.S. (ATSDR, 2003), U.K. (Barclay et al., 1995), Thailand (Sirichakwal et al., 2005) and Greece (Pappa et al., 2006). On the other hand, Se levels in foods from the Amazon (with the exception of *B. excelsa* that is a particular Se-accumulator plant) were considerably lower than Se concentrations measured in foods from seleniferous regions of China (Yang et al., 1983; 1989b). This is consistent with the study of Chang et al. (1995), who has mentioned that soil Se concentrations and availability in the Eastern Amazon, including the Lower Tapajós region, may be high, but not as high as those observed in Se-toxic areas of China. In the present study, Se content of *pupunha* collected in the villages was in the same range as those of *pupunha* of a landrace from the State of Pará (Yuyama et al., 2003).

Some Se-rich food are also bought by villagers at local markets and grocery stores and, for the most part, Se concentrations were lower and in the same range as those from Southern States of Brazil (Ferreira et al., 2002). In SA-1 and Ip, where persons travel daily to the nearby city of Itaituba for commercial proposes and eat market foods more regularly, blood Se was lower compared to persons living in villages regularly consuming local foods. In the present study, the only food from the market with very high Se was beef kidneys. Organ meat is known to be very rich in Se, particularly beef kidneys, and our results are comparable to other studies (Barklay et al., 1995; ATSDR, 2003).

Fish Se concentrations of the Lower Tapajós are highly variable, from 0.05μg/g to 1.01μg/g, and depend not only on fish species, but also on fish habitat and season (Sampaio et al., *personal communication*). These variations probably reflect seasonal, geographic and food sources. In the Madeira River basin (State of Rondônia, Western Amazon), reported fish Se concentrations range from 0.01 to

0.37µg/g, with higher levels in omnivorous species (Dorea et al., 1998). In the Cachoeira do Piriá Municipality (State of Pará, Eastern Amazon), fish Se concentrations varied from 0.10 to 1.21µg/g, with higher levels among omnivorous and piscivorous (Lima et al., 2005). The Se concentrations in fish species from the study region were in the same range as those observed by Lima et al. (2005), who likewise analyzed fish in the State of Pará; however, in the Tapajós, herbivorous fish species had higher Se content compared to omnivorous and piscivorous (Sampaio et al, *personal communication*), which concentrations were higher compared to large fresh water and marine predator fish in other studies, in which Se content is generally higher than 0.50µg/g (Ferreira et al., 2002; Pappa et al., 2006; Sirishakwal et al., 2005).

In the present study, Se levels in drinking water were well below the drinking water standard of 10µg/L (WHO, 1996), and represent a negligible fraction of Se intake compared to dietary sources. Se levels in open wells from VA were low, but high when compared to other local drinking water sources. In general, groundwater has higher Se concentrations than surface water due to higher rock-water interactions (WHO, 1996; Fordyce, 2005). These results suggest that soils Se concentrations in VA may be higher than in the other communities.

The health effects of elevated Se are not known, some studies indicate that there may be risks (Laclaustra et al., 2009a; Stranges et al., 2007; Yang et al., 1983), while other suggest that there are benefits (Valera et al., 2009). In this region of the Amazon, we have observed protective effects of Se on age-related cataractogenesis and motor functions, and this, without evidence of Se-related toxic effects (Lemire, chapitre IV et V).

In this riverside population, high Se intake comes from local diet, which contains mostly organic forms of Se (Whanger, 2002). The toxic effects of organic Se

are less understood and toxicity, if it exists, may occur at higher levels compared to inorganic Se (Rayman et al., 2008). On the other hand, Amazonian population have the highest Hg exposure reported in the world today (Passos and Mergler, 2008) and high exposure to metals, such as Hg, may raise the body's Se requirements to offset Hg-mediated oxidative stress and other toxic effects (Fordyce 2005; Watanabe 2001), and to maintain optimal Se antioxidant enzymes (Rayman, 2008). Thus, in a situation with elevated Hg and Se, there may be less 'excess' of Se and consequently little or no Se toxicity. As Rayman et al. (2008) pointed out that the adequate Se intake depends of several factors including the chemical speciation of Se which is most prominent in the Se source, the adequacy of other nutrients, the presence of additional stressors and the body's ability to make selenoproteins (Rayman et al., 2008). Further studies should examine the relations between biomarkers of Se, Hg and different health outcomes in this region.

# CHAPITRE III

## Aucune évidence de toxicité du Se chez des populations ayant une alimentation riche en sélénium en Amazonie brésilienne

*No evidence of toxicity from selenium-rich diet in the Brazilian Amazon*

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## *Résumé*

Le sélénium (Se) est un oligoélément reconnu pour ses propriétés antioxydantes. Chez les riverains du Bas-Tapajós, les bioindicateurs de Se varient de normal à très élevé. Les noix du Brésil, le poulet, la viande de chasse, les œufs et certaines espèces de poissons sont les principales sources alimentaires de Se. Quelques études ont rapporté des effets toxiques du Se, notamment des problèmes dans la structure de la kératine (cheveux, ongles et peau), des problèmes gastro-intestinaux et de la paresthésie chez des populations ayant un apport alimentaire très riche en Se. L'objectif de cette étude est d'évaluer les symptômes et les signes de toxicité du Se en relation avec le statut de Se chez les riverains du Bas-Tapajós.

Un total de 448 participants, âgés de 15 à 87 ans, ont été recrutés dans 12 communautés. Les concentrations de Se ont été mesurés dans le sang et le plasma par ICP-MS. Une évaluation complète des signes de toxicité du Se au niveau des ongles, des cheveux, de la peau et de l'haleine a été réalisé par une infirmière. Un questionnaire portant sur les données sociodémographiques, l'histoire médicale et les symptômes possibles de la toxicité du Se a aussi été administré.

Chez cette population, les niveaux médians de Se sanguin et de Se plasmatique étaient respectivement de 228.4µg/L (103.3–1500.2µg/L) et de 134.8µg/L (53.6–913.2µg/L). Même si les niveaux de Se sanguin et plasmatique dépassaient les concentrations considérées toxiques, aucun signe ou symptôme de toxicité du Se n'était associé aux bioindicateurs de statut de Se.

Ces résultats mettent en évidence toute l'importance de réévaluer la toxicité du Se en tenant compte d'un certain nombre de facteurs incluant la voie d'exposition au Se (inhalation vs. ingestion), les formes chimiques de l'exposition au Se, le type d'exposition (aigüe vs. chronique), l'exposition concomitante à des éléments toxiques comme le mercure, l'apport alimentaire d'autre éléments nutritifs, la vulnérabilité individuelle et l'adaptation.

## *Abstract*

Selenium (Se) is an essential element and a well-known anti-oxidant. In the Lower Tapajós River inhabitants of the Brazilian Amazon, human status of Se range from normal to very high. Brazil nuts, chicken, game meat, eggs and certain fish species constitute important Se dietary sources. Some studies have reported alterations in keratin structure (hair, nails and skin), gastrointestinal problems and paresthesia in populations with high Se intake. The objective of the present study was to evaluate sentinel symptoms and signs of Se toxicity in relation to Se status in communities along the Tapajós River.

Participants (n = 448), aged 15-87y, were recruited from 12 communities. Se concentrations were measured in blood (B-Se) and plasma (P-Se) by ICP-MS. A nurse performed an examination of hair, nails, skin and breathe for signs of Se toxicity. Interview-administered questionnaires were used to collect information on socio-demographics, medical history and possible symptoms of Se toxicity.

In this population, the median levels of B-Se and P-Se were 228.4μg/L (range 103.3–1500.2μg/L) and 134.8μg/L (range 53.6–913.2μg/L) respectively. Although B-Se and P-Se surpassed concentrations considered toxic, no signs or symptoms of Se toxicity were associated with the biomarkers of Se status.

These findings support the need to re-assess Se toxicity considering a number of factors, including the route of exposure (inhaled vs. ingested), chemical form of Se exposure, the type of exposure (acute vs. chronic), co-exposures to toxic elements such as mercury, intake of other beneficial nutrients, individual vulnerability, and possible adaptation.

## *1. Introduction*

Selenium (Se) is an essential element involved in several body functions through selenoproteins expression, including protection against oxidative stress, redox status, immune and thyroid function. Both deficiency and excess of Se can lead to adverse health outcomes (Rayman, 2000; Papp et al., 2007).

Diet is the principal route of Se intake and food Se levels generally reflect Se concentrations in soils. Natural geophysical and bioavailability of Se in soils, sediments and waters, which vary greatly from one region to another, account for uptake by plants and subsequent levels in fish and mammals (Levander, 1987). Seleniferous soils have been identified in the Great Plains of the USA and Canada, in the Enshi Country of Hubei Province in China and in parts of Ireland, India, Israel, Russia, South Africa, Australia, Colombia and Venezuela (Combs Jr., 2001; Fordyce, 2005). Since seleniferous environments are far less widespread than Se-deficient environments, acute or chronic Se poisoning from local crops is not common in both livestock and human populations (Fordyce, 2005; Rayman, 2008).

The most detailed reports on outbreaks of human Se intoxication (selenosis) are from China, where local populations consumed Se-rich crops grown on soil fertilized with Se-rich coal ashes and where persons were also possibly exposed to Se vapor from domestic heating and cooking with mineral coal (Liu et al., 2007; Yang et al., 1983; 1989a). Signs and symptoms of selenosis in humans have been described as progressive alterations in the keratin structure, causing brittle hair and important hair loss, broken nail walls, nail sloughing and further thickened and stratified nails, skin lesions, garlic odor of the breath, excess dental caries, gastro-intestinal disorders and, in more heavily affected cases, possible neurologic problems, including motor and sensory abnormalities (WHO, 1986).

In studies carried out in China, the first clinical manifestations of Se toxicity were observed on hair and nails at blood Se levels ranging from 1020 to 1854µg/L (WHO, 1986). The U.S. Environmental Protection Agency (EPA) used these data to establish a No Observable Adverse Effect Level (NOAEL) at 1000µg/L and a Low Observable Adverse Effect Level (LOAEL) at 1350µg/L (Poirier, 1994). Yang et al. (1989a) proposed 900µg/L in blood as the limit of homeostatic Se regulation, based on the decline of the ratio of Se concentrations in erythrocytes to plasma. In 2000, the U.S. Institute of Medicine set the tolerable upper intake level (UL) of Se for adults at 400µg/day to prevent the risk of selenosis, and proposed a selenium deficiency guideline at plasma Se levels below 70-90µg/L.

In the Tapajós River region of the Brazilian Amazon, Se status of riverside populations ranges from normal to very high, varying between 142 and 2447µg/L in whole blood (Lemire et al., 2006; 2009; Pinheiro et al., 2005). Important local dietary Se sources, such as Brazil nuts (*Bertholletia excelsa*), domestic chicken, game meat and certain fish species, have been identified (Lemire et al., chapitre II; Sampaio da Silva et al., *in revision*). Along the Tapajós River, significant variations of Se status have been observed between villages and seasons. The highest Se levels were among persons who consume large amounts of Brazil nuts, particularly during the Brazil nuts season, from December to April, when the mature nut capsules fall from the trees (Lemire et al., 2009). In contrast, very low Se concentrations were measured in the drinking water of the communities along the Tapajós and in the waters of the Tapajós and Amazon Rivers (Lemire et al., chapitre II; Fordyce 2005). To our knowledge, no industrial source of Se is present in the Tapajós region.

Se in plasma and/or serum are the favored biomarkers to compare Se status between countries (Thomson, 2004). However, since plasma Se tends to saturate at high Se intake (Hansen et al., 2004; Yang et al., 1989a), most studies on Se toxicity rely on whole blood to evaluate the relation between Se and adverse health effects. Although hair Se levels may also be a good biomarker of Se status (Lemire et al., 2009), there is a possibility of external hair contamination in areas polluted by coal burning as it was the case in the Chinese studies (Yang et al., 1983). Thus, the comparison of hair Se levels between populations with high Se status may be inadequate.

The objective of the present study was to evaluate sentinel symptoms and signs of Se toxicity in relation to plasma and whole blood biomarkers of Se status in communities along the Tapajós River.

## *2. Material and methods*

### Study population

This cross-sectional study is part of a larger project on factors that affect human Hg exposure and its health effects (CARUSO, 2009). In the Lower Tapajós River Basin (State of Pará, Brazil), there are approximately 50 communities of diverse size and origin, with varying access to health care, education, local authorities and cities. Based on sample size calculations for visual outcomes from preliminary studies, we sought a minimum of 400 people to test Hg and Se interaction effects. To reach this number and as well to reflect the diversity of regional populations, social conditions and ecosystems, we selected 12 selected communities (presented in Figure 24). Recruitment of persons 15y and older was carried out in each village through home visits and at village meetings, during which the research project was explained, and villagers were invited to participate on a voluntary basis. No *a priori* conditions were

imposed, however, preference was given to those who had participated in a previous study (Passos et al., 2008).

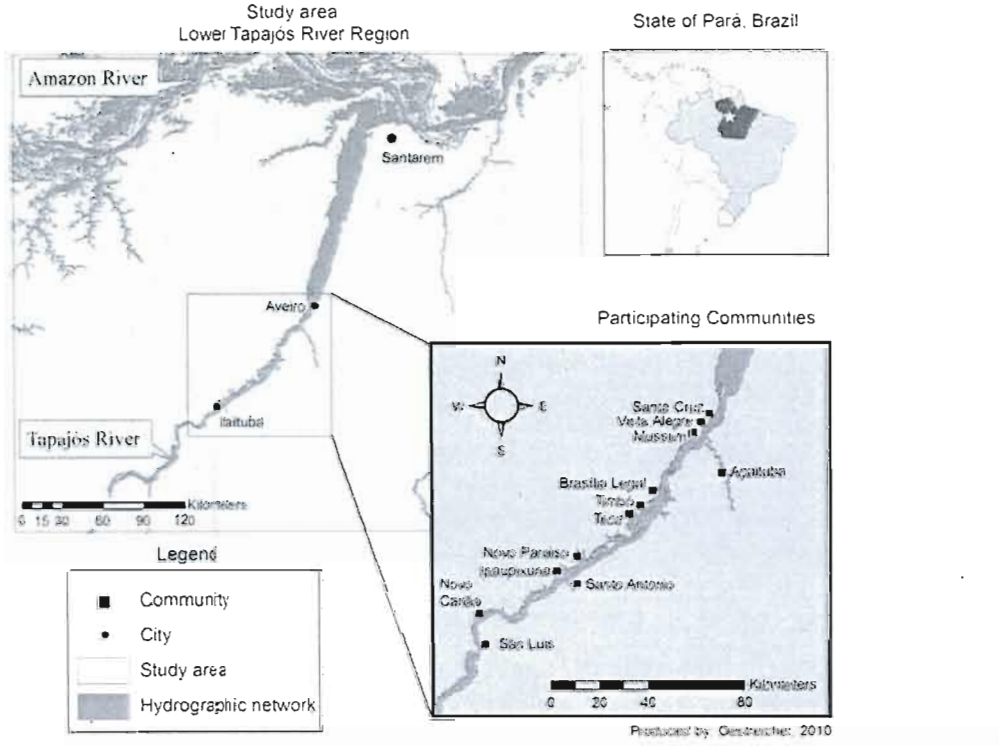


Figure 24 : Study area

The study was carried out from May to August, 2006, in a technical college, located in a nearby city of Itaituba. A total of 448 persons (216 men and 232 women) participated in the present study. Based on a house-to-house survey carried out by our research group in 2003, this represents 25% of the adult population of these villages (27% of all women and 23% of all men) participated in the study. The participation within each village varied from 10 to 67%, with higher relative frequencies in the smaller villages (the smallest consisted of 13 adult residents and the largest, 384 adults). Since recruitment favored participants from our previous studies, younger persons ($\geq$ 15y and < 40y) were underrepresented with respect to the age distribution of the entire population (50% vs. 62%), those between to 40y to 65y were overrepresented (40% vs. 28%), while the oldest group ($\geq$ 65y) was similar to the underlying population (10%).

The study was approved by the Ethics Review Boards of the University of Quebec at Montreal and the Faculty of Pharmaceutical Sciences of the University of São Paulo-Ribeirão Preto. All participants signed an informed consent form, which was read to them. There was no remuneration for study participation.

## Blood sample collection and analysis

For each participant, an experienced Brazilian phlebotomist collected a 6mL blood sample in "trace metal free" evacuated tubes (BD Vacutainer®), containing heparin as anticoagulant. For plasma separation, blood samples were centrifuged (800 x g for 6 minutes). Plasma fractions were then pipetted into previously cleaned Eppendorf tubes (2 mL), and immediately frozen at -20°C. Blood total Hg (B-Hg), blood Se (B-Se) and blood lead (B-Pb), as well as plasma total Hg (P-Hg) and plasma Se (P-Se) were determined by inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer DRC II) according to the method proposed by Batista et al. (2009), at the *Laboratório de Toxicologia e Essencialidade de Metais*, *Universidade de São Paulo*, Ribeirão Preto, SP, Brazil. Quality control was guaranteed by analyzing various secondary reference materials, provided by the New York State Department of Health's Proficiency Testing program for trace elements in whole blood, and from the external quality assessment scheme (EQAS) for trace elements operated by the *Institut National de Santé Publique du Québec*, Canada. Reference materials were analyzed before and after ten ordinary samples. Measured values were always within the provided reference or certified values.

## Clinical signs examination

A Brazilian trained nurse, who had no knowledge of the Se status of the participants, performed an examination of the clinical dermal signs of Se toxicity (hair, nails and skin) and breath. Participants with painted fingernails and toenails were duly identified. Signs of tooth decay and dental cavities were excluded from the present

study because of the non-specificity of these signs for Se toxicity, the lack of dental care and the important number of dental prosthesis in this study population (WHO, 1986; ATSDR, 2003) (Appendice B).

## Questionnaires

Interview-administered questionnaires were used to survey: (i) socio-demographic characteristics including age, sex, smoking status, alcohol consumption, years of education, and medical history; (ii) sentinel symptoms of selenosis reported in the literature (ATSRD, 2003; Longnecker et al., 1991; WHO, 1986; Yang et al., 1983; 1989; Yang and Zhou 1994; Yang and Xia, 1995) and other possible non-specific symptoms that could reflect early Se or metal toxicity (Mergler, 1998) (Appendice B).

## Statistical analyses

General exclusions for the present analyses were pregnant and breastfeeding women ($N$ = 15), reported stroke ($N$ = 14), taking psychotropic medication ($N$ = 7) or missing data for blood or plasma biomarker ($N$ = 5). A total of 407 participants, 204 women and 203 men, were included in the present study.

The study population was grouped into three categories of Se status, based on normative data and by estimating the equivalences between the Se intake, B-Se and P-Se, using the tables and formulas proposed by Yang and Xia (1995): *normal* (B-Se < 560µg/L and P-Se < 328), *high* (B-Se ≥ 560 < 1000µg/L or P-Se ≥ 328 < 520µg/L) and *very high* (B-Se ≥ 1000µg/L or P-Se ≥ 520µg/L). Since very few persons were Se-deficient (P-Se < 70µg/L (n = 6) or B-Se < 89µg/L (n = 0), we did not make a separate category for deficiency.

Signs and symptoms were classified as absent (0) or present (1). Descriptive statistics were used to illustrate the study population's general characteristics, the

distribution of the biomarker's levels and the distribution of reported symptoms and examined signs in the present population.

Since none of the Se biomarker variables displayed a normal distribution, non-parametric analyses of variance (Wilcoxon/Kruskall-Wallis $\chi^2$, Rank Sums Test) were performed when analyzing continuous variables (i.e. biomarkers or age) against categorical variables (gender, current smoking (smoker vs. nonsmoker) and drinking (drinker vs. nondrinker)). Contingency analyses (Likelihood Ratio $\chi^2$ test) were used to evaluate the associations between categories of Se status and (i) socio-demographic and medical data and (ii) the presence/absence of symptoms and signs of Se toxicity.

For contingency analysis, when 20% of cells had an expected count less than 5 or any had less than 5% of the total count, the Likelihood Ratio $\chi^2$ test was considered invalid. When the prevalence of a symptom or a sign was above 50% in the high and/or the very high Se categories, multiple logistic regression models were performed using Se as the explanatory variable, controlling for age (y), gender and smoking and drinking habits. In addition, for each person, the number of signs and symptoms were summed and analyzed as response variables in multiple linear regression models with respect to Se status and controlling for the above-mentioned co-variables.

Multiple factorial analyses were used to portray the structure in the relationships between variables. For both signs and symptoms of Se toxicity, separate multiple correspondence analyses (MCA) were performed to explore the structure and highlight the associations between signs (presence vs. absence) or symptoms (presence vs. absence) and the following categorical variables: Se status, gender, age ($< 40$y and $\geq 40$y), smoking and drinking habits. Symptoms and signs that were reported by less than 5% of persons were not included in the MCA models.

Results were defined as statistically significant at $P \leq 0.05$. Analyses were performed using JMP 8.0.1 software (SAS Institute Inc.) and SPSS version 16 (SPSS Inc., Chicago, IL).

## *3. Results*

The distribution of B-Se and P-Se concentrations is shown in Figure 25 and 26. Median B-Se and P-Se were 228.4µg/L and 134.8µg/L respectively. A total of 23 persons (5.7%) had high levels of B-Se (≥ 560µg/L) and 20 persons (4.9%) had high levels of P-Se (≥ 328µg/L). Twelve persons, all from the same village, presented B-Se ≥ 900µg/L; among these, ten persons (2.5%) had very high levels of B-Se (≥ 1000µg/L), and nine persons (2.2%) had very high levels of P-Se (≥ 520µg/L). The highest B-Se and P-Se levels were 1500.2µg/L and 913.2µg/L respectively. Figure 27 shows the relation between B-Se and P-Se levels.

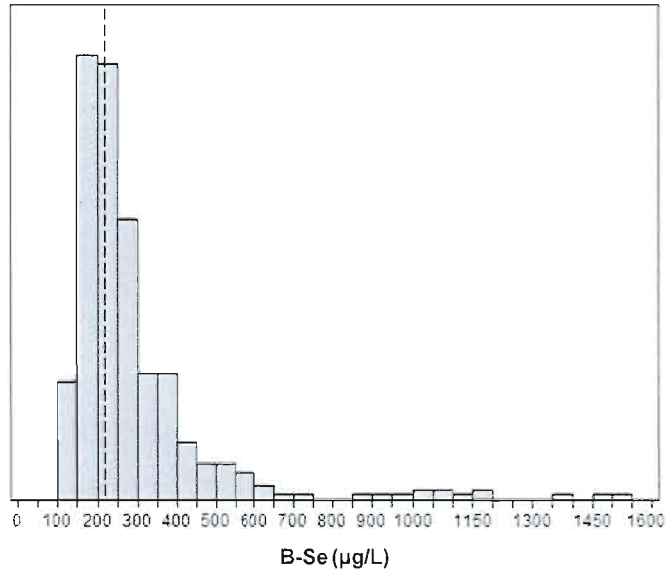


Figure 25 : B-Se distribution
Legend: Pointed line shows the median B-Se level (228µg/L)

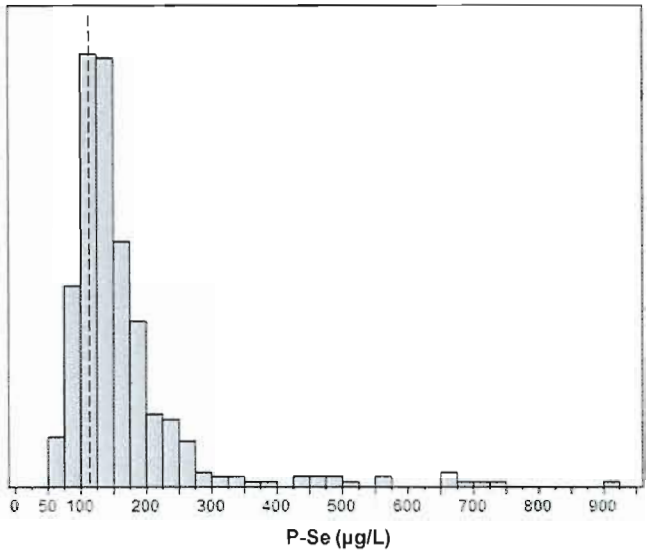


Figure 26 : P-Se distribution
Legend: Pointed line shows the median P-Se level (135µg/L)

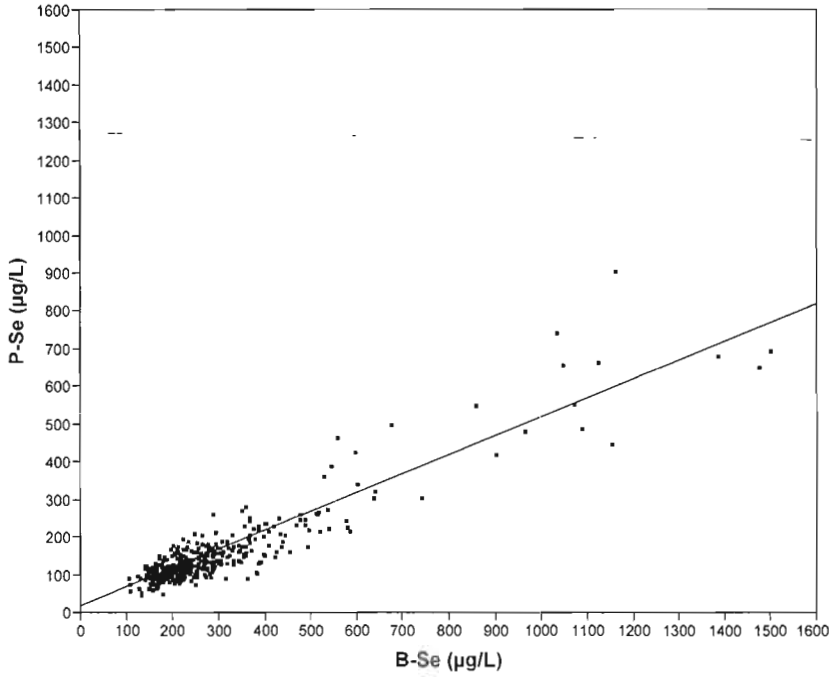


Figure 27 : B-Se and P-Se bivariate plot
Legend: The small pointed line represents the B-Se and P-Se reference values for the UL and the large pointed line represents the B-Se and P-Se references values for the NOAEL. Regression line: P-Se (µg/L) = 18.9 + 0.5 * B-Se (µg/L).

The distribution of socio-demographic characteristics was compared between the three Se status categories. Gender, smoking status, alcohol consumption, education and place of birth were similar across categories. Age, however, was significantly different between the groups: persons with normal Se status were older than those with high and very high Se (*normal*: mean = 42.0 ± 16.4y, range = 15-87y; *high*: mean = 34.0 ± 16.2y, range = 15-70y; *very high*: mean = 35.0 ± 15.0, range = 15-62y; Wilcoxon/Kruskall-Wallis Rank Sum Test $\chi^2 = 5.3$, $P = 0.07$). In the normal Se status group, twenty-one persons (5.2%) were over than 70 years of age, but there were none in the other 2 groups. To ensure similar age distribution between the Se status groups, persons over 70 years of age were excluded from further analysis. When these persons were excluded, all socio-demographic characteristics, presented in Table 9, were similar between Se groups ($P > 0.05$). Overall, a total of 89.1% were born in the State of Pará, while the others came from different areas of Brazil, mostly from the North-eastern states such as Maranhão (6.5%), Ceará (1.3%) and Piauí (0.8%). Few (15.1%) had completed elementary school (8 years) and fewer still (2.6%) had finished high school (11 years), while 6.2% had no formal education.

Tableau 9 : Socio-demographic characteristics of the study population ($N = 386$)

| | Se status | | |
|---|---|---|---|
| | Normal<br>*N* (%) | High<br>*N* (%) | Very high<br>*N* (%) |
| Gender | | | |
| Women | 180 (50.0) | 7 (46.7) | 6 (54.5) |
| Men | 180 (50.0) | 8 (53.3) | 5 (45.5) |
| Born in the region (State of Pará) | 320 (88.9) | 13 (86.7) | 11 (100) |
| Drinks alcohol | 204 (56.7) | 6 (42.9) | 7 (63.6) |
| Current smoker | 95 (26.4) | 2 (13.3) | 4 (36.4) |
| Age (y) (mean ± SD) | 39.9 ± 14.3 | 34.0 ± 16.2 | 35.0 ± 15.0 |
| Education (y) (mean ± SD) | 5.0 ± 3.4 | 5.1 ± 3.1 | 4.7 ± 2.6 |

Table 10 shows the prevalence of the signs of possible selenosis for those with normal, high and very high Se status. No sign was more prevalent in the high and/or very high Se groups compared to those with normal Se (Likelihood Ratio $\chi^2$ Test, $P > 0.05$). Several women had painted fingernails (N = 63) and/or painted toenails (N = 105). When these women were excluded from the analysis, the results remained similar. Since no signs were observed in more than 50% in persons within the high Se groups, we did not perform multiple logistic analyses.

The prevalence of sentinel symptoms and other possible non-specific symptoms of Se toxicity, presented in Table 11, were likewise similar across the three groups (Likelihood Ratio $\chi^2$ Test, $P > 0.05$). Certain reported neurologic symptoms (difficulty to identify an object by touch, hand tremor, muscular twitches and frequent depression) tended to be less prevalent or absent in the high Se groups. Very few participants reported diabetes (N = 3), and all of them had Se status within the normal range. Symptoms that were reported by more than 50% of the persons in the high Se groups were examined in multiple logistic regression models controlling for co-variables; no significant relation with Se status was observed ($P > 0.05$).

Tableau 10 : Prevalence of signs associated with Se toxicity

| Signs | Se status | | |
|---|---|---|---|
| | Normal *N* (%) | High *N* (%) | Very high *N* (%) |
| **HAIR/BODY HAIR** | | | |
| Absence of hair shine | 11 (3.1) | 0 (0.0) | 0 (0.0) |
| Damage with split hairs | 78 (21.7) | 4 (26.7) | 1 (9.1) |
| Dry and brittle, and easily broken at the scalp | 2 (0.6) | 0 (0.0) | 0 (0.0) |
| Sparse head hair | 21 (5.8) | 1 (6.7) | 0 (0.0) |
| Sparse body hair | 163 (45.3) | 9 (60.0) | 4 (36.4) |
| **NAILS** | | | |
| Abnormal fingernails | 149 (41.4) | 7 (46.7) | 5 (45.5) |
| Presenting at least one fingernail whitlow | 52 (14.4) | 1 (6.7) | 2 (18.2) |
| Presenting at least one toenail whitlow | 11 (3.1) | 1 (6.7) | 1 (9.1) |
| **Specific fingernail signs** | | | |
| Longitudinal streaks | 4 (1.1) | 0 (0.0) | 1 (9.1) |
| Transversal streaks | 3 (0.8) | 0 (0.0) | 0 (0.0) |
| Darkening | 5 (1.4) | 0 (0.0) | 0 (0.0) |
| Yellowish or reddish discoloration | 1 (0.3) | 0 (0.0) | 0 (0.0) |
| Abnormal cuticle | 2 (0.6) | 0 (0.0) | 0 (0.0) |
| Break on the wall and nail sloughing | 3 (0.8) | 0 (0.0) | 0 (0.0) |
| Symmetric thickening and stratifying | 8 (2.2) | 0 (0.0) | 0 (0.0) |
| Deformed and brittle (maybe hard but breaks easily) | 8 (2.2) | 0 (0.0) | 0 (0.0) |
| **Specific toenail signs** | | | |
| Longitudinal streaks | 5 (1.4) | 0 (0.0) | 0 (0.0) |
| Transversal streaks | 1 (0.3) | 0 (0.0) | 0 (0.0) |
| Darkening | 3 (0.8) | 1 (6.7) | 1(9.1) |
| Symmetric thickening and stratifying | 7 (1.9) | 0 (0.0) | 0 (0.0) |
| Break on the wall and nail sloughing | 2 (0.6) | 0 (0.0) | 0 (0.0) |
| **SKIN** | | | |
| General irritation or mycosis on the body | 84 (23.3) | 4 (26.7) | 3 (27.3) |
| Yellowish or reddish pigmentation of the skin | 1 (0.3) | 0 (0.0) | 0 (0.0) |
| Discoloration of the skin | 1 (0.3) | 0 (0.0) | 0 (0.0) |
| Red and swollen skin and/or blistered and eruptive: | | | |
| Back of the hands | 4 (1.1) | 0 (0.0) | 0 (0.0) |
| Back of the feet | 14 (4.0) | 0 (0.0) | 0 (0.0) |
| Outer side of the limbs | 19 (5.3) | 1 (6.7) | 0 (0.0) |
| Back of the neck | 3 (0.8) | 0 (0.0) | 0 (0.0) |
| Forehead and/or eyebrows | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| **BREATH** | | | |
| Garlic breath | 56 (15.6) | 3 (20.0) | 1 (9.1) |

Tableau 11 : Prevalence of relevant reported symptoms

| Symptoms | Se status | | |
|---|---|---|---|
| | Normal *N* (%) | High *N* (%) | Very high *N* (%) |
| **HAIR** | | | |
| Dry hair | 215 (59.7) | 10 (66.7) | 6 (54.5) |
| Irritation/Itchiness of the scalp | 208 (57.8) | 11 (73.3) | 5 (45.5) |
| Important hair loss | 89 (24.7) | 4 (26.7) | 4 (36.4) |
| Body hair loss | 16 (4.4) | 0 (0.0) | 0 (0.0) |
| **NAILS** | | | |
| Weak finger nails | 163 (45.3) | 6 (40.0) | 3 (27.3) |
| Weak toenails | 3 (0.8) | 1 (6.7) | 0 (0.0) |
| Break on the wall of the nail | 14 (3.9) | 0 (0.0) | 0 (0.0) |
| Abnormal growth (rough, thick and striped) | 23 (6.4) | 0 (0.0) | 0 (0.0) |
| **SKIN** | | | |
| Irritations and/or eruptions | 97 (26.9) | 2 (13.3) | 2 (18.8) |
| Irritation of the eyebrows | 48 (13.3) | 2 (13.3) | 3 (27.3) |
| Yellowish or reddish pigmentation on the outer side of the limbs | 12 (3.3) | 1 (6.7) | 0 (0.0) |
| Discoloration of the skin | 26 (7.2) | 0 (0.0) | 2 (18.2) |
| **BREATH** | | | |
| Metal taste in the mouth | 105 (29.2) | 5 (33.3) | 3 (27.3) |
| Garlic breath | 26 (7.2) | 1 (6.7) | 1 (9.1) |
| **GASTRO-INTESTINAL DYSFUNCTIONS** | | | |
| Gastric reflux | 185 (51.4) | 8 (53.3) | 6 (54.5) |
| Abdominal cramp | 44 (12.2) | 1 (6.7) | 1 (9.1) |
| Antiacid use | 107 (29.7) | 6 (40.0) | 2 (18.2) |
| Frequent diarrhea | 29 (8.1) | 1 (6.7) | 0 (0.0) |
| **NERVOUS SYSTEM DYSFUNCTIONS** | | | |
| Difficulty to identify an object by touch | 24 (6.7) | 2 (13.3) | 0 (0.0) |
| Tingling in hands, foot and/or mouth | 163 (45.3) | 4 (26.7) | 4 (36.4) |
| Tiredness in legs and/or arms | 198 (55.0) | 6 (40.0) | 5 (45.5) |
| Pain in legs | 125 (34.7) | 6 (40.0) | 5 (45.5) |
| Pain in arms | 74 (20.6) | 4 (26.7) | 2 (18.2) |
| Hand tremor | 104 (28.9) | 5 (33.3) | 0 (0.0) |
| Muscle twitches and/or cramps | 203 (56.4) | 9 (60.0) | 3 (27.3) |
| Joint pain | 237 (65.8) | 11 (73.3) | 8 (72.7) |
| Easy tiredness | 198 (55.0) | 6 (40.0) | 5 (45.5) |
| Memory problems | 235 (65.3) | 9 (60.0) | 8 (72.7) |
| Frequent headache | 69 (19.1) | 2 (13.3) | 2 (18.2) |
| Frequent dizziness | 28 (7.2) | 2 (13.3) | 0 (0.0) |
| Frequent depression | 35 (9.7) | 0 (0.0) | 0 (0.0) |
| Diabetes | 3 (0.8) | 0 (0.0) | 0 (0.0) |

The total number of signs and reported symptoms was calculated and examined with respect to Se status. The average sum of signs was 2.6 ± 1.7 (range 0 – 11), while

the average sum of symptoms was 8.4 ± 4.3 (range 0 – 23). There was no difference in the sum of signs and symptoms between Se categories (Wilcoxon/Kruskall-Wallis Rank Sum $\chi^2 = 2.3$, $P = 0.31$ and $\chi^2 = 1.1$, $P = 0.58$). In multiple regression models, the sum of positive signs was higher for women (Estimate = 0.39, 95%CI [0.22 – 0.55], $P < 0.0001$), increased with age (Estimate = 0.037, 95%CI [0.025 – 0.049], $P < 0.0001$) and was higher for those who drink alcohol (Estimate = 0.20, 95%CI [0.03 – 0.38], $P = 0.02$); there was a tendency towards fewer signs for those with very high Se status compared to the others (Estimate = -1.06, 95%CI [-2.30 – 0.17], $P = 0.09$). The sum of positive symptoms was also higher for women (Estimate = 1.13, 95%CI [0.70 – 1.57], $P < 0.0001$) and increased with age (Estimate = 0.076, 95%CI [0.046 – 0.105], $P < 0.0001$), but no association was observed for alcohol consumption or Se status.

Figure 28 shows the scatterplot of the MCA for reported symptoms, Se status, socio-demographic and lifestyle variables. The explained variance on the two first dimensions was 27.8% (Dimension 1: Cronbach's $\alpha = 0.80$, Eigenvalue = 4.26, explained variance accounted for 20.3%; Dimension 2: Cronbach's $\alpha = 0.39$, Eigenvalue = 1.59; explained variance accounted for 7.5%). The symptom categories were clearly discriminated along the first dimension (main axis of variance). All the categories for the presence of symptoms were positioned in the two right quadrants of the scatterplot while the categories for the absence of symptoms scored on the left quadrants. In addition, a greater dispersion was observed for the presence of symptoms on the right side of the scatterplot compared to the absence of symptoms on the left part. The presence of symptom appears to be divided into 2 clusters between the upper and lower right quadrants (Figure 5). The first cluster in the upper right quadrant included: gastric reflux, metal taste in the mouth, antiacid use, hand tremor and itchiness of the scalp, and the cluster in the lower right quadrant consisted of: frequent headache, tiredness in the legs and/or arms, easy tiredness and pain in the legs.

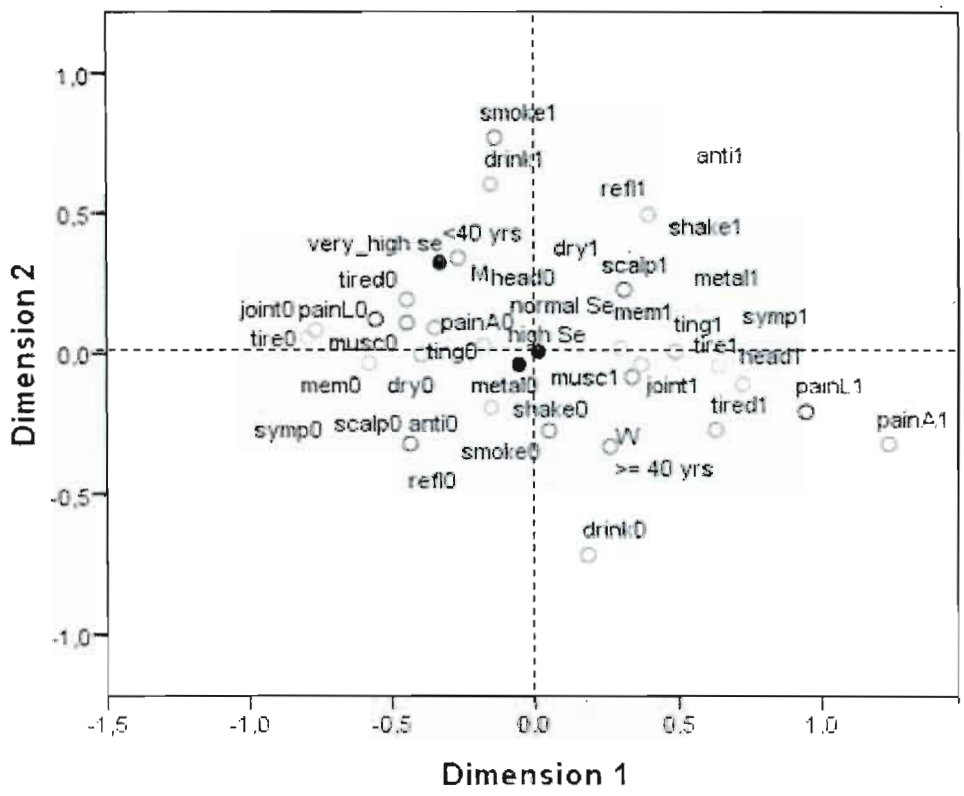


Figure 28 : MCA analysis scatterplot for reported symptoms

Legend: Symptoms variables were coded as presence (1) and absence (0). The following variables were included in the model: dry hair (dry), irritation/itchiness of the scalp (scalp), metal taste in the mouth (metal), gastric reflux (refl), antiacid use (anti), tingling in hands, foot and/or mouth (ting), tiredness in legs and/or arms (tire), pain in legs (painL), pain in arms (painA), hand tremor (shake), muscle twitches and/or cramps (musc), joint pain (joint), easy tiredness (tired), memory problems (mem), frequent headache (head), drinking status (drink), smoking status (smoke), sum of symptoms (<8: symp0 and ≥8: symp1), age (<40yrs and ≥40yrs), sex (M and W) and Se status (black dot: normal Se, high Se and very high Se).

The central position in the scatterplot of the categories of normal and high Se status showed the general characteristics of the study population. Normal and high Se status was not related to the presence or absence of any symptoms. In the upper left quadrant, very high Se status was associated with men younger than 40 years old, and to lesser extent, with the absence of tiredness in the legs and/or arms, pain in the legs, pain in the arms and muscular twitches.

Since the "presence" categories were observed in less than 5% of persons for most signs, we did not perform a MCA for signs recorded by the nurse.

## *4. Discussion*

In this population, there were no apparent signs and symptoms of selenium toxicity, although for many, Se status surpassed concentrations considered toxic. The absence of a relationship between sentinel signs and symptoms of selenosis and high or very high Se status was confirmed by using different statistical approaches. Indeed, multiple regression models with the sums of signs and the MCA scatterplot for reported symptoms showed that high Se status was associated more with beneficial rather than toxic effects. Indeed, those with high and very high Se status reported fewer symptoms of tactile and motor problems. These findings are consistent with other results in this population showing better motor performances with increasing Se status (Lemire et al., chapitre IV) and a lower prevalence of age-related cataracts among those with elevated Se (Lemire et al., chapitre V).

Several reasons could explain why the present findings differ from previous studies showing Se toxicity at similar blood and/or plasma Se concentrations. First, in some studies, Se exposure included inhaled inorganic fumes, which are known to be more toxic (WHO, 1986; Ryan and Terry, 1996). Second, Se compounds in manufactured supplements are highly variable and may be quite different from those in food (Dumont et al., 2006; Rayman et al., 2008; Uden et al., 2003). Third, Se ingested in this region of the Amazon could be toxic, but at higher concentrations than those observed here. Fourth, the population in the present study is highly exposed to mercury (Passos and Mergler, 2008), and, in this context, there may be less 'excess' Se and consequently little or no Se toxicity.

The guidelines on Se toxicity issued by World Health Organization (WHO, 1986), U.S. Institute of Medicine (2000) and U.S. Environmental Protection Agency (EPA, 2002) are mostly based on reports of cases of human chronic selenosis in the 1960's among residents of Enshi County, Hubei Province, in China. Excessive Se exposure resulted from high Se in crops, which were grown on seleniferous soils fertilized with coal ash highly rich in Se (Yang et al., 1983; 1989a), and also from combustion of Se-rich coal for domestic heating and cooking (Liu et al., 2007). Coal combustion is a dirty process that, in addition to Se, can emit arsenic, fluorine, mercury, lead, acidic gases and organic compounds (Finkelman, 2007). These outbreaks were partly due to a failure in rice crops caused by a drought, forcing villagers to consume high-Se maize and vegetables and less protein-rich foods. Locally produced foods contained the highest concentrations of Se ever reported in corn (28.5μg/g), soybean (22.2μg/g) and rice (20.2μg/g) (Yang et al., 1983), in which selenomethionine (SeMet) was the main Se species (Beilstein et al., 1991). Even the drinking water, which leached through seleniferous coal seams, contained unusually high concentrations of Se (from 117 to 159μg/L), almost totally in the form of selenate (Yang et al., 1983). Thus, several factors such as protein and nutrient deficiency, exposure to inorganic Se vapors from coal combustion and/or inorganic Se exposure from drinking water may have contributed to toxic Se effects observed in China, initially attributed primarily to high organic Se in local crops (Whanger, 1989; Longnecker et al., 1991; Poirier, 1994; ATSDR, 2003). In areas of China where high-Se coal is still used for heating and cooking, cases of selenosis are still reported (Liu et al., 2007). Se toxicity has also been reported in occupational settings (involving metal processing, micro-electronics, agro-chemicals and/or fossil fuel burning), where workers are exposed to inorganic Se. Inhaled inorganic Se fumes, directly entering through the bloodstream, are more acutely toxic than ingested Se, which undergoes homeostatic regulation for selenoprotein synthesis (Barceloux, 1999; WHO, 1986; Ryan and Terry, 1996).

There is increasing evidence suggesting that different toxicological profiles are associated to each form of ingested Se. The inorganic forms (selenite, selenate, etc.) appear to be more toxic than organic forms, and some metabolites may be even more toxic than the original ingested form (Rayman, et al., 2008). Recent studies on Se toxicity mostly refer to excessive Se intake from nutritional supplements, ingestion of nutritional supplements with Se formulation error or inorganic Se through drinking water (Ryan and Terry, 1996; Reid et al., 2004; Sutter et al., 2008; Schuh and Jappe, 2007; Vinceti et al., 1995). Severe cases of acute selenosis, but without neurologic disorders, were reported from Se supplements at levels below those observed in the Chinese outbreak described above (Yang et al., 1983; 1989), with serum Se levels between 352μg/L and 534μg/L (Sutter et al., 2008; Schuh and Jappe, 2007). Se supplements contain sodium selenite or commercial selenized yeast. In yeast, most of the Se is bound to proteins (mostly as SeMet species), however depending on the quality of the manufactured product, it may contain high levels of sodium selenite rather than SeMet (Uden et al., 2003). Certain authors consider that Se content on the product label may be severely understated and, in many studies, no information is available regarding supplements' chemical Se formula (Pedrero and Madrid, 2009; Dumont et al., 2006).

The advisability of supplementing individuals with already-replete Se status has recently been reconsidered by several authors (Laclaustra et al., 2009a; Lippman et al. 2009; Rayman, 2009). Two years ago, the Selenium and Vitamin E Cancer Prevention Trial (SELECT) reported an association between long-term supplementation with Se-enriched yeast (P-Se > 121.6μg/L) and the incidence of self-reported type 2 diabetes (Stranges et al., 2007). Laclaustra et al. (2009b) showed an association between persons from the general US adult population with serum Se levels above 147μg/L and incidence of type 2 diabetes. In this latter study, more than 60% used Se dietary supplements with unknown Se composition. High selenium status has also been linked with hypertension in the U.S. (Laclaustra et al., 2009a) and hypercholesterolemia in the U.S. and the U.K. population (Bleys et al., 2008; Stranges

et al, 2010). It should be noted that clinical trials on Se supplementation do not necessarily consider pre-morbid Se status and possible selenoprotein genotypes (Rayman, 2009) as well as the exact composition of Se supplements.

Reports on Se toxicity resulting from local crops grown in rich or very rich-Se areas are scarce and contradictory. In a seleniferous area of Venezuela, a few cases of mild selenosis (hair and nails) were reported in children who consumed local crops; their blood Se concentrations did not exceed 813μg/L (Bratter et al., 1991; Jaffe et al., 1972). The authors considered this high dietary Se intake to be safe (Bratter et al., 1991). In western South Dakota and Eastern Wyoming, where human cases of selenosis might have occurred in the 1930's (Fan and Kizer, 1990), no evidence of Se toxicity was observed at Se dietary intake as high as 724 μg/day (approx. 800μg/L of B-Se) (Longnecker et al., 1991). In the Nawan Shar District of Punjab in India, where Se intake from local crops may be as high as 1200μg/day, some signs of selenosis in hair and nails were reported, although the clinical data presented were not detailed (Hira et al., 2004).

Chemical forms of Se can vary markedly in foods. Several organic forms of Se are synthesized by plants, mostly SeMet, but also selenocysteine (SeCys), Se-methyl-SeCys, γ-glutamyl-Se-methyl-SeCys, and others, depending on the plant species. Plants can also directly accumulate selenate ($Se^{6+}$) from soils. In animal tissues, the most important Se compounds are SeMet and SeCys, although there appears to be high variation between species (Whanger, 2002). Although there are several publications on Se speciation in foods and analytical techniques have appeared in the last years, isolation, accurate characterization and quantification of Se species remains a challenge (Pedredo and Madrid, 2009; Rayman et al., 2008).

Certain edible plant species such as *Lecythidaceae* family and *Brassica* species have the ability to accumulate high to very high levels of Se. In the communities along the Tapajós River, Brazil nuts are the most important source of Se, with Se content

varying between 0.4 and 158.4µg/g (Lemire et al., chapitre II). SeMet appears to be the major Se compound in Brazil nuts (see reviews by Rayman et al., 2008 and Dumont et al., 2006). Other less important sources include some fish species and other local foods such as chicken, eggs, game meat and meat. They also contain considerable levels of Se (0.2–1.4µg/g) (Sampaio et al., manuscript in preparation; Lemire et al., chapitre II) and represent a good dietary source of a mixture of organic Se species.

Se toxicity has been associated with the consumption of other *Lecythidaceae* species. Kerdel-Vegas (1966) described symptoms of acute intoxication such as fever, vomiting, dizziness, followed by temporary alopecia (abundant hair, body hair and nail loss) in persons who had consumed monkey's nuts (*Lecythis ollaria* Loefl) from the state of Portuguesa in Venezuela; no information was available on biomarkers of Se status. Unlike Brazil nuts, selenocystathionine was the main form of Se identified in these nuts (Aronow and Kerdel-Vegas 1965; Ferri et al, 2004), and in *Lecythis minor* nuts (Dernovics et al., 2007). A case of selenosis with similar signs and symptoms was reported in Honduras by a scientist who had consumed several nuts of *Lecythis elliptica* H.B.K. (Dickson. 1968). While no information was available on the quantity of Se in the nuts ingested in the latter intoxication, monkey's nuts can contain up to 5,800µg/g of total Se (Andrade et al., 1999), which is considerably higher than the maximum concentrations for Brazil nuts assessed in the present study. The highest reported concentration in Brazil nuts is 512.0µg/g (Chang et al., 1995). In light of these studies, the absence of signs and symptoms of Se toxicity in the present study on the Tapajós suggests that although Se in food might be toxic, it would require much higher concentrations than those that were measured herein. The 'primary Se-accumulator plants', such as the *Lecythis* species implicated in the intoxications described above, are known to synthesize by-products of Se to avoid toxic effects of Se by excessive incorporation of SeMet and SeCys into plant proteins (Fordyce, 2005). These plants are well-known to be toxic for livestock; it is not clear whether toxicity results from very high Se (> 1000µg/g) or their particular Se compounds (Tinggi, 2003; Rayman et al., 2008).

Populations living in the far North have a diet that is exceptionally rich in Se from consumption of marine mammals, seabirds, fish, and the whale skin delicacy, *muktuk*, whose Se content can reach 47.9μg/g (Dietz et al., 1997). Compared to diets based on cereal or rice crops, Se of marine origin probably contains a greater fraction of SeCys (Rayman, 2008). Epidemiologic studies indicate normal to very high Se status (ranging from 80 to 3100μg/L in whole blood) (Bélanger et al., 2006; Grandjean et al., 1992; Hansen and Pedersen 1986; Hansen et al., 2004; Muckle et al., 2001). This large range of blood Se is similar to the concentrations that we observed in the Brazilian Amazon. It is noteworthy that Hansen et al. (2004) pointed out that there are no recorded signs of Se toxicity in Greenland despite high blood Se, suggesting that high Se intake from marine diet can be tolerated at much higher levels than the daily allowances normally accepted.

There are some similarities between the Northern and Amazonian populations. The most important one is that both groups are exposed to high levels of dietary mercury (Mergler et al., 2007). Populations with high mercury may require higher levels of dietary Se to offset Hg-mediated oxidative stress and/or to maintain optimal Se antioxidant enzymes and other Se physiological activities (Watanabe, 2001, 1999a,b; Raltson et al., 2008a; Fordyce, 2005). In a situation with elevated Hg and Se, there may be less 'excess' of Se and consequently little or no Se toxicity (Khan and Wang, 2009). Furthermore, Se-rich food sources have always been part of their diet and it is possible that through homeostatic adjustment of this essential element, persons chronically exposed to Se may be able to adapt to higher Se status (ATSDR; 2003; WHO, 1986), and may even develop mechanisms to take advantage of such high Se intake (Mattson, 2008).

One of the differences, however, between the present study and those in Greenland is that in the Amazonian communities, there was a linear relation between whole blood Se and plasma Se, while Hansen et al. (2004) observed a plateau effect around 150μg/L in blood in Greenland populations. These differences may be due to

the fact that the most important Se species in Inuit diet is SeCys (Rayman et al., 2008), while, in the Tapajós populations, most of the Se dietary intake is probably SeMet, which undergoes non-specific accumulation in blood protein constituents rather than being excreted. Contrary to SeCys intake, SeMet may not entirely go through the homeostatic regulation processes; SeMet can be either reduced to hydrogen selenide for selenoprotein synthesis or nonspecifically replace methionine into proteins of plasma (mainly in albumin) and blood (mainly in erythrocytes) (U.S. Institute of Medicine, 2000), with unknown physiological activity (Laclaustra et al., 2009a). This nonspecific accumulation of Se may also act as a storage pool of Se, which can be slowly released during protein turnover to maintain Se requirements over a longer period (Xia et al., 2005). However, it is unclear whether SeMet is first accumulated into the methionine pool and further released or if SeMet nonspecific accumulation only occurs when dietary intake is high and the organism's requirements for selenoprotein synthesis have been met.

It is not clear at which levels food Se intake may become unsafe. Our data in the Lower Tapajós region shows no deleterious effect of Se, and even beneficial effects of high Se status on health. These results support the need to re-assess Se toxicity taking into account a number of factors, including the route of exposure (inhaled versus ingested), chemical form of Se exposure, co-exposures to elements such as mercury and lead, other dietary intakes, notably anti-oxidants, the type of exposure (acute versus chronic), individual vulnerability and possible adaptation.

# CHAPITRE IV

## Les effets bénéfiques du sélénium venant des aliments sur les fonctions motrices en Amazonie brésilienne

## *Beneficial effects of dietary selenium on motor functions in the Brazilian Amazon*

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## *Résumé*

Le sélénium (Se) est un oligoélément qui constitue le centre actif de plusieurs sélénoenzymes antioxydantes qui sont indispensables pour le cerveau. La déficience a été associée à des problèmes cognitifs et l'excès de Se, à la paresthésie. Chez les riverains du Bas-Tapajós, le statut de Se varie de normal à très élevé et d'importantes sources locales, telles que les noix du Brésil, le poisson, le poulet et la viande de chasse, ont été identifiées. Les communautés riveraines d'Amazonie ont actuellement l'exposition au Hg la plus élevée rapportée dans le monde. De plus, une exposition élevée au plomb (Pb) a récemment été identifiée chez ces riverains. De nombreuses études animales soutiennent que le Se peut interagir avec le Hg ou le Pb, et contrer les effets toxiques de ces derniers. Les quelques études sur les effets du Se chez les populations exposées au Hg sont toutefois mitigées. L'objectif de la présente étude est d'examiner les relations entre les bioindicateurs de Se et les fonctions motrices, en tenant compte des covariables et des bioindicateurs d'exposition au Hg et au Pb.

Un total de 448 participants, âgés de 15 à 87 ans, ont été recrutés dans 12 communautés. Les concentrations de Se ont été mesurés dans le sang, le plasma, les cheveux et l'urine par ICP-MS. Les niveaux sanguins de Hg et de Pb ont aussi été mesurés par ICP-MS. Un questionnaire portant sur les données sociodémographiques et l'histoire médicale a été administré. Tous les participants ont reçu un examen visuel complet et réalisé une batterie de tests moteurs incluant le mouvement alterné de Branches, le *Santa Ana*, le dynamomètre et le *Grooved Pegboard.*

Les niveaux de Se sanguins variaient entre 103.3–1500.2µg/L (médiane 228.4µg/L), de Se plasmatique entre 53.6–913.2µg/L (médiane 134.8µg/L), de Se dans les cheveux entre 0.4–3.8µg/g (médiane 0.7µg/g) et de Se dans l'urine entre 2.3 – 1375.0µg/g creat. (médiane 33.6µg/g creat.). Les médianes de Hg et Pb sanguins étaient de 42.5µg/L et de 113.0µg/L respectivement. Les bioindicateurs de Se étaient positivement reliés à de meilleures performances pour tous les tests moteurs dans les analyses multivariées tenant compte des variables socio-démographiques et des bioindicateurs de Hg et de Pb. Les coefficients de régression pour les bioindicateurs de Se étaient considérablement plus élevés lorsque les modèles étaient contrôlés pour le Hg sanguin, et dans un cas pour le Pb sanguin. Toutefois, aucune interaction entre les bioindicateurs de Se, Hg et Pb n'a été observée. Le Se dans le plasma était le meilleur bioindicateur associé aux performances motrices pour tous les tests.

Cette étude est la première à rapporter des effets bénéfiques du Se sur les fonctions motrices. L'apport alimentaire élevé de Se pourrait être essentiel aux les fonctions cérébrales lorsque l'exposition au Hg est élevée. Les associations étaient principalement observées avec les niveaux de Se dans le plasma, ce qui suggère que le Se dans le plasma ou les sélénoprotéines plasmatiques peuvent constituer de meilleurs bioindicateurs d'effet du Se sur ces fonctions de l'organisme comparé aux autres bioindicateurs.

## *Abstract*

Selenium (Se) is an essential element and a well-known anti-oxidant with a critical role in the proper functioning of the nervous system. Se deficiency and excess have been associated with cognitive impairment and paraesthesia, respectively. In the Lower Tapajós Region of the Brazilian Amazon, Se status of riverside populations ranges from normal to very high. Important local dietary Se sources, such as Brazil nuts, fish, chicken and game meat, have been identified. These fish-eating communities have among the highest mercury (Hg) exposures reported in the world today, and elevated lead (Pb) exposure was recently been identified. Experimental studies suggest that Se intake can interact with mercury (Hg) and/or lead (Pb) to protect against their toxicity. However, epidemiological data from human studies is inconsistent. The objective of the present study was to examine the relations between biomarkers of Se and motor functions, taking into account co-variables and biomarkers of exposure to Hg and Pb.

Participants (n = 448), aged 15-87y, were recruited from 12 communities along the Tapajós River. Se concentrations were measured in blood (B-Se), plasma (P-Se), hair (H-Se) and urine (U-Se) by ICP-MS. Blood Hg (B-Hg) and Pb (B-Pb) concentrations were also measured by ICP-MS. Interview-administered questionnaires served to collect information on socio-demographics and medical history. All participants underwent a complete visual examination and performed tests of motor functions (Branches Alternate Movement Task, Santa Ana, Dynamometer and Grooved Pegboard).

B-Se varied from 103.3–1500.2µg/L (median 228.4µg/L), P-Se from 53.6–913.2µg/L (median 134.8µg/L), H-Se from 0.4–3.8µg/g (median 0.7µg/g) and U-Se from 2.3–1375.0µg/g creat. (median 33.6µg/g creat.). Median B-Hg and B-Pb levels were 42.5µg/L and 113.0µg/L respectively. In multivariable analysis, Se biomarkers were positively related to a better performance on all motor tests, taking into account socio-demographic co-variables and B-Hg and B-Pb levels. Indeed, regression coefficients for Se biomarkers were considerably stronger when controlling for B-Hg, and in one case for B-Pb, although no interaction between Se, Hg or Pb biomarkers were observed. P-Se was the biomarker which was consistently better related to improved performances on all tests.

This is the first human study to report beneficial effects of Se on motor functions. High dietary Se intake may be critical for brain functions when Hg exposure is high. The associations were mostly observed with P-Se, suggesting that P-Se or plasmatic selenoproteins may be better biomarkers than other Se biomarkers for these outcomes.

## *1. Introduction*

In the last decade, several authors have suggested that Se may be a potent protective agent for neurons through selenoproteins' expression in the brain (Chen et Berry, 2003; Rayman et al., 2008; Schweizer et al., 2004a; Whanger, 2001). Selenoproteins are mostly involved in antioxidant defense, thyroid homeostasis and regulation of redox status under physiological conditions (Papp et al., 2007).

Both Se deficiency and excess can lead to neurologic disorders. Low Se status in the elderly from French and Chinese populations has been associated with senility and accelerated cognitive decline (Akbaraly et al., 2007; Berr et al., 2000; Gao et al., 2007). Since the brain is deficient in catalase, antioxidant selenoenzymes may be essential in removing peroxidation products such as $H_2O_2$ and lipid peroxides (Rayman, 2005). Conversely, in Chinese populations, severe cases of selenosis were characterized by sensory and motor loss (Yang et al., 1983). The oxidation of the excessive endogenous Se may lead to the production of reactive oxygen species, with related deleterious health effects (Barceloux, 1999). Adequate Se intake appears to play a critical role in the proper functioning of the nervous system and the physiologic functions of Se in the human brain. There is a need to better understand this role in order to determine optimal Se intake (Rayman et al., 2008; Chen et Berry, 2003).

On one hand, experimental models have shown that insufficient brain Se supply, resulting in reduced selenoproteins production, can exacerbate neuronal loss and dysfunctions induced by endogenous or exogenous stimuli, trauma and other neurodegenerative conditions such as heavy metal exposures (Schweizer et al., 2004a; Chen et Berry, 2003). On the other hand, animal studies suggest that high Se intake may alleviate mercury (Hg) and/or lead (Pb) toxicity in the brain and/or reduce Hg- and Pb-mediated oxidative stress (Ralston et al., 2008; Ganther et Sunde, 2007;

Watanabe et al, 1999a,b; Ahamed et Siddiqui, 2007). However, epidemiological data in populations exposed to Hg and/or Pb is inconsistent (Choi et al., 2008; Steuwerwald et al, 2000; Després et al, 2005; Saint-Amour et al, 2006).

In riverside populations of the Brazilian Amazon, Se status range from normal to very high (Soares de Campos et al., 2002; Lemire et al., 2006; 2009; Pinheiro et al., 2005), and important local dietary Se sources, such as Brazil nuts, chicken, game meat, fish, eggs and beef, have been identified (Lemire et al., chapitre II). These fish-eating populations also have among the highest reported mercury (Hg) exposures in the world today (Passos et Mergler, 2008). Visual and motor decline have been related to this chronic exposure (Dolbec et al., 2000; Lebel et al., 1996, 1998). In the Lower Tapajós region, recent findings have also revealed elevated lead (Pb) exposure (Barbosa et al. 2009).

Since plasma Se levels tend to saturate at high Se intake (Hansen et al, 2004; Yang et al, 1989b), most studies have used whole blood to evaluate the relation between Se and health effects when Se status tend to be elevated. However, there is little information available on plasma Se, urine Se and hair Se levels in relation to health outcomes in such conditions. The objective of the present study is to examine the relations between different biomarkers of Se and motor functions in riverside population of the Lower Tapajós region, taking into account co-variables and environmental exposures to Hg and Pb.

## *2. Methods*

### Study population

This cross-sectional study is part of a larger project on factors that affect human Hg exposure and its health effects (CARUSO, 2009). In the Lower Tapajós River Basin (State of Pará, Brazil), there are approximately 50 communities of

diverse size and origin, with varying access to health care, education, local authorities and cities. Based on sample size calculations for visual outcomes from preliminary studies, we sought a minimum of 400 people to test Hg and Se interaction effects. We selected 12 communities to obtain this sample size as well to reflect the diversity of regional populations, social conditions and ecosystems (presented in Figure 29). Recruitment of persons 15y and older was carried out in each village through home visits and village meetings, during which the research project was explained and villagers were invited to participate on a voluntary basis. No *a priori* conditions were imposed, although preference was given to those who had participated in a previous study (Passos et al., 2008).

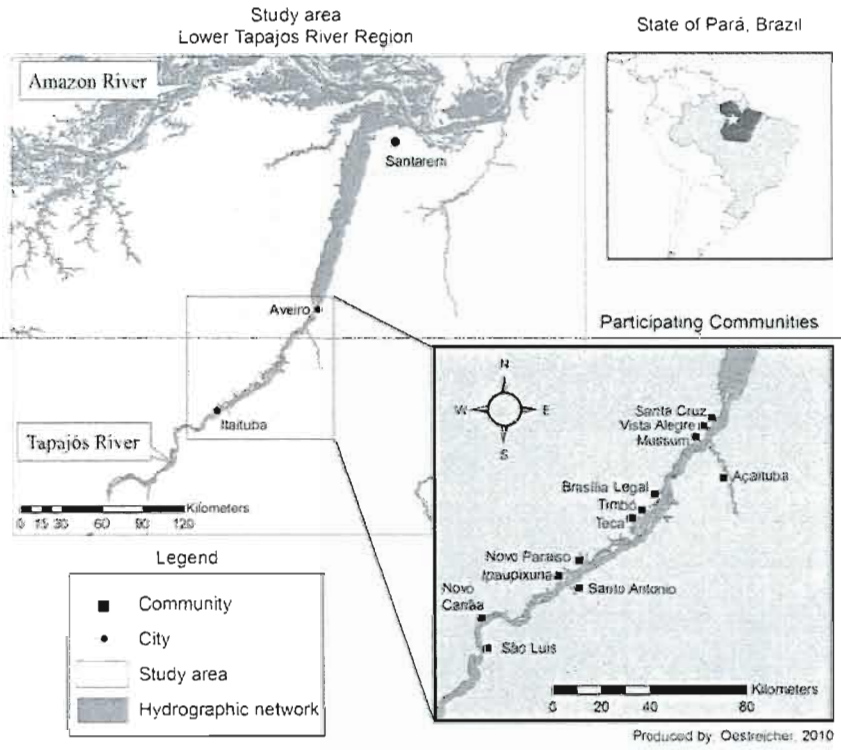


Figure 29 : Study area

The study was carried out from May to August, 2006, in a technical college, located in the nearby city of Itaituba. A total of 448 persons (216 men and 232 women) participated in the present study. Based on a house-to-house survey carried out by our research group in 2003, this represents 25% of the adult population in

these villages (27% of all women and 23% of all men) participated in the study. The participation within each village varied from 10 to 67%, with higher relative frequencies in the smaller villages (the smallest consisted of 13 adult residents and the largest, 384 adults). Since recruitment favored participants from our previous studies, younger persons (≥ 15y and < 40y) were underrepresented with respect to the age distribution of the entire population (50% vs. 62%), those between to 40y to 65y were overrepresented (40% vs. 28%), while the oldest group (≥ 65y) was similar to the underlying population (10%).

The study was approved by the Ethics Review Boards of the University of Quebec at Montreal and the Faculty of Pharmaceutical Sciences of the University of São Paulo-Ribeirão Preto. All participants signed an informed consent form, which was read to them. There was no remuneration for study participation.

## Socio-demographic characteristics

All participants went through an interview of approximately one hour. Trained interviewers administered a questionnaire to collect socio-demographic information, occupational and residential history. A nurse administered the questionnaire on medical history. Research assistants visited each participant in their homes and noted the names of all currently used medication (Appendice B).

## Biomarkers' assessment

*Blood.* For each participant, an experienced Brazilian phlebotomist collected a 6mL blood sample in "trace metal free" evacuated tubes (BD Vacutainer®), containing heparin as anticoagulant. For plasma separation, blood samples were centrifuged (800 x g for 6 minutes). Plasma fractions were then pipetted into previously cleaned Eppendorf tubes (2 mL), and immediately frozen at -20°C. Blood total Hg (B-Hg), blood Se (B-Se) and blood lead (B-Pb), as well as plasma total Hg

(P-Hg) and plasma Se (P-Se) were determined by inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer DRC II) according to the method proposed by Batista et al. (2009), at the *Laboratório de Toxicologia e Essencialidade de Metais, Universidade de São Paulo*, Ribeirão Preto, SP, Brazil. Quality control was guaranteed by analyzing various secondary reference materials, provided by the New York State Department of Health's Proficiency Testing program for trace elements in whole blood, and from the external quality assessment scheme (EQAS) for trace elements operated by the *Institut National de Santé Publique du Québec*, Canada. Reference materials were analyzed before and after ten ordinary samples. Measured values were always within the provided reference or certified values.

*Hair.* Hair has often been used as a biomarker for current and retrospective exposure to Se (Lemire et al 2009). Two hair strands from the occipital region were cut next to the scalp with stainless steel scissors and then placed in plastic bags, with the root end stapled. One hair strand (first 2 cm) was used to determine total hair Se concentration (H-Se) by inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer DRC II) according to the method proposed by Rodrigues et al. (2008), at the *Laboratório de Toxicologia e Essencialidade de Metais, Universidade de São Paulo*, Ribeirão Preto, SP, Brazil. Quality control was guaranteed by analyzing the Standard Reference Material (Human Hair 086), provided by the International Atomic Energy Agency (IAEA). Reference material was analyzed before and after ten ordinary samples. Measured values were always within the provided reference or certified values.

*Urine.* Urine was collected in sterile metal-free plastic recipients, and then kept frozen at -20°C until analysis. Urinary total Se (U-Se) was determined ICP-MS (Perkin Elmer DRC II) according to the biomonitoring method proposed by Heitland and Koster (2006), at the *Laboratório de Toxicologia e Essencialidade de Metais, Universidade de São Paulo*, Ribeirão Preto, SP, Brazil. Quality control was guaranteed by analyzing the Standard Reference Material (NIST 2670a Toxic Metals

in Urine), provided by the National Institute of Standards and Technology (NIST), USA. The Urine Reference Material was analyzed before and after ten ordinary samples. Measured values were always within the provided reference or certified values. Creatinine was determined in urine by spectrophotometric flow-injection analysis with using the Jaffe reaction in accordance with the method proposed by Sakai et al. (1995). The World Health Organization (WHO) recommends that if a sample is too diluted (creatinine concentration < 30 mg/dL) or too concentrated (creatinine concentration > 300 mg/dL), the urine sample should be excluded (WHO, 1996). For correction purposes, U-Se levels were divided by the creatinine individual value.

*Guidelines for Se status.* Se deficiency has been set at P-Se < 70μg/L, when supply for selenoprotein synthesis began to be limiting (U.S. Institute of Medicine, 2000). The Tolerable Upper Intake level (UL) has been set at 500μg/day (U.S. Institute of Medicine, 2000), corresponding to 560μg/L in whole blood (Yang and Xia, 1995). The No Observable Adverse Effect Level (NOAEL) has been set at 1000μg/L of B-Se (Poirier, 1994). The reported normal range for H-Se is between 0.1 and 5.0 μg/g (WHO, 1994). For urine, the absence of symptoms of toxicity is usually associated with U-Se < 100μg/L, while > 400–700μg/L are considered excessive (WHO, 2001).

## Ocular health and near visual acuity assessment

Ocular health examinations were carried out by clinical optometrists from the School of Optometry of the University of Montreal. The anterior segment of the eye was examined using slit-lamp biomicroscopy. The posterior segment of the retina and its periphery were examined during pupillary dilation using two mydriatic agents (tropicamide 1% and phenylephrine 2.5%) with Volk lenses, through binocular indirect and direct ophthalmoscopy. Iridocorneal angles were noted and intra-ocular pressure was measured with a Perkins tonometer under topical anaesthesia. The eye

examination served to identify ocular pathologies and trauma for *post hoc* exclusion. Near visual acuity (NVA) at 40 cm was assessed using the Allen Chart and far visual acuity (FVA) at 6 m was assessed using the E directional chart. For the purpose of statistical analysis, NVA and FVA were log transformed to obtain the minimum angle of resolution (logMAR).

## Motor functions assessment

Testing was carried out at the technical school of Itaituba, the main urban center of the region. Tests were administered by trained Brazilian students, who did not know the participants' Se, Hg and Pb levels. The evaluation was carried out in the same order for each participant. The dominant hand was designated as that which the person used to hammer, to fish and/or to pick up small items.

*Branches Alternate Movement Task (BAMT).* This test was developed by Dr. Fernando Branches to assess motor coordination in mercury exposed patients (Lebel et al., 1998). After receiving an explanation of how to perform the test, participants had the opportunity to practice the movement before being tested. The number of repetitions in a 30 second trial was registered.

*Santa Ana manual dexterity test.* The Santa Ana test (Helsinki version) was used to determine manual dexterity (Hänninen and Lindstrom, 1979). The task consisted of lifting up square pegs with circular tops, turning them 180 degrees and replacing them in their holes as rapidly as possible. Following a training period, participants went through two trials of 30 seconds for each hand. The number of pegs successively turned was recorded. The number of pegs successfully turned 180 degrees in 30 seconds for the two trials were averaged for the dominant and non dominant hand.

*Dynamometer test.* Maximum grip strength was assessed for both hands with a dynamometer (Lafayette Instruments, Model 78010) over two trials. Mean values for

the two trials were calculated for each hand. Participants were required to maintain maximum grip strength and performance was registered in kg.

*Grooved Pegboard test.* The Grooved Pegboard test (Lafayette Instruments, model 32025) was used to assess manual dexterity and fine eye-hand movement. The participant was asked to place 25 pins into holes, as quickly as possible. The pins were key-shaped and had to be rotated to fit into the holes. The test was performed first with the dominant hand and then with the non dominant hand for 2 trials. The time required by the participant to complete this test was recorded. Mean time for the two trials constituted the score for each hand. If the participant did not complete the test within 5 minutes, the scores were not considered for the present analyses. Since the test requires fine eye-hand coordination, persons with near visual acuity greater or equal to 20/40 for at least one eye were excluded from analyses, as were participants diagnosed with ophthalmic abnormalities and visual diseases that affect the macula.

## Statistical analyses

General exclusions were: pregnant and breastfeeding women ($N$ = 15), reported stroke ($N$ = 14), taking psychotropic medication ($N$ = 7) and missing data for blood or plasma biomarker ($N$ = 5). A total of 407 participants, 204 women and 203 men, were included in the present study.

Descriptive analyses were performed to characterize the population, biomarkers of Se, Hg and Pb and performances on each test. One-way ANOVA (F ratio) were used to test the difference of age distribution between genders. Non-parametric Wilcoxon/Kruskall-Wallis tests ($\chi^2$), non-parametric correlational statistics (Spearman's $\rho$) and contingency tables ($\chi^2$ Likelihood Ratio) were used to evaluate the associations between continuous (eg. biomarker levels) and binary variables (eg. socio-demographic variables) when some variables did not display a normal distribution.

Biomarker variables were likewise log-transformed (log10). Pearson correlations and simple regression models were used to evaluate the relationship between neurofunctional test performances and biomarkers of Se status. In addition, multiple linear regression and nominal logistic regression analyses were used to evaluate the relationship between biomarkers of Se and neurofunctional test performances, taking into account relevant co-variables. Age, sex, smoking status, alcohol consumption, and years of formal education were included in the first model (Model 1). In the Model 2, we also controlled for B-Hg levels, while in the Model 3, we controlled Model 1for B-Pb levels. In the Model 4, both B-Hg and B-Pb levels were added to the first one. The relations between neurofunctional test performances and the interactions term between Se biomarker variables and B-Hg or B-Pb variables were likewise tested in these models. Studentized residuals greater than 3 and lower than - 3 were considered as outliers, and excluded from the analysis. Results were defined as statistically significant at $P < 0.05$. Analyses were performed using JMP 5.0.1 software (SAS Institute Inc.).

## *3. Results*

Characteristics of the study population are presented in Table 12. Mean age of the participants was 41.5 years old and no difference in age distribution was observed between women and men (40.3y [95% CI: 38.1 to 42.6] and 42.6y [95% CI: 40.4 to 44.9] respectively; F ratio = 2.0, $P = 0.16$). Few participants (11.8%) had completed elementary school (8 years) and fewer still (2.5%) had finished high school (11 years), while 7.9% reported no formal education.

Tableau 12 : Characteristics of the study participants and neurofunctional test parameters

| | *N* | Mean (95% CI) | Range |
|---|---|---|---|
| Age, y | 407 | 41.5 (39.1 to 43.1) | 15 to 87 |
| Gender, *N* (%) | | | |
| Women | 204 (50.1) | | |
| Men | 203 (49.9) | | |
| B-Se, µg/L | 407 | 287.6 (269.0 to 306.1) | 103.3 to 1500.2 |
| P-Se, µg/L | 407 | 163.3 (153.2 to 173.5) | 53.6 to 913.2 |
| H-Se, µg/g | 393 | 0.84 (0.79 to 0.89) | 0.38 to 3.81 |
| U-Se, µg/g creatinine | 319 | 57.0 (45.7 to 68.3) | 2.3 to 1375.0 |
| B-Hg, µg/L | 407 | 51.0 (47.5 to 54.6) | 1.7 to 288.9 |
| B-Pb, µg/L | 407 | 132.7 (124.3 to 141.0) | 5.9 to 483.1 |
| BAMT, no. of repetitions | 320 [a] | 58.7 (57.4 to 60.0) | 20 to 90 |
| Santa Ana, no. of pegs | | | |
| Dominant hand | 399 [a] | 18.0 (17.6 to 18.4) | 3.5 to 27.0 |
| Non dominant hand | 396 [a] | 17.9 (17.5 to 18.3) | 7.5 to 28.0 |
| Dynamometer, kg | | | |
| Dominant hand | 396 [a] | 28.8 (28.1 to 29.6) | 7.5 to 51.5 |
| Non dominant hand | 398 [a] | 28.3 (27.5 to 29.1) | 6.0 to 53.5 |
| Grooved pegboard, sec | | | |
| Dominant hand | 174 [a] | 58.7 (57.6 to 60.0) | 41.5 to 84.5 |
| Non dominant hand | 176 [a] | 63.1 (61.8 to 64.4) | 47.0 to 94.5 |
| Education, y | 405 | 4.9 (4.5 to 5.2) | 0 to 16 |
| Alcohol consumption, *N* (%) | 406 | | |
| Drinkers | 221 (54.4) | | |
| Nondrinkers | 185 (45.6) | | |
| Smoking habit, *N* (%) | 407 | | |
| Current smoker | 107 (26.3) | | |
| Non- or exsmoker | 300 (73.7) | | |

[a] Number of persons after exclusions for specific criterias of each test and studentized residuals outliers (> 3 or < -3) in multiple regression models involving B-Se or P-Se biomarkers.

In this population, very few (N = 6) had deficient P-Se levels, with P-Se levels varying between 53.6 and 70.0µg/L. Most (94.3%) had B-Se levels in the normal range, however, 13 participants (3.2%) had between B-Se levels between 560 and 1000µg/L and 10 individuals (2.5%) had B-Se levels over 1000µg/L. All participants had H-Se levels between 0.1 and 5.0 µg/g and 291 participants (71.0%) had U-Se levels below 100 µg/g creatinine, while 30 participants (7.3%) had U-Se over 400 µg/g creatinine.

Biomarkers of Se did not vary with gender, smoking habits and the years of education. B-Se and H-Se levels decreased with age (Spearman's $\rho = -0.11$, $p = 0.03$ and $\rho = -0.13$, $p = 0.008$ respectively), although this was not the case for P-Se and U-Se levels ($\rho = -0.07$, $p = 0.18$ and $\rho = -0.06$, $p = 0.20$ respectively). Se levels were also higher for alcohol consumers: B-Se (Wilcoxon $\chi^2 = 4.0$, $p = 0.05$), P-Se ($\chi^2 = 8.3$, $p = 0.004$), H-Se ($\chi^2 = 6.3$, $p = 0.01$), except for U-Se ($\chi^2 = 1.4$, $p = 0.23$).

The associations between biomarker variables are shown in Table 13. B-Se, P-Se and H-Se levels were highly correlated. U-Se levels and other Se biomarkers were also correlated, but to a lesser extent. B-Hg and B-Pb levels were positively correlated, and both were also positively correlated to B-Se and P-Se levels. However, B-Hg levels were negatively correlated to U-Se levels.

Tableau 13 : Spearman's ρ correlation between Se, Hg and Pb biomarkers

| | B-Se | P-Se | H-Se | U-Se | B-Hg | B-Pb |
|---|---|---|---|---|---|---|
| B-Se | | | | | | |
| P-Se | 0.76 *** | | | | | |
| H-Se | 0.77 *** | 0.70 *** | | | | |
| U-Se | 0.44 *** | 0.34 *** | 0.45 *** | | | |
| B-Hg | 0.19 *** | 0.22 *** | 0.09 † | - 0.14 ** | | |
| B-Pb | 0.13 ** | 0.11 * | ns | ns | 0.40 *** | |

$P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.0001$, ns : non-significant

Results of simple and multiple analyses between the different Se biomarkers and neurofunctional test outcomes are presented in Table 14. For the BAMT, B-Se entered significantly into the model only when B-Hg was included with the other variables (Models 2 and 4). On the other hand, P-Se levels were positively correlated with performance on the BAMT and when controlling for age, gender, education, smoking and alcohol consumption (Model 1). When B-Hg was included in the model, the β estimate increased by approximately 30% (Model 2). In all of these models, B-Hg was negatively associated with BAMT ($P < 0.01$). B-Pb levels were not related to the outcome ($P > 0.05$) and did not influence B-Se and/or P-Se estimates in these models.

Tableau 14 : Results of simple correlation, simple regression and multiple regression models between Se biomarkers and motor tests outcomes

| Outcomes | Log-Se biomarkers, µg/L | | | |
|---|---|---|---|---|
| | B-Se | P-Se | H-Se | U-Se |
| **BAMT** | | | | |
| *r* Pearson | 0.09 | 0.13 * | 0.02 | 0.09 |
| $\beta$ crude | 5.2 | 8.2 * | 1.6 | 3.0 |
| (95% CI) | (-1.3/11.7) | (1.3/15.1) | (-6.1/9.4) | (-1.0/7.0) |
| Model 1 $\beta$ [a] | 5.4 † | **7.8 *** | 3.2 | 1.5 |
| (95% CI) | (-0.4/11.2) | **(1.7/13.9)** | (-3.8/10.1) | (-2.0/4.0) |
| $R^2$ | 0.25 | **0.26** | | |
| Model 2 $\beta$ [b] | **7.5 *** | **10.1 **** | 4.6 | 1.3 |
| (95% CI) | **(1.6/13.4)** | **(3.9/16.3)** | (-2.4/11.7) | (-2.2/4.7) |
| $R^2$ | **0.27** | **0.28** | | |
| Model 3 $\beta$ [c] | 5.6 † | **8.1 *** | 3.2 | 1.5 |
| (95% CI) | (-0.3/11.5) | **(1.9/14.4)** | (-2.8/12.3) | (-2.0/5.0) |
| $R^2$ | 0.25 | **0.26** | | |
| Model 4 $\beta$ [d] | **7.3*** | **9.9 **** | 4.4 | 1.2 |
| (95% CI) | **(1.4/13.2)** | **(3.7/16.2)** | (-2.6/11.5) | (-2.3/4.7) |
| $R^2$ | **0.27** | **0.28** | | |
| **Santa Ana** | | | | |
| **Dominant hand** | | | | |
| *r* Pearson | 0.13 ** | 0.15 ** | 0.13 * | 0.21 ** |
| $\beta$ crude | 2.8 ** | 3.4 ** | 3.1 * | 2.4 ** |
| (95% CI) | (0.7/4.9) | (1.2/-5.6) | (0.6/5.6) | (1.1/-3.6) |
| Model 1 $\beta$ [a] | 0.09 | 1.6 † | -0.62 | 1.1 † |
| (95% CI) | (-0.09/2.7) | (-0.3/-3.6) | (-1.5/2.8) | (-0.1/-2.3) |
| $R^2$ | | 0.31 | | 0.30 |
| Model 2 $\beta$ [b] | 1.4 | **2.2 *** | 1.0 | 1.1 † |
| (95% CI) | (-0.4/3.3) | **(0.2/-4.2)** | (-1.2/3.2) | (-0.1/-2.3) |
| $R^2$ | | **0.32** | | 0.31 |
| Model 3 $\beta$ [c] | 1.0 | 1.7 † | 0.7 | 1.2 † |
| (95% CI) | (-0.8/2.9) | (-0.3/-3.6) | (-1.5/2.9) | (-0.2/-2.4) |
| $R^2$ | | 0.31 | | 0.31 |
| Model 4 $\beta$ [d] | 1.4 | **2.2 *** | 1.0 | 1.1 † |
| (95% CI) | (-0.4/3.3) | **(0.2/-4.2)** | (-1.2/3.2) | (-0.1/-2.3) |
| $R^2$ | | **0.32** | | 0.31 |
| **Non dominant hand** | | | | |
| *r* Pearson | 0.09 † | 0.06 | 0.06 | 0.10 † |
| $\beta$ crude | 1.6 | 1.2 | 1.2 | 1.0 |
| (95% CI) | (-0.2/3.5) | (-0.7/3.2) | (-1.0/3.5) | (-0.1/2.1) |
| Model 1 $\beta$ [a] | 0.2 | 0.1 | -0.6 | 0.1 |
| (95% CI) | (-1.4/1.8) | (-1.6/1.8) | (-2.5/1.4) | (-1.0/1.2) |
| Model 2 $\beta$ [b] | 0.5 | 0.4 | -0.4 | 0.1 |
| (95% CI) | (-1.2/2.2) | (-1.4/2.2) | (-2.4/1.6) | (-1.0/1.2) |
| Model 3 $\beta$ [c] | 0.5 | 0.3 | -0.4 | 0.2 |
| (95% CI) | (-1.2/2.1) | (-1.4/2.1) | (-2.3/1.6) | (-0.9/1.3) |
| Model 4 $\beta$ [d] | 0.6 | 0.5 | -0.3 | 0.2 |
| (95% CI) | (-1.1/2.3) | (-1.3/2.3) | (-.23/1.7) | (-0.9/1.3) |
| **Dynamometer** | | | | |
| **Dominant hand** | | | | |
| *r* Pearson | 0.10 † | 0.10 * | 0.07 | 0.12 * |
| $\beta$ crude | 3.7 † | 4.1 * | 3.3 | 2.7 * |
| (95% CI) | (-0.1/7.5) | (0.1-8.1) | (-1.3/7.8) | (0.3/5.1) |

| Outcomes | Log-Se biomarkers, µg/L | | | |
|---|---|---|---|---|
| | B-Se | P-Se | H-Se | U-Se |
| Model 1 $\beta$ [a] | 2.0 | 2.5 | 2.1 | 1.6 |
| (95% CI) | (-0.9/5.0) | (-0.6/5.6) | (-1.4/5.6) | (-0.4/3.6) |
| Model 2 $\beta$ [b] | 2.8 † | **3.4 *** | 2.7 | 1.6 |
| (95% CI) | (-0.2/5.9) | **(0.2/6.7)** | (-0.8/6.3) | (-0.4/3.5) |
| $R^2$ | 0.47 | **0.47** | | |
| Model 3 $\beta$ [c] | 1.8 | 2.3 | 1.8 | 1.5 |
| (95% CI) | (-1.2/4.8) | (-0.9/5.5) | (-1.8/5.4) | (-0.4/3.5) |
| Model 4 $\beta$ [d] | 2.6 † | 3.1 † | 2.5 | 1.4 |
| (95% CI) | (-0.4/5.6) | (-0.1/6.4) | (-0.1/6.1) | (-0.5/3.4) |
| $R^2$ | 0.47 | 0.47 | | |
| **Non dominant hand** | | | | |
| *r* Pearson | -0.07 | -0.07 | -0.06 | -0.09 |
| $\beta$ crude | 3.2 | 3.7 † | 2.0 | 3.4 ** |
| (95% CI) | (-0.9/7.3) | (-0.6/8.0) | (-2.8/6.9) | (1.0/5.9) |
| Model 1 $\beta$ [a] | 1.0 | 1.1 | -0.3 | 1.3 |
| (95% CI) | (-2.0/4.0) | (-2.0/4.3) | (-3.9/3.2) | (-0.6/3.2) |
| Model 2 $\beta$ [b] | 1.6 | 1.8 | 0.2 | 1.3 |
| (95% CI) | (-1.4/4.7) | (-1.5/5.1) | (-3.5/3.8) | (-0.6/3.2) |
| Model 3 $\beta$ [c] | 0.8 | 0.9 | -0.6 | 1.3 |
| (95% CI) | (-2.2/3.8) | (-2.2/4.1) | (-4.2/3.0) | (-0.6/3.2) |
| Model 4 $\beta$ [d] | 1.4 | 1.6 | -0.1 | 1.2 |
| (95% CI) | (-1.6/4.5) | (-1.7/4.9) | (-3.7/3.6) | (-0.7/3.1) |
| **Grooved pegboard** | | | | |
| **Dominant hand** | | | | |
| *r* Pearson | 0.05 | 0.01 | 0.03 | 0.06 |
| $\beta$ crude | 2.0 | 0.5 | 1.5 | 1.2 |
| (95% CI) | (-3.6/7.7) | (-5.6/6.5) | (-5.3/8.3) | (-2.1/4.5) |
| Model 1 $\beta$ [a] | 1.2 | -0.7 | 1.3 | 0.5 |
| (95% CI) | (-4.0/6.4) | (-6.3/4.9) | (-5.0/7.6) | (-3.1/4.0) |
| Model 2 $\beta$ [b] | 0.1 | -2.0 | 0.1 | 0.3 |
| (95% CI) | (-5.5/5.6) | (-7.8/3.8) | (-6.5/6.6) | (-3.3/3.8) |
| Model 3 $\beta$ [c] | 0.9 | -1.2 | 0.9 | 0.4 |
| (95% CI) | (-4.4/6.2) | (-6.9/4.5) | 9-7.5/5.3) | (-3.1/4.0) |
| Model 4 $\beta$ [d] | 0.1 | -2.1 | 0.1 | 0.3 |
| (95% CI) | (-5.4/5.5) | (-7.9/3.8) | (-6.5/6.6) | (-3.3/3.8) |
| **Non dominant hand** | | | | |
| *r* Pearson | -0.08 | -0.07 | -0.02 | 0.07 |
| $\beta$ crude | -3.3 | -3.2 | -1.2 | 1.6 |
| (95% CI) | (-9.8/3.1) | (-10.1/-3.7) | (-9.0/6.7) | (-2.2/5.4) |
| Model 1 $\beta$ [a] | -5.2 † | -6.1 † | -3.1 | -1.2 |
| (95% CI) | (-11.3/1.0) | (-12.7/-0.5) | (-10.7/4.4) | (-5.3/2.9) |
| $R^2$ | 0.18 | 0.19 | | |
| Model 2 $\beta$ [b] | **-6.6 *** | **-7.4 *** | -4.6 | -1.4 |
| (95% CI) | **(-13.0/-0.2)** | **(-14.3/-0.6)** | (-12.4/3.2) | (-5.5/2.7) |
| $R^2$ | **0.19** | **0.20** | | |
| Model 3 $\beta$ [c] | -6.1 † | **-7.1 *** | -4.1 | -1.3 |
| (95% CI) | (-12.2/0.1) | **(-13.8/-0.5)** | (-11.7/3.4) | (-5.3/2.8) |
| $R^2$ | 0.20 | **0.20** | | |
| Model 4 $\beta$ [d] | **-6.7 *** | **- 7.7 *** | -4.8 | -1.4 |
| (95% CI) | **(-13.1/-0.4)** | **(-14.6/-0.9)** | (-12.6/3.0) | (-5.5/2.7) |
| $R^2$ | **0.20** | **0.21** | | |

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$

[a] Model 1 was controlled for age, gender, education, smoking and alcohol consumption

[b] Model 2 corresponds to model 1 also controlled for Log BHg

[c] Model 3 corresponds to model 1 also controlled for Log BPb

For the Santa Ana and Dynamometer tests, better performances were associated to increasing Se status for the dominant hand. In the multiple regression models, P-Se was the only Se biomarker that entered significantly, particularly when B-Hg was included (Model 2 and/or 4). Indeed, for both tests, B-Hg was significantly related to the outcome ($P < 0.05$), while B-Pb was not ($P \geq 0.05$).

For the Grooved Pegboard test, better performance was significantly associated the Se levels for the non dominant hand. The β estimate for B-Se and P-Se status were significant only in the multiple regression models controlled for B-Hg and/or B-Pb levels, although in the model with B-Se biomarker controlled for B-Pb levels, it was only a trend. In these models, B-Hg and B-Pb levels were not related to the outcome for this test ($P > 0.05$).

P-Se biomarker had consistently the most significant influences on the motor performances compared to other Se biomarkers. When the relation with P-Se status and the outcome was particularly strong, B-Se biomarker was also related to the outcome, but to a lesser extent. For Santa Ana test, increasing U-Se levels also tended to be related to a better performance. In these regression models controlled for co-variables but not for the metal exposures to Hg and Pb (Model 1), the positive influence of the P-Se biomarker remained, even if it was not always significant ($P < 0.12$).

In all of the models described in Table 3, the interaction terms between Se biomarkers and B-Hg, as well as between Se biomarkers and B-Pb were not statistically significant ($P > 0.05$). Moreover, when the persons presenting high Se status were excluded from these models, the β estimate for Se biomarkers remained similar.

## *4. Discussion*

This is the first human study to report beneficial effects of Se on motor functions. Saint Amour et al. (2006) reported opposing effects of Hg and Se on visual evoked potentials (VEP) in Inuit preschool children, but the interpretation of the results was unclear since B-Hg was associated with decreased latency, and Se with increased latency. In this same cohort, some neuromotor functions were inversely related to B-Hg and B-Pb, however, no effects of B-Se were observed (Després et al., 2005). In the Faroe Islands, where Se likewise comes primarily from marine diet, cord blood Se did not modified the Hg-induced neurological deficits in neonates (Steuerwald et al., 2000) nor the Hg-induced neurobehavioral changes in children at 7 years old (Choi et al., 2008). However, these studies did not provided information on Se effect independently of Hg exposure. The relationships between Hg and Pb exposures and motor functions in this present study population are detailed elsewhere.

In the present study population, since the Se status varies from normal to very high, we were expecting to observe adverse Se effects on motor outcomes. In naturally rich-Se area of China, B-Se status above 1000µg/L was associated with hair and nail loss, skin lesions, garlic breath as well as gastro-intestinal disorders (Yang et al., 1983). In a particularly heavily affected village, where B-Se levels ranged from 1300 to 7500µg/L, polyneuropathy and paraesthesia were reported. In this Amazonian population, there was no evidence of the signs and symptoms of selenosis (Lemire et al., chapitre III), on the contrary, motor performance improved with Se status.

Several factors may account for these differences. First, in the present study, Se intake is mostly from diet (Lemire et al., chapitre II), which probably contains a mixture of Se organic forms (Whanger, 2002). In China, persons not only had high dietary intake but were also heavily exposed to Se inorganic fumes (Yang et al.,

1983; Liu et al., 2008) which may be more toxic than the organic forms (Rayman et al., 2008).

Second, Amazonian populations have high concurrent exposure to Hg (Passos and Mergler, 2008) and higher levels of dietary Se may be required to offset Hg-induced toxic effects, Hg-mediated oxidative stress and/or to maintain optimal Se antioxidant enzymes (Watanabe, 2001; Fordyce, 2005; Khan et Wang, 2009; Raltson et al., 2008). Animal studies have suggested that specific selenoproteins such as selenoprotein P (SelP) may protect against Hg toxicity by binding to Hg and reducing his availability for target proteins and organs (Yoneda et Suzuki, 1997). Conversely, some authors have suggested that Hg has a very high affinity for selenol groups in the active site of selenoenzymes, thereby inhibiting their enzymatic functions (Seppanen et al., 2004; Raltson et al., 2008; Ganther et Sunde, 2007). Hg might then cause functional Se deficiency which is the root cause of Hg toxic effects in the brain (Ganther et Sunde, 2007; Ralston et al., 2008; Khan et Wang, 2009). Indeed, high Se intake have been shown to restore selenoprotein activities and alleviate Hg toxic effects in the foetus and rat brain (Watanabe et al., 1999a,b; Ralston et al., 2008; Ganther et Sunde, 2007; Floven et al., 2009). A recent *in vitro* study showed that organic Se (selenomethionine) can also contribute to mitigating Hg-induced oxidative damage in neural cell lines (Kaur et al., 2009). This raises the question of whether more Hg can be tolerated in the brain without related-toxic effects. In the cat brain, Ganther et Sunde (2007) observed that, contrarily to liver, not all Hg is complexed to Se. The authors therefore suggest that Hg and Se relationship may be more complex than a direct molecular interaction and that Se do not necessarily offset all toxic effects of Hg in the brain.

In the present study, Pb had little influence on the beneficial effects associated with P-Se. There is much less literature on Pb and Se interface. Some authors have suggested that Se can contribute to reducing Pb-mediated oxidative stress in the brain

(Ahamhed et Siddiki, 2007). It has also been suggested that Pb could bind, like Hg, to selenoproteins and induce a functional Se deficiency (Kasperczyk et al., 2009).

Another explanation regarding the absence of Se toxicity is that Se-rich food such as Brazil nuts has always been part of the Amazonian diet. It is possible that through homeostatic adjustment of this essential element, persons chronically exposed to Se may be able to adapt to higher Se status (ATSDR, 2003; WHO, 1986), and may even develop mechanisms to take advantage of such high Se intake (Mattson, 2008).

Selenium exerts its biological functions in the form of selenoproteins, which are composed of a varying number of selenocysteine (SeCys). The human genome encodes for 25 different selenoproteins, including different forms of glutathione peroxidases (GPx), thyroid hormone deiodinases and thioredoxin reductases (Reeves et Hoffman, 2009). SelP is an extracellular heparin-binding glycoprotein containing 10 SeCys residues, which originates largely in the liver, is also produced in the brain and other tissues, and is secreted either into plasma or interstitial fluids (Burk et Hill, 2005). Indeed, SelP contains 40-50% of the total Se in plasma and is believed to be involved in Se transport and delivery to target tissues (Hill et al., 2003; Schomburg et al., 2003). Recent animal studies confirmed that plasma SelP, derived from hepatocytes, is the main transport form of Se to support essential physiological functions of Se in kidney, testis and brain (Renko et al., 2008).

Several animal studies show an important role of selenoproteins on brain functions. Many regions of the mice brain are exceptionally rich in several selenoprotein gene expressions, suggesting that neurons are key functional site for Se (Zhang et al., 2008). Although specific roles for many of these proteins have not been elucidated, they may help mitigate neurodegeneration (Chen et Berry, 2003). For example, it has been shown that cellular GPx deficiency enhance the sensitivity to transient brain ischemia, while increased GPx activity, as a result of increased Se

supply or overexpression, ameliorates brain functions (Crack et al., 2001; Ishibashi et al., 2002).

SelP is the selenoprotein more highly expressed, and this, in most regions of the brain (Zhang et al., 2008). In addition to its role in Se transport into and within the brain, there is increasing evidences also suggesting that locally produced SelP may play a physiological role in the brain (Renko et al., 2008). Experimental studies found that SelP deficiency in mice fed with low Se diet leads to: premature death (i.e. before weaning) (Schweizer et al., 2004b); motor disorders with spontaneous seizures and ataxia (Hill et al., 2004); brainstem axonal degeneration (Valentine et al., 2005) and; disrupted spatial learning and altered synaptic transmission (Peters et al., 2006). In experimental studies with human brain tissues, SelP has been shown to protect cultured astrocytes against oxidative damage (Steinbrenner et al., 2006). SelP expression has been associated with Alzheimer's pathology in human cortex, where oxidative damage is strongly involved in the progression of the disease. Therefore, human SelP may provide Se for biosynthesis of other antioxidant selenoproteins in the brain, but also may simultaneously act as an antioxidant to prevent oxidative stress and promote neuronal cell survival (Bellinger et al., 2008).

In the present study, P-Se was the biomarker best related to motor outcomes. There is increasing evidence demonstrating the important role of SelP for Se supply to the brain and antioxidant functions in neurons. SelP also happens to be the most important selenoprotein in plasma. P-Se may be the most biologically active fraction of Se in blood in comparison to other blood constituents, hair and urine biomarkers. Higher P-Se status has also been associated to a lower prevalence of age-related cataracts in this population (Lemire et al, chapitre V). These findings suggest that P-Se or plasmatic selenoproteins may be a better Se biomarker than B-Se to evaluate the relations between Se status and health outcomes.

There is growing evidences suggesting that when considering the effects of beneficial nutrients in food, adverse effects of Hg on neurologic and cardiovascular systems become more apparent (Choi et al., 2008b; Rice, 2008; Valera et al., 2009). The present findings show that likewise, when the negative effects of Hg and Pb exposures are considered, we better estimated the benefits of Se on the same neuromotor outcome. This was especially true with Hg exposure, and even when Hg did not show a significant negative relation on the outcome. Interestingly, Hg and Pb exposures did not show a modifier effect on the relationship between Se and motor performances. Since the relationships between Se biomarkers and the outcomes were better improved with Hg exposure than with Pb exposure, it can be suggested that maybe the underlying counteracting mechanisms of Se on Hg toxicity are more widespread in the brain than for Pb toxic effects. Without adjustments for Hg and Pb exposures, the Se effect was still present, although not always highly significant. However, it is not clear whether Se beneficial effects on motor functions would be observed if high Hg exposure was not present in this population.

The evaluation of optimal Se intake in human populations depends on several factors including : the chemical speciation of Se which is most prominent in the Se source; the adequacy of other nutrients; the presence of additional stressors and the body's ability to make selenoproteins (Rayman et al., 2008). The present results suggest that in populations exposed to Hg, high dietary Se intake may be critical for adequate brain functions and to offset the deleterious effects of Hg.

# CHAPITRE V

## Les influences opposées du sélénium et du mercure sur les cataractes séniles en Amazonie brésilienne

## *Selenium and mercury in the Brazilian Amazon: opposing influences on age-related cataracts*

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## *Résumé*

Les cataractes séniles sont une cause importante de cécité dans les pays en voie de développement. L'exposition à des contaminants environnementaux, comme le mercure (Hg) ou le plomb (Pb), peut contribuer à la formation de cataractes séniles alors que certains antioxydants, comme le sélénium (Se), peuvent aider l'organisme à se prévenir contre le stress oxydatif causant cette pathologie. Chez les communautés riveraines du Bas-Tapajós (Amazonie brésilienne), une exposition élevée au Hg et au Pb a été rapportée et les niveaux de Se sanguins varient de normal à très élevé (> 1000µg/L). La présente étude a pour objectif d'examiner la prévalence de cataractes séniles en relation avec ces éléments chez des adultes (≥ 15ans) venant de 12 communautés du Bas-Tapajós.

Les participants ($N$ = 396 après exclusions) ont fourni des échantillons de sang et complété un examen oculaire exhaustif. Les concentrations de Hg, Pb et Se ont été mesurées dans le sang ou le plasma par ICP-MS.

Chez les adultes de 40 ans et plus, 32.7% présentaient des cataractes séniles. Les rapports de cote, ajustés pour l'âge et la consommation de cigarette, ont montré un effet bénéfique du Se dans le plasma à des concentrations ≥ 111µg/L et des effets néfastes du Hg dans le sang à des concentrations ≥ 25µg/L: 0.25 [0.10–0.69] et 4.52 [1.35–15.16], respectivement. Le groupe de personnes le plus à risque de présenter des cataractes avait des niveaux faibles de Se plasmatiques et des niveaux élevés de Hg dans le sang. Lorsque les concentrations de Se plasmatiques étaient supérieures à 111µg/L, aucune association entre le Hg et la prévalence de cataractes séniles n'a été observée. Par ailleurs, aucune association n'a été observée avec le Se et le Pb sanguin.

Chez cette population exposée à des niveaux élevés de Hg, le Se pourrait atténuer les effets néfastes du Hg sur la formation de cataractes. Le Se pourrait jouer un rôle préventif contre les cataractes séniles par le biais de ses activités antioxydantes. Un apport élevé de Se pourrait aussi être essentiel pour contrer les effets néfastes du Hg en restaurant l'activité optimale de sélénoprotéines ou les concentrations de GSH dans l'organisme.

## *Abstract*

Age-related cataracts (ARC) are an important cause of blindness in developing countries. Environmental contaminants, such as mercury (Hg) and lead (Pb), may contribute to ARC formation, while antioxidants, such as selenium (Se), may be part of the body's defense to prevent ARC. In fish-eating populations of the Lower Tapajós Region (Brazilian Amazon), elevated exposure to Hg and Pb has been reported, and blood Se ranges from normal to very high (> 1000µg/L). The present study sought to examine ARC in relation to these elements among adults (≥ 15y) from 12 Tapajós riverside communities.

Participants ($N$ = 396 after exclusions) provided blood samples and underwent an extensive ocular examination. ICP-MS was used to assess Hg, Pb and Se in blood and/or plasma.

For those over 40y, 32.7% presented ARC. ORs, adjusted for age and smoking, showed a protective effect of plasma Se at concentrations ≥ 111µg/L and a negative effects of B-Hg at concentrations ≥ 25µg/L: 0.25 [0.10–0.69] and 4.52 [1.35–15.16], respectively. The group with the highest risk had low P-Se and high B-Hg. No association with B-Hg was observed when P-Se concentrations were above 111µg/L. No association was observed with blood Se and Pb.

In this population with elevated Hg, Se may offset the cataractogenic effects of Hg. Se may play an important preventive role for ARC disease through its antioxidant enzymatic activities and/or higher Se intake may be essential to counter Hg-induced cataracts by restoring the selenoenzymes and/or the GSH pool.

## *1. Introduction*

Age-related cataract (ARC) is a leading cause of impaired vision among elderly populations, particularly in developing nations where there is little access to surgical procedures (WHO, 1991). ARC is generally characterized by a gradual painless loss of vision, resulting from lens protein damage, accumulation, aggregation and precipitation, causing a partial or complete progressive opacification of the lens that prevents light from reaching the retina. The lens' proteins are unusually long lived and since there is little protein turnover, opacification is, for the most part, irreversible (Horwitz, 2003). Between 65 and 75 years of age, ARC prevalence increases approximately from 5% up to 50% (Chiu and Taylor, 2007).

The eye's lens is avascular; it depends mostly on passive diffusion, active transport and intra-lens protein synthesis. Compared to other tissues, the lens contains important levels of locally synthesized glutathione (GSH) (Reddy and Giblin, 1984), whose concentration decreases with age (Harding, 1970). *Reduced* GSH, the "active" form of GSH, is part of the GSH enzymatic complex involved in the antioxidant defense system and possibly acts as the first line of protection against cataract formation (Fernandez and Afshari, 2008).

Observational and clinical trials have evaluated the protective effect of nutrients such as vitamin C and E, carotenoids and selenium (Se) on lens tissues (Chiu and Taylor, 2007; Bunce et al., 1990). Since the 60's, several authors have suggested that specific selenoenzymes, such as glutathione peroxidases (GPx), may be part of the body's defense to prevent or delay the progression of ARC (Pirie, 1965). Animal studies have shown that chronic Se deficiency or GPx depletion can lead to cataract formation, while Se excess, at levels that may be below those that produce acute systemic toxicity, can induce pro-oxidant conditions involved in

cataractogenesis (for review see Flohé, 2005). However, the role of Se status on cataract formation in human populations remains unclear (Flohé, 2005; Li et al., 2009).

ARC pathology is believed to result from a combination of factors acting over many years, including smoking, UV-light and ionizing radiation, exposure to heavy metals, including cadmium, mercury (Hg) and lead (Pb), as well as the use of steroids and gout medication (reviewed by Head, 2001). For many of these factors, oxidative damage and/or unbalance in *reduced* GSH concentrations may be the underlying process leading to developmental or degenerative opacities of the lens (Head, 2001; Truscott, 2005).

At low latitudes, where solar UV exposure is high, there is an elevated prevalence of ARC (Sasaki et al., 2003). The Tapajós River, a major tributary of the Amazon, is situated near the equator and inhabitants spend most of the day outdoors, involved in subsistence activities such as traditional fishing, slash-and-burn agriculture and washing clothes and dishes in the river (Mertens et al., 2005). The fish-eating populations from this region have among the highest reported Hg exposure in the world today (Passos and Mergler 2008). In addition, a recent study in this area showed elevated blood Pb levels (Barbosa Jr et al., 2009). On the other hand, Se status in these same communities ranges from normal to very high (Lemire et al., 2006; 2009; Pinheiro et al., 2005). The objective of the present study was to examine the prevalence of ARC in relation to environmental exposures to Hg and Pb and to Se status.

## 2. Materiel and methods

### Study population

This cross-sectional study is part of a larger project on factors that affect human Hg exposure and its health effects (CARUSO, 2009). In the Lower Tapajós River Basin (State of Pará, Brazil), there are approximately 50 communities of diverse size and origin, with varying access to health care, education, local authorities and cities. Based on sample size calculations for visual outcomes from preliminary studies, we sought a minimum of 400 people to test Hg and Se interaction effects. To reach this number and as well to reflect the diversity of regional populations, social conditions and ecosystems, we selected 12 selected communities (presented in Figure 30). Recruitment of persons 15y and older was carried out in each village through home visits and at village meetings, during which the research project was explained, and villagers were invited to participate on a voluntary basis. No *a priori* conditions were imposed, however, preference was given to those who had participated in a previous study (Passos et al., 2008).

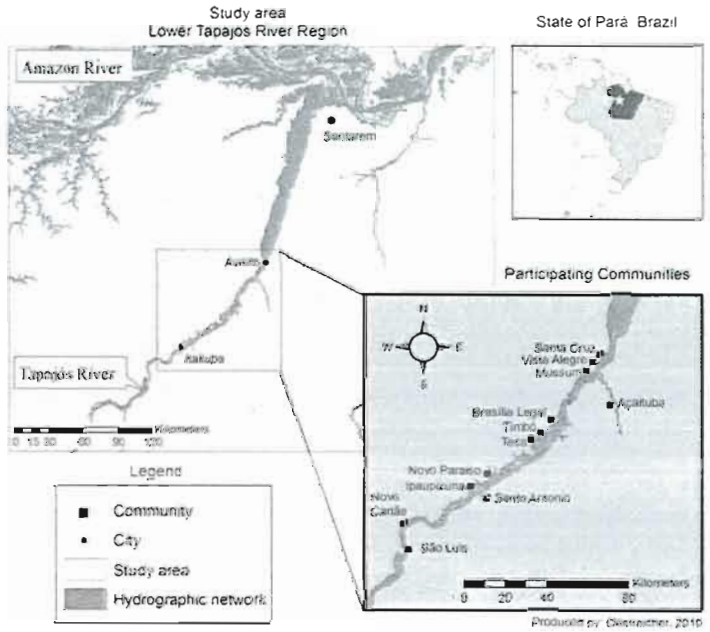


Figure 30 : Study area

A total of 448 persons (216 men and 232 women) participated in the present study. Based on a house-to-house survey carried out by our research group in 2003, this represents 25% of the adult population of these villages (27% of all women and 23% of all men) participated in the study. The participation within each village varied from 10 to 67%, with higher relative frequencies in the smaller villages (the smallest consisted of 13 adult residents and the largest, 384 adults). Since recruitment favored participants from our previous studies, younger persons (≥ 15y and < 40y) were underrepresented with respect to the age distribution of the entire population (50% vs. 62%), those between to 40y to 65y were overrepresented (40% vs. 28%), while the oldest group (≥ 65y) was similar to the underlying population (10%).

The study was approved by the Ethics Review Boards of the University of Quebec at Montreal and the Faculty of Pharmaceutical Sciences of the University of São Paulo-Ribeirão Preto. All participants signed an informed consent form, which was read to them. There was no remuneration for study participation.

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## Ocular health assessment

The study was carried out from May to August, 2006, in a technical college, located in a nearby city. Ocular health examinations were performed by clinical optometrists from the School of Optometry of the University of Montreal. The anterior segment of the eye was examined using slit-lamp biomicroscopy. The posterior segment of the retina and its periphery were examined during pupillary dilation using two mydriatic agents (tropicamide 1% and phenylephrine 2.5%). In addition, irido-corneal angles were noted. Intra-ocular pressure was measured with a Perkins tonometer under topical anaesthesia.

All lens opacities were noted and ARC was distinguished from other lens pathologies, such as cataracts typical of trauma, which were noted for *post hoc* exclusions in the present analysis. A severity score, based on opalescence, color and lens opacity, derived from the Lens Opacities Classification System III (LOCS III) (Chylack Jr et al. 1993), was applied. The score ranged from Grade 1 (lens with few opacities and a light yellowish nucleus) to Grade 4 (brownish nucleus with more opacities). The four optometrists were trained prior to the field study to minimize inter-observer bias. All examinations were made under dilation, with the same slit-lamp, with normalized slit section and maximal, constant luminal intensity. Over the period of the study, two groups of 2 optometrists worked together. For each team, in cases of doubt in diagnosis, they consulted each other and arrived at a consensus. Statistical comparison of cataract cases diagnosed by the 2 teams showed a similar rate (N = 37/218 (17.0%) versus N = 39/230 (17.0%)).

For the present analyses, each person was classified with respect to the presence or absence of ARC in at least one eye. For opacity categorization, the most severe grade was attributed when there was a difference in the 2 eyes.

## Blood sample collection and analysis

For each participant, an experienced Brazilian phlebotomist collected a 6mL blood sample in "trace metal free" evacuated tubes (BD Vacutainer®), containing heparin as anticoagulant. For plasma separation, blood samples were centrifuged (800 x g for 6 minutes). Plasma fractions were then pipetted into previously cleaned Eppendorf tubes (2 mL), and immediately frozen at -20°C. Blood total Hg (B-Hg), blood Se (B-Se) and blood lead (B-Pb), as well as plasma total Hg (P-Hg) and plasma Se (P-Se) were determined by inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer DRC II) according to the method proposed by Batista et al. (2009), at the *Laboratório de Toxicologia e Essencialidade de Metais*, *Universidade de São Paulo*, Ribeirão Preto, SP, Brazil. Quality control was guaranteed by analyzing various secondary reference materials, provided by the New York State Department of Health's Proficiency Testing program for trace elements in whole blood, and from the external quality assessment scheme (EQAS) for trace elements operated by the *Institut National de Santé Publique du Québec*, Canada. Reference materials were analyzed before and after ten ordinary samples. Measured values were always within the provided reference or certified values.

## Questionnaires and medication

Socio-demographic characteristics including age, sex, current smoking habits and alcohol consumption, years of education and medical history, were surveyed using an interview-administered questionnaire. Research assistants visited each

participant in their homes and noted the names of all currently used medication (Appendice B).

## Statistical analyses

Descriptive statistics were used to illustrate the study population's general characteristics, the distribution of the biomarkers, the prevalence of ARC and the distribution of ARC grading. Since none of the biomarker variables displayed a significant normal distribution, nonparametric analyses were carried out. The Rank Sums Test (Wilcoxon/Kruskall-Wallis $\chi^2$ Approximation) was performed to compare groups, while non-parametric Spearman's $\rho$ were used to evaluate the correlations between two continuous variables. 2-Tail Fisher's Exact Test was retained for contingency tables analyses.

Multiple logistic regression models (Estimate, Wald $\chi^2$ and Odds Ratio [CI 95%]) were used to examine, separately and jointly, the influence of co-variables, such as age, sex, years of formal education, and current smoking habit (Y or N) and alcohol consumption (Y or N), on ARC. For the association between biomarkers and ARC, no *a priori* information was available to indicate where a 'cut-off' effect could occur. Thus, we proceeded in several steps. First, the biomarkers were categorized by quartiles and logistic regression models were used to examine, for each biomarker, the relation with ARC, taking into account the relevant co-variables. The results of these analyses were used to determine binary categories, based on the most significant inter-quartile differences. This allowed us to test the biomarkers (as binary variables) in the same model. Since possible modifying effects of Se on Hg and Pb toxicity has been suggested (Amhed and Siddiki 2007; Kang and Wang 2009), the interaction term between biomarker variables was likewise tested in these models. Results were defined as statistically significant at $P < 0.05$. Analyses were performed using JMP 8.0.1 and SPSS 16.0 software (SAS Institute Inc.).

## *3. Results*

The following exclusions were applied *post hoc*: pregnancy and breastfeeding ($N$ = 15), reported stroke ($N$ = 14), reported diagnosed diabetes ($N$ = 5), eye trauma ($N$ = 1), psychotropic and steroid medication ($N$ = 15). None of the participants reported gout or gout-related medication. Two participants with intraocular lens (cataract surgery) in both eyes were also excluded. One participant had had cataract surgery on the right eye, but since he presented an ARC of grade 4 on the left eye, he was not excluded. A total of 396 participants, 196 women and 200 men, were included in the present analyses.

Participants' age ranged from 15 to 87 years (mean: 41.2 ± 16.3y; median: 40y). Consistent with our knowledge on ARC, participants were categorized into 3 age groups: < 40y ($N$ = 197), 40-65y ($N$ = 164) and ≥ 65y ($N$ = 35). The characteristics of the study participants for each age group are shown in Table 15. Gender distribution was not different between the age groups and within each age group; age distribution was similar between men and women. Overall, the mean number of years of formal education was 4.9 ± 3.4y (median: 4y, range 0-16y), and decreased from the youngest to oldest age groups(Wilcoxon/Kruskall-Wallis $\chi^2$ = 70.9, $P < 0.0001$). Fifty-five percent of the participants reported drinking alcohol; the prevalence decreased with age (Likelihood Ratio $\chi^2$ = 21.1, $P < 0.0001$). Among the participants, 26.8% reported currently smoking, and there was no significant difference in the proportion of current smokers in the three age categories. Overall, smoking frequency was low (median: 5 cigarettes/day).

Tableau 15 : Characteristics of the study population

| | < 40y (*N* = 197) | | ≥ 40 < 65y (*N* = 164) | | ≥ 65y (*N* = 35) | |
|---|---|---|---|---|---|---|
| | *N* | Median (Range) | *N* | Median (Range) | *N* | Median (Range) |
| Women, *N* (%) | 105 (53.2) | | 76 (46.3) | | 15 (42.9) | |
| Men, *N* (%) | 92 (46.7) | | 88 (53.7) | | 20 (57.9) | |
| Age, y | 197 | 28 (15 – 39) | 164 | 50 (40 – 64) | 35 | 73 (65 – 87) |
| Women, y | 105 | 28 (15 – 39) | 76 | 49 (40 – 62) | 15 | 73 (65 – 84) |
| Men, y | 92 | 28 (15 – 39) | 88 | 52 (40 – 64) | 20 | 72 (65 – 87) |
| Education, y | 197 | 6 (0 – 16) | 163 | 4 (0 – 16) | 34 | 2 (0 – 8) |
| Alcohol drinkers, *N* (%) | 128 (65.0) | | 80 (48.8) | | 10 (28.6) | |
| Current smokers, *N* (%) | 46 (23.3) | | 51 (31.1) | | 9 (25.7) | |
| ARC, *N* (%) | 1 (0.01) | | 35 (21.3) | | 30 (85.7) | |
| Women, *N* (%) | 1 (0.01) | | 14 (18.4) | | 14 (93.3) | |
| Men, *N* (%) | 0 (0.0) | | 21 (23.9) | | 16 (80.0) | |
| ARC of Grade 4, *N* (%) | 0 (0.0) | | 4 (2.4) | | 21 (60.0) | |
| Women, *N* (%) | 0 (0.0) | | 1 (1.3) | | 11 (73.3) | |
| Men, *N* (%) | 0 (0.0) | | 3 (3.4) | | 10 (50.0) | |

ARC in at least one eye was diagnosed in 66 persons (16.6%). As expected, ARC prevalence increased with age (Table 1). Only one person under 40 years of age presented ARC, while for those 40 years and more, it rose to 32.7% (N = 65 / 199). For persons between 40 and 65y, ARC prevalence was 21.3% and for those over 65y, 85.7% presented ARC pathology. The difference between these age groups was highly significant (Likelihood Ratio $\chi^2$: 145.5, $p < 0.0001$). No difference for ARC prevalence was observed between men and women, overall or within the three age groups.

ARC grading over the age groups revealed that the participant with ARC in the youngest group (< 40y) was classified Grade 1. For those 40-65y, close to half of those presenting ARC pathology were Grade 2 (48.6%, *N* = 17/35), and very few

were Grade 3 and 4 (8.6%, $N$ = 3/35 and 11.4%, $N$ = 4/35 respectively). For those ≥ 65y, most participants with ARC (70.0%, $N$ = 21/30) were classified as Grade 4.

Since there was only one person with ARC under 40 years of age, further analyses were performed with those aged 40 years and more. For this age group, the distribution of Se, Hg and Pb biomarkers is shown in Table 16. Positive, but weak correlations were observed between some of the biomarkers: B-Se and P-Se were positively correlated with B-Hg (Spearman's ρ = 0.14; $P$ = 0.05; Spearman's ρ = 0.16; $P$ = 0.02, respectively), but not to P-Hg. B-Se, but not P-Se, was also positively correlated to B-Pb (Spearman's ρ = 0.17; $P$ = 0.02).

Tableau 16 : Biomarker of Se, Hg and Pb levels for those of 40y and more ($N$ = 199)

| Biomarkers | Mean ± SD | Min. | 25$^{th}$ | 50$^{th}$ | 75$^{th}$ | Max. |
|---|---|---|---|---|---|---|
| B-Se, µg/L | 264.1 ± 150.7 | 123.9 | 185.1 | 222.2 | 293.6 | 1500.2 |
| P-Se, µg/L | 153.1 ± 89.1 | 57.2 | 111.1 | 134.8 | 169.1 | 913.2 |
| B-Hg, µg/L | 53.1 ± 37.5 | 4.3 | 25.3 | 43.9 | 75.6 | 288.9 |
| P-Hg, µg/L | 8.1 ± 6.5 | 0.2 | 3.3 | 6.5 | 11.3 | 40.0 |
| B-Pb, µg/L | 131.1 ± 77.8 | 10.1 | 76.1 | 113.8 | 169.4 | 483.1 |

Table 17 presents the associations between biomarkers and co-variables. Biomarkers of Se status did not vary with gender, smoking habits and educational level, but decreased with age and were higher for alcohol consumers. B-Hg levels tended to be higher in men and to decrease with years of education but this was not the case for P-Hg. Biomarkers of Hg exposure were higher for those who reported smoking. B-Pb levels were higher in men compared to women, decreased with education and were higher for smokers. Biomarkers of Hg and Pb exposure did not vary with age and drinking status.

Tableau 17 : Relations between biomarkers and co-variables for those of 40y and more ($N = 199$)

| | B-Se, µg/L | P-Se, µg/L | B-Hg,µg/L | P-Hg, µg/L | B-Pb, µg/L |
|---|---|---|---|---|---|
| Age, y | -0.27 *** | -0.26 ** | 0.05 | -0.05 | 0.05 |
| Gender (W vs. M) | 0.01 | 0.07 | 3.6 † | 0.72 | 28.0 *** |
| Education, y | 0.09 | 0.07 | -0.12 † | -0.05 | -0.18 * |
| Drinking alcohol (no vs. yes) | 8.28 ** | 14.0 ** | 1.6 | 0.68 | 1.5 |
| Smoking (no vs. yes) | 0.42 | 0.01 | 5.6 * | 5.7 * | 4.6 * |

Spearman's ρ correlation was used for continuous variables and Wilcoxon/Kruskall-Wallis $\chi^2$ categorical variables with continuous variables.
*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.0001$

Table 18 presents the results of the simple and multiple logistic regression analyses for the co-variables with ARC for the group 40y and more. In the bivariate models, ARC increased with age (entered as a continuous variable), while those who reported drinking alcohol present a lower ARC. In the multiple logistic model, ARC increased significantly with age, with similar estimates, and current smokers presented higher ARC compared to non-smokers; drinking did not enter significantly into the model. Age and smoking were retained to examine the associations with biomarkers.

Tableau 18 : Simple and multiple logistic regression analyses for ARC with respect to co-variables for those of 40y and more ($N = 199$)

| | Estimate | S.E. | Wald χ2 | *P* | OR [CI 95%] |
|---|---|---|---|---|---|
| Simple regression models | | | | | |
| Age (y) | 0.20 | 0.03 | 47.1 | 0.0001 | 1.23 [1.16 – 1.30] |
| Gender (M vs. W) | -0.16 | 0.30 | 0.27 | 0.60 | 0.85 [0.47 – 1.55] |
| Drinking alcohol (no vs. yes) | -0.80 | 0.32 | 6.38 | 0.01 | 0.44 [0.84 – 0.24] |
| Smoking (no vs. yes) | 0.26 | 0.33 | 0.62 | 0.43 | 1.29 [0.68 – 2.44] |
| Education (y) | -0.28 | 0.07 | 16.2 | 0.0001 | 0.78 [0.66 – 0.87] |
| Multiple regression model | | | | | |
| Age (y) | 0.21 | 0.03 | 40.6 | 0.0001 | 1.23 [1.16 – 1.31] |
| Alcohol habit (no vs. yes) | -0.43 | 0.45 | 0.95 | 0.33 | 0.65 [0.27 – 1.56] |
| Smoking habit (no vs. yes) | 0.96 | 0.48 | 3.99 | 0.05 | 2.61 [1.02 – 6.70] |
| Education (y) | 0.00 | 0.08 | 0.00 | 0.99 | 1.00 [0.85 – 1.17] |

Table 19 contains the results of the multiple logistic regression analyses for ARC with respect to biomarker concentrations in quartiles, taking into account age and smoking status. Significantly lower ORs were observed for the first quartile of P-Se with respect to the second and the fourth. A significantly higher OR was observed for B-Hg between the third compared to the first quartile. In all models, age continued to enter significantly into the model, with little change in estimates (0.21 - 0.23), while smoking only showed a tendency (Estimates = 0.75 – 0.84). B-Se, P-Hg and B-Pb quartiles did not enter significantly into the models.

Tableau 19 : Multiple logistic regression analyses for ARC with respect to biomarker quartiles, adjusted for age and current smoking habits, for those of 40y and more ($N$ = 199)

| | | Estimate | S.E. | Wald $\chi 2$ | $P$ | OR [CI 95%] |
|---|---|---|---|---|---|---|
| B-Se quartiles | | | | 2.23 | 0.53 | |
| | 1st vs. 2nd | -0.08 | 0.57 | 0.02 | 0.89 | 0.92 [0.30 – 2.84] |
| | 1st vs. 3rd | -0.80 | 0.62 | 1.64 | 0.20 | 0.45 [0.13 – 1.53] |
| | 1st vs. 4th | -0.01 | 0.60 | 0.00 | 0.99 | 0.99 [0.30 – 3.23] |
| P-Se quartiles | | | | 7.26 | 0.06 | |
| | 1st vs. 2nd | -1.45 | 0.59 | 6.01 | 0.01 | 0.23 [0.07 – 0.75] |
| | 1st vs. 3rd | -0.87 | 0.61 | 2.05 | 0.15 | 0.42 [0.13 – 1.38] |
| | 1st vs. 4th | -1.27 | 0.60 | 4.59 | 0.03 | 0.21 [0.28 – 0.90] |
| B-Hg quartiles | | | | 5.34 | 0.15 | |
| | 1st vs. 2nd | 1.01 | 0.67 | 2.25 | 0.13 | 2.73 [0.74 – 10.15] |
| | 1st vs. 3rd | 1.51 | 0.66 | 5.33 | 0.02 | 4.54 [1.26 – 16.42] |
| | 1st vs. 4th | 0.99 | 0.67 | 2.22 | 0.14 | 2.69 [0.73 – 9.92] |
| P-Hg quartiles | | | | 4.74 | 0.19 | |
| | 1st vs. 2nd | 1.17 | 0.67 | 3.55 | 0.06 | 3.23 [0.95 – 10.95] |
| | 1st vs. 3rd | 0.44 | 0.65 | 0.46 | 0.50 | 1.55 [0.43 – 5.56] |
| | 1st vs. 4th | 1.09 | 0.60 | 3.29 | 0.07 | 2.98 [0.92 – 9.68] |
| B-Pb quartiles | | | | 0.58 | 0.90 | |
| | 1st vs. 2nd | -0.51 | 0.69 | 0.55 | 0.46 | 0.60 [0.16 – 2.32] |
| | 1st vs. 3rd | -0.40 | 0.73 | 0.30 | 0.59 | 0.67 [0.16 – 2.80] |
| | 1st vs. 4th | -0.31 | 0.71 | 0.19 | 0.67 | 0.74 [0.19 – 2.94] |

On the basis of the results obtained above, the upper quartiles of P-Se (≥ 111µg/L) were grouped and compared to the first quartile (< 111µg/L), the adjusted OR for P-Se was 0.30 [0.12 – 0.75] (Table 20). Two groupings were made for B-Hg: the upper quartiles vs. the first quartile (< 25 µg/L) and at the median (44µg/L); the adjusted ORs were 3.25 [1.06 – 9.93] and 2.00 [0.85 – 4.68] respectively. When P-Se and B-Hg were included together (Model 2, Table 6), P-Se above 111µg/L is protective: OR = 0.25 [0.10 – 0.69], while the adjusted for OR blood Hg (≥ 25µg/L) is 4.52 [1.35 – 15.16]. The same pattern is observed in the second model with respect to median concentrations of B-Hg (44µg/L), but the OR for elevated Hg is lower and just above significance level. B-Pb did not enter into or modify any of these models.

Tableau 20 : Multiple logistic regression models for ARC with respect to P-Se and B-Hg, adjusted for age and current smoking habits, for those of 40y and more ($N$ = 199)

| | Estimate | S.E. | Wald $\chi^2$ | $P$ | OR [CI 95%] |
|---|---|---|---|---|---|
| *Model 1* [a] | | | | | |
| P-Se (< 111 vs. ≥ 111µg/L) | -1.21 | 0.47 | 6.60 | 0.01 | 0.30 [0.12 – 0.75] |
| B-Hg (< 25 vs. ≥ 25µg/L) | 1.18 | 0.57 | 4.27 | 0.04 | 3.25 [1.06 – 9.93] |
| B-Hg (< 44 vs. ≥ 44µg/L) | 0.69 | 0.43 | 2.56 | 0.10 | 2.00 [0.85 – 4.68] |
| *Model 2* [b] | | | | | |
| P-Se (< 111 vs. ≥ 111µg/L) | -1.45 | 0.50 | 8.46 | 0.004 | 0.24 [0.09 – 0.62] |
| B-Hg (< 25 vs. ≥ 25µg/L) | 1.51 | 0.62 | 5.97 | 0.02 | 4.52 [1.35 – 15.16] |
| P-Se (< 111 vs. ≥ 111µg/L) | -1.32 | 0.49 | 7.45 | 0.006 | 0.25 [0.10 – 0.69] |
| B-Hg (< 44 vs. ≥ 44µg/L) | 0.84 | 0.45 | 3.43 | 0.06 | 2.32 [0.95 – 5.65] |

[a] Model 1 includes P-Se and B-Hg separately
[b] Model 2 includes both P-Se and B-Hg

When the interaction term P-Se * B-Hg was entered into the above models, a tendency was observed for the interaction (Estimate = -2.01, $P$ = 0.09), B-Hg (≥ 25µg/L) remained significant (Estimate = 2.72, $P$ = 0.005), while P-Se (≥ 111µg/L) was no longer significant (Estimate = 0.10, $P$ = 0.92), suggesting possible synergy. To further examine the interactions between P-Se and B-Hg with respect to ARC, potential 'risk groups' were examined with respect to the lowest risk group (P-Se ≥

111µg/L (high Se) and B-Hg < 25µg/L (low Hg)). The OR for high Se/high Hg is 2.03 [0.49 – 8.49], low Se/low Hg: 0.91 [0.12 – 6.90] and for low Se/high Hg: 13.78 [2.57 – 73.81]. The same pattern was observed when the B-Hg cut-off was at the median (44µg/L), the ORs are: 1.54 [0.55 – 4.32], 1.80 [0.46 – 7.01], 11.57 [2.68 – 49.98] for the 3 groups respectively.

Because of the high prevalence of ARC among the older participants (85.7%), we examined ARC prevalence in the 40-65 year olds and those ≥ 65 years separately. For the 40-65y group, we included all grades, while in the older group, we considered those with Grade 4 (compared to all of the others). The median value of B-Hg (44µg/L) was used to ensure a better distribution in each quadrant. Figure 31A shows the prevalence of ARC for these categories of P-Se and B-Hg for those between 40 and 65y. When controlling for age and smoking habits, the adjusted OR for P-Se ≥ 111µg/L was 0.20 [0.07 – 0.54] and for B-Hg ≥ 44µg/L, the OR was 2.68 [1.04 – 7.48]. With respect to the risk categories, in comparison to the low risk group (high Se/low Hg), the adjusted OR for high Se/high Hg was 2.19 [0.67 – 7.11], low Se/low Hg: 3.62 [0.78 – 16.83] and for low Se/high Hg: 14.55 [3.04 – 69.73].

For those of 65 years and more, P-Se and B-Hg did not enter significantly into the logistic regression model ($P = 0.13$ and $P = 0.51$ respectively). Figure 31B shows the distribution of prevalence of ARC of Grade 4 along B-Hg group for each of the P-Se categories.

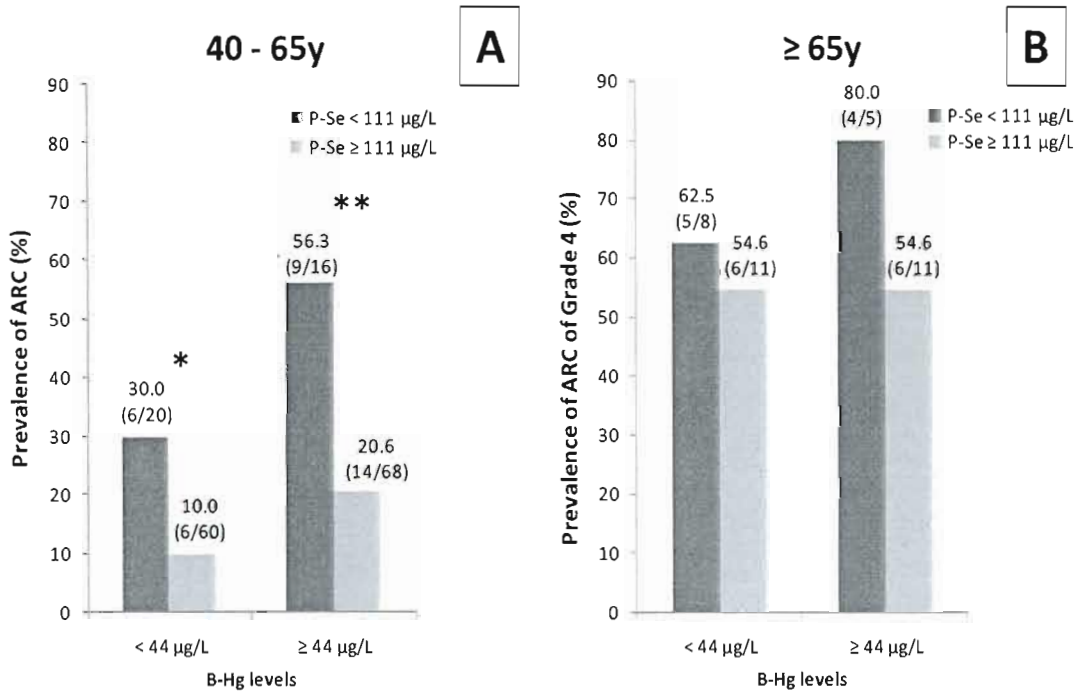


Figure 31 : (A): Prevalence of ARC for the 40-65y group at low and high P-Se with median B-Hg levels; (B): Prevalence of ARC of Grade 4 for the ≥ 65y group at low and high P-Se with median B-Hg levels
Legend: $P < 0.05$: *, $P < 0.01$: **

A small group of persons ($N = 6$) had P-Se levels below 70µg/L, considered Se deficient (U.S. Institute of Medicine, 2000). Of these, two were below 40 years old and did not present ARC; three were between 40 and 65 years old of whom two presented ARC; the remaining person, who was 73 years old, presented Grade 4 ARC. Exclusion of these persons from the above analysis did not change any of the results.

## *4. Discussion*

For this population of the Lower Tapajós Region in the Brazilian Amazon, where environmental exposure to Hg is high compared to the rest of the world (Passos and Mergler, 2008), the prevalence of ARC varied in opposite directions with P-Se and B-Hg. The highest risk group presented elevated B-Hg and low P-Se levels, below 111µg/L. For those with P-Se concentrations above this level, there was no association between B-Hg and ARC. Recent evidence suggests that the Se intake required to optimize all different selenoproteins would be at P-Se concentrations around 125µg/L (Burk et al., 2006).

The increase in ARC with B-Hg for those with lower P-Se is a new finding. Few studies have examined cataracts in relation to Hg exposure, although some reports suggest that Hg accumulates in the lens and may be involved in cataract formation (Gabal and Raslan, 1995; Kairada et al., 1988; Winder et al., 1980). Reddy and Giblin (1984) showed that pre-treatment of human and rabbit lenses with methylmercury decreased *reduced* GSH concentration by 75%; the lenses were also less effective in hydrogen peroxide conversion, which can result in lens opacification. Indeed, both *in vitro* and *in vivo*, Hg molecules have a high affinity to SH groups of small molecules, such as *reduced* GSH and cysteine proteins (for review see Clarkson and Magos, 2006). The binding of Hg to GSH molecules leads to the conversion of *reduced* GSH to its oxidized form (GSSG) and to a depletion of *reduced* GSH concentrations and related antioxidant defenses. However, since SH groups are ubiquitous within the cells, GSH linking to Hg diverts Hg cations from binding to other target proteins and promote Hg efflux from the cell using GSH carriers. Some authors have suggested that Hg can also bind to Se in the active site of selenoenzymes, such as GPx, thereby inhibiting their enzymatic functions (Seppänen et al., 2004).

The literature on the association between Se and ARC in human populations is inconsistent. Low levels of Se were reported for cataractous human lens tissues (Swanson and Truesdale, 1971). Others have reported lower Se concentrations in the aqueous humor of patients with ARC, but no differences in Se lens content (Karaküçük et al., 1995). Some epidemiological studies indicate that Se status (serum or estimated intake) has a positive effect on reducing ARC (Karaküçük et al., 1995; Valero et al., 2002), while one study indicates an increase in cataracts with B-Se (Jacques et al., 1988). Akesson et al. (1987) observed no relation with P-Se levels in men of 68 years old (for those with and without cataracts, mean P-Se levels were 86.1 ± 16.6μg/L and 84.5 ± 11.8μg/L respectively). Li et al. (2009) reported no difference in ARC prevalence between persons living in a poor and a rich-Se area. Several factors may explain these differences: the range of Se status in these populations, the biomarkers used to assess Se status, the chemical form of the Se intake (inorganic versus organic), as well as confounding factors and concomitant environmental and/or occupational exposures.

In the present study, it is not clear whether the Se effect is independent of Hg-related lens damage. A high prevalence of ARC was observed with B-Hg exposure among those with P-Se status under 111μg/L, but the prevalence of ARC did not increase with Hg exposure for whose P-Se was equal to or above this concentration. Several selenoproteins may be involved in lens protection against reactive oxygen species that causes protein cross-linking and lipid peroxidation in the lens (for review see Flohé, 2005). Cytosolic GPx (GPx-1) uses GSH as reducing substrate and has been shown to play a central role in hydrogen peroxide detoxification and consequently, against cataract formation. Together with glutathione reductase and glutathione synthase, GPx-1 is involved in *reduced* GSH pool regeneration in the lens. Extracellular GPx (GPx-3), which plays a role in the regulation of extracellular hydrogen peroxide, has also been identified in the eye (Flohé, 2005). Thioredoxin, which has also been isolated in the lens, participates in the repair process of lens

proteins/enzymes damaged by oxidative stress (Lou, 2003). This small ubiquitous dithiol protein, together with the selenoprotein thioredoxin reductases and NADPH, is involved in redox regulation (Bjornstedt et al., 1997). The selenoprotein R, a methionine sulfoxide reductase, may also be involved in lens cell viability and oxidative stress protection (Marchetti et al., 2005).

The results of the present study suggest that once there is sufficient P-Se for ARC protection, there is no further benefit for ARC from higher P-Se concentrations, which would be more than required for selenoproteins activity. When Se dietary intake is high, excess Se is accumulated nonspecifically in the form of selenomethionine in the proteins of plasma (mainly in albumin) and blood (mainly in erythrocytes) (U.S. Institute of Medicine, 2000), with unknown physiological activity (Laclaustra et al, 2009a). Some authors have suggested that this nonspecific accumulation of Se acts as a storage pool of Se into protein structure that is released during protein turnover (WHO, 1986).

Two hypotheses may explain the findings of the present study. First, Se may play an important preventive role for ARC disease through its antioxidant enzymatic activities and second, higher Se intake may be essential to counter the effects of Hg-induced cataracts by restoring the selenoenzymes and/or the GSH pool. The second hypothesis is more probable, although a combination of both is also possible. Since in the present study, no one is exempt from Hg exposure, we are unable to conclude whether higher Se status (in the absence of Hg) can prevent or delay the progression of ARC. As expected, the prevalence of cataracts increased with age. It is noteworthy that, those with low Se and high Hg in the 40-65y age category displayed a similar ARC prevalence to those above 65 years old, suggesting that in these conditions, the lens may undergo premature aging.

Cataracts have been induced in experimental models with high concentrations of inorganic Se (selenite), at doses below those causing acute Se systemic toxicity (Flohé, 2005). In the present study, no adverse effects were observed although Se concentrations were very high, reaching 1500µg/L for B-Se and 913µg/L for P-Se. In this riverside population, high Se intake comes from local diet, which contains mostly organic forms of Se (Lemire et al., 2009). The toxic effects of organic Se are less understood and toxicity, if it exists, may occur at higher levels compared to inorganic Se (Rayman et al., 2008). On the other hand, concomitant exposure to metals, such as Hg, may raise the body's Se requirements to offset toxic effects (Fordyce, 2005; Watanabe, 2001), and to maintain optimal Se antioxidant enzymes and other Se physiological activities (Rayman, 2009). Thus, in a situation with elevated Hg and Se, there may be less 'excess' of Se and consequently little or no Se toxicity.

For Hg exposure, B-Hg showed a better association with ARC compared to P-Hg. Hg accumulates in the erythrocytes (Clarkson and Magos, 2006) and the largest fraction of Hg is measured in whole blood. On the other hand, P-Se concentration was the Se biomarker best related to ARC prevalence. P-Se reflects recent intake (Combs Jr., 2001), and in this population, despite some seasonal variations, Se remains high throughout the year (Lemire et al. 2009). More than 40% of the Se in human plasma is bound to selenoprotein P (SelP) (Persson Moschos, 2000). Plasma SelP is the main transport form of Se for delivery and supporting essential physiological functions of Se in kidney, testis and brain. There is also increasing evidence suggesting a physiological role of SelP locally produced in the brain (Renko et al., 2008). However, its role in Se transport to the lens and expression within the lens remains to be established. The lens is an isolated system where most of the proteins, such as selenoproteins and GSH, are probably locally synthesized (Head, 2001). In patients with cataract-removal surgery, no correlation was found between P-Se levels and GPx activity in the lens or between GPx activity in the lens and in the erythrocytes, although P-Se and GPx activity in the erythrocytes was positively

correlated (Belpoliti et al., 1993). Since the relations between elements in the blood and in the lens are not well understood and it is unclear whether different blood biomarkers reflect the status of these factors in the lens itself. The mechanism of entry into the lens may also differ between essential elements and toxic metals (Belpoliti et al., 1993).

Some authors suggest that Pb can accumulate in some ocular tissues and that Pb precipitation in the lens may contribute to lens protein denaturation (Erie et al., 2005; Cekic, 1998). In the present study, although B-Pb levels are relatively high, no relation was observed between Pb concentrations and ARC. Pb exposure in this region has been linked primarily to the metal plates used in the processing of manioc flour (Barbosa Jr. et al., 2009). Although little information in available, it is possible that Pb exposure may be relatively recent because the metal plates, which need to be changed approximately every five years, are commercially produced and there may be different suppliers and different manufacturing processes over time.

This cross-sectional study suggests that there are opposing effects of P-Se and B-Hg on ARC, however, longitudinal and case-control studies would be useful to confirm these findings and establish causality. In the Amazon, intense sunlight is omnipresent in daily activities and since there is little inter-individual variability, it would be impossible to control for this important etiological factor in ARC disease (Head, 2001).

Habitants of the Lower Tapajós region regularly eat a great diversity of fruits, and, when available, vegetables complete the daily food intake (Passos et al, 2001). Fruits and green leafy vegetables can contain high concentrations of beneficial antioxidant nutrients (Se, vitamins C, E, β-carotene), as well as other phytonutrients including polyphenols, flavonoids, anthocyanins and carotenoids (Feeney, 2004). There is increasing evidence suggesting that high consumption of fruits and

vegetables nutrients, particularly vitamin C and lutein and zeaxanthin carotenoids, may help prevent or delay age-related eye diseases including cataracts (Bélanger and Johns, 2008; Chiu and Taylor, 2007; Fernandez and Afshari, 2008). These nutrients were not considered here and further studies in this region should address this issue.

ARC prevalence in this Amazonian adult population (32.7%) is similar to that reported for Amazonian riverside indigenous communities of the Rio Negro (36.6%) (de Paula et al., 2006). A study of persons over 80 years of age living in Rio Grande do Sul, the southernmost state of Brazil reported that 85.6% suffered from cataracts (Romani, 2005). This proportion is similar to those in the present study whose age ranged from 65 – 80 years (81.5%), suggesting that the conditions in the Amazonian region may put the population at higher risk for ARC. However, comparisons of ARC prevalence between studies are difficult to interpret because of differences in diagnostic methods, definitions of lens opacities, age distributions, geography, environment, genetics, climate, economy, and culture (Li et al., 2009).

In the Amazon, riverside populations have little access to surgical cataract repair and severe ARC is an important cause of blindness among older persons. Public health interventions to alleviate this disease need to consider the risks and benefits of consumption of fish (the major source of Hg) and local foods that are high in Se.

# CONCLUSION

Les suppléments alimentaires sont à la mode. Plusieurs prétendent que le Se peut prévenir la formation de cancers et plusieurs autres maladies chroniques, même la perte des cheveux! Dans les dernières années, plusieurs compagnies pharmaceutiques ont mis de l'avant les bienfaits des suppléments alimentaires de Se. Inévitablement, ceci a entrainé une augmentation importante de la consommation de suppléments de Se et des multi-vitamines, surtout dans les pays industrialisés. Le bilan de plusieurs études montre néanmoins que de tels effets protecteurs ne sont pas nécessairement fondés (Rayman et al, 2009). Plusieurs études récentes aux États-Unis, en France et en Angleterre suggèrent même que la consommation accrue de suppléments de Se pourrait être en lien avec une augmentation du diabète, de l'hypercholestérolémie et de l'hypertension, et ce, à partir des niveaux de Se plasmatique aussi bas que 95µg/L (en revue dans Stranges et al, 2010). Soulignons que cette concentration est bien inférieure aux niveaux de Se plasmatique chez les riverains du Bas-Tapajós (médiane de 135µg/L). Ces études récentes sur la toxicité du Se, insoupçonnée jusqu'en 2005 (Hercberg et al., 2005), mettent en lumière toute l'importance de comprendre les effets, tant bénéfiques que néfastes, du Se provenant de diverses sources sur plusieurs aspects de la santé chez des populations à travers le monde.

Les travaux de cette thèse contribuent de façon originale aux connaissances sur les sources alimentaires de Se dans le Bas-Tapajós, en Amazonie brésilienne, et mettent en évidence, pour la première fois, des effets bénéfiques d'un statut normal-élevé de Se sur la prévalence de cataractes séniles et sur les fonctions motrices des riverains, exposées à des niveaux élevés de Hg et de Pb.

Les résultats des chapitres I et II montrent que les noix du Brésil constituent la source principale de Se dans cette région. En effet, les concentrations de H-Se chez les riverains étaient en général plus élevées à la saison de la crue des eaux, l'époque de l'année où les noix du Brésil arrivent à maturité et tombent des arbres. Toutefois, les niveaux de B-Se ne variaient pas entre les saisons suggérant qu'il y a d'autres sources de Se dans l'alimentation quotidienne. Ces résultats soulèvent aussi l'hypothèse que lorsque le statut est normal-élevé, le B-Se est un bioindicateur qui reflète les niveaux de Se à plus long terme que les segments séquentiels de H-Se. En effet, l'apport alimentaire principal de Se en Amazonie est probablement sous forme de SeMet, ce qui pourrait entraîner une accumulation non-spécifique élevée de SeMet dans les protéines sanguines (U.S. Institute of Medicine, 2000), et particulièrement dans les érythrocytes qui ont une durée de vie d'environ 120 jours. Ainsi, chez les populations où le statut varie de normal à élevé et lorsque la contamination externe au Se est inexistante (shampoing ou vapeurs de charbon), les segments de H-Se peuvent constituer un bioindicateur utile pour évaluer les variations temporelles et le statut mensuel de Se.

L'étude des niveaux de Se dans les aliments au chapitre II montre qu'en effet il y a plusieurs sources de Se dans l'alimentation des riverains, notamment le poulet domestique, la viande de chasse, les œufs, les abats, le bœuf et certains poissons. De façon générale, les aliments riches en Se qui proviennent des communautés renfermaient plus de Se que ceux des marchés locaux. Toutefois, selon le type d'aliment, les niveaux de Se ne suivaient pas les mêmes profils de variation entre les communautés, ce qui rend l'apport alimentaire de Se presque impossible à prédire.

Malgré d'autres sources de Se, les variations du statut de Se étaient fortement reliées à la consommation régulière des noix du Brésil. À chaque phase de l'étude (2003, 2004 et 2006), le statut de Se étaient systématiquement plus élevé chez la famille élargie de SA-2, où les noix du Brésil contiennent des niveaux plus élevés de

Se que celles des autres villages. Toutefois, les analyses montrent que les noix des villages étudiés contiennent aussi des niveaux élevés de Ba et de Sr. Selon la littérature, ces noix peuvent aussi contenir des quantités importantes de Ra et d'aflatoxines, mais aussi de plusieurs nutriments comme la vitamine E et les acides gras mono et polyinsaturés. Il faut plus de recherches sur l'ensemble des composantes présentes dans les noix du Brésil et leurs effets sur la santé des riverains avant d'en faire la promotion comme un aliment clé contre la toxicité du Hg. Le projet PLUPH (Poor Land Use and Poor Health, 2009), la suite du projet CARUSO dans la région, fait néanmoins la promotion de la plantation d'arbres à noix du Brésil, non seulement pour les noix qui ont une haute valeur commerciale mais aussi pour les propriétés écologiques de cette plante qui favorisent la reforestation et l'équilibre des écosystèmes (Wadt et al., 2005).

Les résultats du troisième chapitre montrent que, dans la région d'étude, il n'y a pas d'évidences de toxicité chronique du Se tel que rapporté en Chine. Trois hypothèses peuvent expliquer ces différences. Les formes de Se dans l'alimentation du Bas-Tapajós sont en grande majorité des composés organiques, qui sont possiblement moins toxiques que les différentes formes de Se inorganiques et organiques présentes dans l'air et dans les aliments en Chine. Puisque les communautés du Bas-Tapajós sont exposées à des niveaux élevés de Hg et de Pb, il se peut que la déficience fonctionnelle de Se causée par l'exposition à ces toxiques réduise l'excès de Se et explique le peu ou l'absence de toxicité du Se. Les noix du Brésil sont endémiques au bassin Amazonien et il se peut que ces populations soient adaptées, et aient même développé des mécanismes pour tirer avantage de cet apport alimentaire élevé de Se.

Cette étude sur la toxicité du Se a été conçue avant la publication de la série d'articles sur le diabète, l'hypercholestérolémie et l'hypertension en lien avec le statut normal-élevé de Se. Toutefois, seulement trois cas de diabète (0.8%) ont été rapportés

chez la population à l'étude. Les résultats préliminaires sur la variabilité du rythme cardiaque et la pression sanguine montrent que le statut de Se ne semble pas les influencer (Philibert et al., *en préparation*). Le profil lipidique complet (cholestérol total, lipoprotéines de haute et de basse densité, etc.) de cette population n'a pas été évalué. Chez les populations du Nunavik, où les concentrations de Se sanguin sont aussi élevées sinon plus qu'en Amazonie, on a rapporté un effet bénéfique du Se sur la pression sanguine (Valera et al., 2009). Il est possible que, tant chez les populations nordiques qu'en Amazonie, les effets bénéfiques du Se relèvent en partie de l'exposition élevée au Hg ou au Pb. Soulignons que pour ces deux populations, le Se provient des aliments et non des suppléments, comme c'est majoritairement le cas dans les études populationnelles mentionnées ci-haut. Il serait important de mieux connaître les conditions dans lesquelles le Se peut avoir des effets bénéfiques ou néfastes sur le diabète, la santé cardiovasculaire et le métabolisme des lipides.

Les résultats des chapitres IV et V montrent des effets bénéfiques du Se sur les fonctions motrices et sur la prévalence de cataractes séniles et corroborent cette hypothèse. De plus, dans ces deux études, lorsqu'on tient compte des effets de l'exposition au Hg, les effets bénéfiques du Se sur le système moteur et les cataractes deviennent plus apparents. Aucune interaction statistique n'a été observée entre le Se et le Hg. Ces résultats suggèrent que les mécanismes d'interactions entre le Se et le Hg ou d'atténuation des effets toxiques du Hg par le Se sont complexes et impliquent possiblement plusieurs voies métaboliques. Des études menées chez cette même population amazonienne suggèrent que les sélénoprotéines et le Se soient intimement liés aux processus métaboliques de la toxicité du MeHg dans la région du Bas-Tapajós en Amazonie (Grotto et al., 2010; Jacob-Ferreira et al, 2009). La figure 32 résume les interrelations métaboliques possibles entre le Se et Hg dans l'organisme.

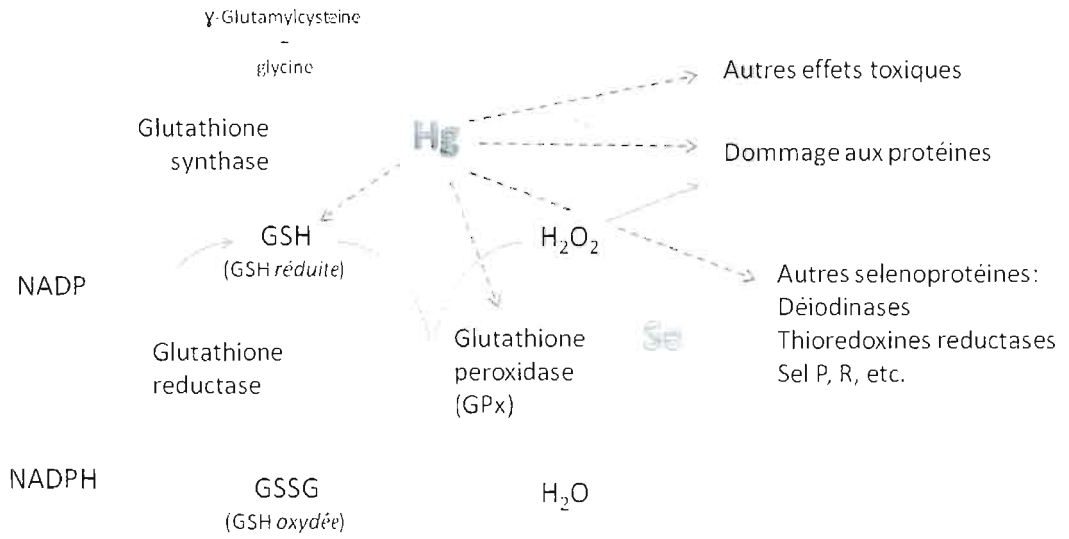


Figure 32 : Schéma simplifié des interrelations possibles entre le Se et le Hg

Les résultats des études sur les fonctions motrices et les cataractes ne permettent toutefois pas de dire si les effets bénéfiques du Se seraient observés chez cette population en l'absence de l'exposition au Hg. L'effet bénéfique du Se sur la prévalence de cataractes séniles n'était pas observé lorsque les niveaux de Hg sanguin étaient plus faibles. Toutefois, il faut noter que même ces niveaux faibles sont élevés comparativement à d'autres populations.

Chez cette population, le Pb avait peu d'influence sur les effets du Se. Ceci suggère qu'il ne semble pas avoir d'interrelations entre ces deux éléments. Les mécanismes de toxicité du Pb sont fort différents du Hg et impliquent peut-être moins de processus oxydatifs pouvant être atténués par un apport élevé de Se.

L'équipe de Nick Ralston fait la propagande qu'un apport alimentaire de Se élevé, venant surtout des poissons marins, au-delà d'un ratio moléculaire Hg:Se 1:1, peut contrecarrer les effets du MeHg sur les sélénoprotéines et sur la santé (Kaneko

and Ralston, 2007; Ralston et al, 2008b; Berry and Ralston, 2008). Ces auteurs disent pouvoir prédire les bénéfices du Se et les risques du Hg pour la santé à partir de ce ratio et proposent d'utiliser cet indice comme critère de risque pour la consommation de poissons et fruits de mer. Dans cette même lignée, Pinheiro et al. (2009) ont récemment suggéré d'utiliser le statut de Se dans le sang comme bioindicateur de susceptibilité au Hg chez les populations exposées au Hg. Les résultats de cette thèse montrent que les interrelations entre le Se et le Hg sont possibles, mais qu'elles sont beaucoup plus complexes qu'un simple ratio molaire. Le plus grand danger d'une approche réductrice est de laisser croire qu'une grande quantité de Hg peut être toléré dans l'organisme sans effets toxiques. Le stress oxydatif n'est pas le seul mécanisme de toxicité du Hg, notamment lors de l'exposition intra-utérine. Il se peut que le Se n'ait pas d'effet bénéfique sur certains effets toxiques du Hg. En effet, les études aux Îles Féroé n'ont pas montré d'effet du Se du sang de cordon ombilical sur les effets chez de l'exposition au Hg *in utero* chez les nouveau-nés et les enfants (Steuwerwald et al., 2000; Choi et al., 2000a).

Cette thèse est la première étude à montrer une relation linéaire entre le Se dans le sang et le plasma, et ce, même lorsque le statut de Se est élevé. Les principales sources alimentaires de Se varient selon les populations, et les différentes formes de Se ingérées pourrait expliquer cette différence entre les études. Par ailleurs, tant pour les fonctions motrices que les cataractes séniles, le Se dans le plasma était systématiquement le meilleur bioindicateur des effets du Se sur la santé. Le Se dans le plasma chez cette population est possiblement la fraction la plus « active » du Se dans le sang, probablement via la SelP et les autres sélénoenzymes qu'il renferme. Chez des populations ayant un statut élevé de Se, les résultats de cette thèse suggèrent que le Se dans le plasma ou les sélénoprotéines plasmatiques peuvent constituer de meilleurs bioindicateurs d'effet du Se sur la santé comparé au Se dans le sang total.

Cette thèse ouvre de nouvelles directions de recherche sur le rôle du Se en lien avec l'exposition au Hg. L'approche écosystémique utilisée dans la présente thèse a permis de caractériser les voies d'exposition, une partie des effets du Se sur la santé et de mettre en évidence la complexité des interrelations possibles entre le Se et le Hg. Chez des populations exposées à des niveaux élevés de Hg, un statut adéquat, et même élevé de Se, pourrait jouer un rôle crucial pour atténuer les effets toxiques du Hg et maintenir l'activité optimale des sélénoprotéines. Plus d'études sont nécessaires pour comprendre dans quelles conditions le Se dans les aliments affecte la santé chez des populations avec une exposition faible ou modérée au Hg.

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# APPENDICE A

## L'approche participative et les différentes phases d'intervention du projet CARUSO dans les communautés du Bas-Tapajós

### *L'origine du volet alimentation au sein du projet CARUSO*

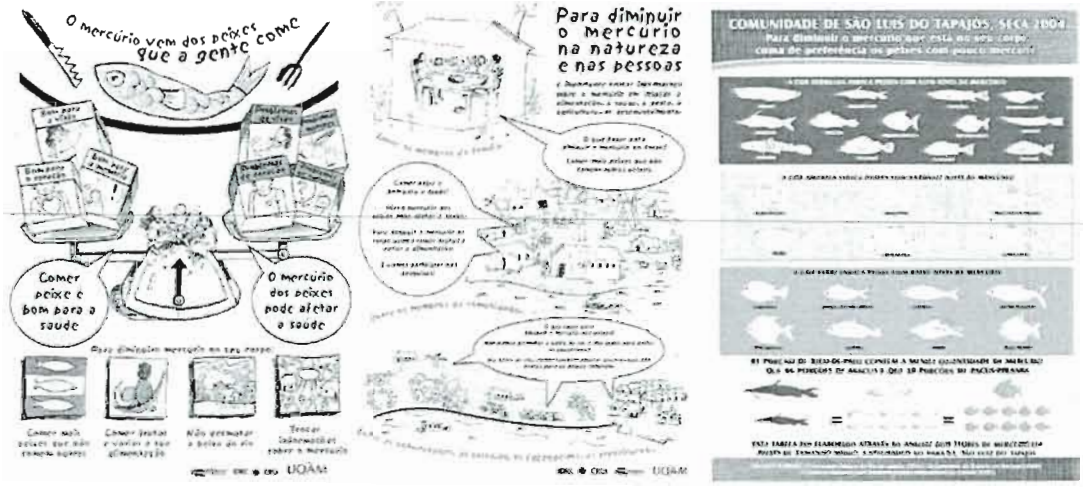
Le projet CARUSO a débuté en 1994 à Brasília Legal. Les deux premières phases de l'étude avaient pour but d'évaluer les sources et les voies de transmission du Hg des sources jusqu'aux riverains et d'étudier les effets neurologiques et cytotoxiques du Hg chez les riverains. Avant chaque terrain de recherche, des réunions communautaires ont eu lieu pour discuter de la problématique du Hg et expliquer le but des collectes d'échantillons réalisées. En 1998, lors d'une réunion communautaire pour présenter les résultats des recherches en cours, les femmes du village ont demandé à l'équipe du CARUSO s'il existait des aliments qui pourraient diminuer les effets du Hg sur leur santé, celle de leurs maris et de leurs enfants. Ce questionnement nous a mené à étudier différents facteurs dans l'alimentation locale tel que : les fruits (Passos et al 2003; 2008), les oméga-3 (Philibert et al, soumis) et le Se (Lemire et al, 2005), en lien avec l'exposition au Hg et, plus récemment, au Pb.

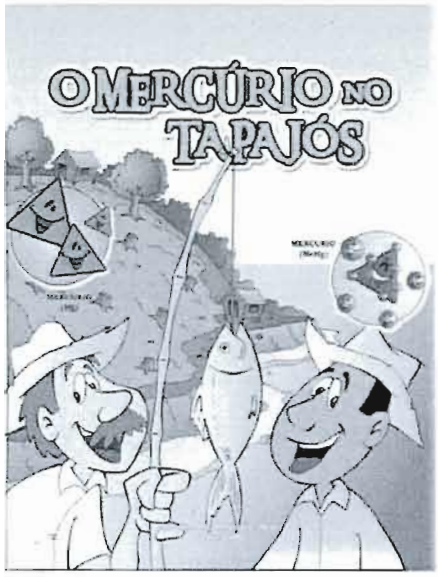
### *Le projet CARUSO de 2003 à 2005*

La troisième phase, l'étape de régionalisation du projet, a démarré en 2002. L'étude du statut de Se des riverains a d'abord été réalisé lors de la saison de la décrue des eaux en 2003 puis à la saison de la crue des eaux en 2004. Six communautés ont été impliquées tout au long de la recherche sur le Se. Cette étude a été réalisée en parallèle aux études suivantes: (i) le Hg dans les écosystèmes aquatiques et les poissons; (ii) le Se dans les poissons; (iii) la consommation de fruits et l'exposition au Hg; (iv) la santé cardiovasculaire et la qualité de vie en lien avec

l'exposition au Hg et (v) les réseaux sociaux sur la dissémination de l'information sur le Hg. Sept autres communautés ont également été impliquées dans l'étude des fruits et l'exposition au Hg. Les résultats de l'étude sur le statut de Se en 2003 sont présentés dans Lemire et al. (2005). Le suivi saisonnier du statut de Se (2003 à 2004) correspond à la PHASE 1 de cette thèse, présentée en détail dans la section Populations à l'étude ainsi qu'au Chapitre I.

Des affiches sur les effets du Hg sur la santé, les réseaux sociaux et les niveaux de Hg dans les poissons des six communautés, ont été conçues par l'équipe en 2004, puis présentées et distribuées lors des réunions communautaires réalisées par les équipes de recherche.






Par ailleurs, le produit de l'ensemble des résultats de la phase trois du projet a été présenté et discuté avec les riverains en 2005 sous plusieurs formes. Une bande dessinée (BD) a été l'outil de communication privilégié par l'équipe de recherche. L'ensemble des résultats des membres de l'équipe a d'abord été intégré puis expliqué dans un langage humoristique et accessible aux riverains. Des graphistes de la région ont conçu les personnages et l'histoire entre les différentes capsules d'information scientifique. À l'été 2005, une tournée de réunions communautaires a été réalisée par l'équipe de recherche dans l'ensemble des communautés impliquées dans les études du CARUSO, dans les hôtels de ville de Aveiro et Itaituba ainsi auprès les professeurs des écoles primaires et secondaires de la région. Ces réunions avaient pour but d'échanger sur la problématique du Hg dans la région et d'informer les gens des nouveaux résultats de l'équipe de recherche, avec la BD comme support visuel. Des tables sur les niveaux de Hg dans les poissons de leur communauté (réalisées en 2004) ont été distribuées en même temps que la BD. Lors de ces réunions, des groupes de discussion ont également été réalisés pour mieux comprendre les aspects de santé qui préoccupent les groupes de femmes et d'hommes des communautés. Les éléments soulevés lors de ces discussions nous ont aidés à préparer la quatrième phase du projet CARUSO. Les autorités locales ont aussi été rencontrées à cette fin.

Dans l'année suivant chaque phase de l'étude en 2003 et 2004, les participants ont reçu leurs résultats personnels de Hg et Se (sang et cheveux), qui leur a été expliqué lors d'une rencontre individuelle et confidentielle.

## *Le projet CARUSO de 2006 à 2008*

La quatrième phase du projet comportait quatre volets. Une étude approfondie des effets du Hg, du Se et des acides gras oméga-3 sur la santé oculaire, visuelle, cardiovasculaire et neurologique a été réalisée dans 12 communautés, incluant Brasília Legal et la plupart des communautés de la troisième phase. Cette dernière a été réalisée dans l'École Technique de Itaituba. Avant de débuter l'étude, des rencontres avec les autorités locales ont été organisées pour informer et demander l'autorisation aux autorités de santé avant de donner suite à l'étude. Dans chacune des 12 communautés, des réunions ont aussi été organisées pour informer les riverains sur la nature de l'étude et les inviter à participer sur une base volontaire. Une visite porte-à-porte par les étudiantes de terrain a aussi été effectuée pour réexpliquer le projet, inviter les gens à participer et montrer la grille horaire de visite-voyage à Itaituba pour l'étude.

Lors de cette étude, des examens visuels ont été réalisés. Les prescriptions des participants ayant besoin de lunettes ont été envoyées chez un optométriste d'Itaituba. En 2006, peu de temps après l'étude, lorsque l'ensemble des lunettes ont été livrées, ces dernières ont été remises en main propre aux riverains dans chacune des communautés.

Une étude des niveaux de Se dans les aliments produits dans quatre communautés (aussi impliquées dans l'étude ci-haut et dans le CARUSO de 2003-2005) a été réalisée en parallèle à l'étude sur la santé. Des réunions communautaires ont d'abord été réalisées avant de commencer l'échantillonnage d'aliments, suivi d'une visite porte-à-porte pour administrer les questionnaires sur la production agricole et les fréquences de consommation des aliments riche en Se et la collecte d'échantillons d'aliments locaux.

Une étude de suivi des pratiques agricoles et une étude des réseaux sociaux sur la dissémination de l'information sur le Hg ont aussi été réalisées dans les six communautés impliquées dans le CARUSO en 2003-2005.

Pour éviter de faire attendre les riverains jusqu'à ce que l'ensemble de l'équipe ait complété l'analyse des résultats, une campagne de retour des résultats personnels de Hg et de Se (sang et cheveux) a été organisée en 2007. Lors de ces rencontres individuelles, les niveaux de Hg et de Se ont été expliqués, accompagnés d'une nouvelle copie de l'affiche de santé et des tables de poissons conçues par l'équipe en 2004.


Une nouvelle version améliorée de la BD a été réalisée en 2008. Les membres de l'équipe de recherche ont veillé à mettre davantage les femmes au cœur de l'histoire, qui sont les personnes les plus importantes pour la diffusion de l'information sur le Hg dans les communautés (Mertens et al, 2005). Des personnages de tous les âges ont aussi été introduits dans l'histoire afin d'être plus près de la réalité et de toucher un plus grand public. Un travail important de vulgarisation a été réalisé pour

expliquer les effets du Hg sur la santé de façon claire et réaliste, afin que les riverains, une fois l'information scientifique en main, soient en mesure de faire leurs propres choix. Puisque l'étude du Hg dans les poissons n'a pas été réalisée lors de cette quatrième phase du projet, au lieu de redistribuer les tables de poissons qui ne sont pas adaptées à toutes les communautés, deux grands concepts ont été développés pour les riverains : (i) *Mangez plus de poissons qui ne mangent pas d'autres poissons* et (ii) *Favoriser la consommation de poisson qui ne viennent pas d'écosystèmes perturbés, entre autres par la déforestation*. L'ensemble du texte a été révisé par un linguiste de la région.

La façon de présenter les résultats sur le Se dans les communautés a suscité plusieurs discussions au sein de l'équipe de recherche. Les résultats bénéfiques du Se sont fort intéressants et importants pour la santé des riverains. Les noix du Brésil sont une excellente source de Se et facilement accesssibles lorsqu'elles sont en saison. Cependant, les niveaux de Se dans les noix sont hautement variables et difficilement prévisibles. Par ailleurs, d'autres éléments peuvent être présents en grande quantité dans les noix, tels le Ba, le Sr, le Ra et les aflatoxines. Leurs effets sur la santé des riverains du Bas-Tapajós demeurent, à ce jour, inconnus. D'autres études sont en cours. L'équipe à choisi de favoriser une alimentation locale et variée et d'attendre d'en savoir davantage avant de recommander spécifiquement la consommation de noix du Brésil.

Une tournée de réunions communautaires a été réalisée par l'équipe de recherche dans l'ensemble des communautés impliquées dans les différentes phases du CARUSO pour discuter avec les riverains des nouveaux résultats et présenter la nouvelle version de la BD. Un séminaire d'une journée avec plusieurs acteurs locaux (syndicats, ONG, organismes gouvernementaux, autorités municipales de la santé et de l'environnement) a aussi été organisé à Itaituba afin de discuter des enjeux concernant la déforestation, le Hg, le Pb et la santé des riverains. Des séances

d'information publiques ont été organisées à Itaituba et Aveiro. Des rencontres privées avec les maires d'Itaituba et Aveiro ont aussi été réalisées afin de les informer des dernières découvertes sur le Hg, et des nouveaux résultats sur le Pb dans la région. Le suivi de l'influence des différentes campagnes d'intervention communautaires sur les niveaux de Hg et la santé des riverains sera abordé en détail dans le chapitre IV de la thèse de Myriam Fillion.

# APPENDICE B

## Questionnaire de l'équipe santé

Universidade de Quebec em Montreal
Universidade Federal do Rio de Janeiro
Projeto Caruso – Fase IV– Itaituba 2006

Caruso 4 : Uma pesquisa participativa sobre os impactos do mercúrio sobre a saúde humana na Amazônia brasileira a fim de construir soluções que maximizam os benefícios do consumo de peixe e minimizam os riscos toxicos ligados ao seu consumo

Nome do participante: _____
ID Comunidade: _____
ID Unidade Familiar: _____
ID Participante: _____
Número de tubo: _____
Número de relógio Polar: _____

### LISTA DE VERIFICAÇÃO

Caro(a) colega, esta lista destina-se ao registro das atividades que já foram realizadas pelo participante do estudo. Por favor, queira marcar com um « X » referente à atividade pela qual você esta responsável, tão logo esta tenha sido realizada.

| | Realizado! | Hora |
|---|---|---|
| Termo de esclarecimento da pesquisa | | |
| Questionário de consumo alimentar | | |
| Questionário medical | | |
| Medidas antropométricas | | |
| Pressão arterial | | |
| Sintomas de toxicidade au selênio | | |
| Testes visuais | | |
| Teste de sensibilidade ao contraste | | |
| Teste de cores | | |
| Teste Santa Ana | | |
| Teste do dinamômetro | | |
| Teste do Groove Pegboard | | |
| Teste de discriminação sensorial | | |
| Exame clínico | | |

Título do Projeto de Pesquisa: Uma pesquisa participativa sobre os impactos do mercúrio sobre a saúde humana na Amazônia brasileira a fim de construir soluções que maximizam os benefícios do consumo de peixe e minimizam os riscos toxicos ligados ao seu consumo.

Coordenadores da Pesquisa: Jean Rémy Davée Guimarães, PhD, UFRJ-Brasil
Donna Mergler, PhD, UQAM-Canadá (514) 987-3000 ramal 3355

Endereço do Pesquisador Responsável no Brasil:
**Lab. de Traçadores, Inst. de Biofísica, Universidade Federal do Rio Janeiro**
**Bloco G, CCS, Ilha do Fundão, Rio de Janeiro RJ CEP 21949-900 Brasil**
Fones : 55 21 25615339 - 55 21 25626651
Fax : 55 21 22808193
E-mail: jeanrdg@biof.ufrj.br

**TERMO DE ESCLARECIMENTO DA PESQUISA**

O objetivo maior do projeto é realizar e avaliar soluções viáveis e sustentáveis para a redução da contaminação mercurial na população ribeirinha do Tapajós, assegurando a saúde do ecossistema. Neste sentido temos a esclarecer os seguintes itens:

O estudo está relacionado com os efeitos do mercúrio nas funções do sistema nervoso, do sistema cardiovascular e do sistema imunológico. Esta pesquisa será realizada por pesquisadores do Laboratório de Radioisótopos da UFRJ, Rio de Janeiro-Brasil, do grupo de pesquisa CINBIOSE, Universidade de Quebec, Montreal-Canadá e da Bloomberg School of Public Health da Johns Hopkins University, Baltimore, U.S.A.

A natureza do estudo envolve os seguintes elementos: a) avaliação de funções do sistema nervoso, usando testes não-invasivos do desempenho motor, visual e sensorial; b) avaliação de funções cardiovasculares, usando testes não-invasivos; c) coleta de amostras de sangue, cabelo e urina serão efetuadas para determinar os níveis de mercúrio, selênio e outros metais ligados à exposição ambiental, de ácidos graxos (benéficos para saúde) e de indicadores de neurotoxidade e de imunotoxicidade.

Todas as precauções necessárias serão tomadas para minimizar as inconveniências e riscos à pessoa pesquisada.

Os benefícios advindos desta pesquisa resultam particularmente do estudo para diagnosticar e prevenir a contaminação mercurial envolvendo inclusive trabalhos de orientação à saúde e educação ambiental.

O sujeito da pesquisa tem a liberdade para decidir participar ou não da pesquisa e ainda retirar-se da mesma sem nenhum prejuízo próprio.

As instituições envolvidas na pesquisa (Universidade Federal do Rio de Janeiro e Universidade de Quebec em Montreal) comprometem-se a amparar quaisquer danos ao

sujeito, desde que devidamente comprovados serem decorrentes de qualquer negligências ou erros da pesquisa.

A informação obtida através de questionários, testes ou análises dentro do contexto da pesquisa são confidenciais e devem ser resguardados e mantidos em sigilo no Laboratório de Radioisótopos da UFRJ, no Laboratório CINBIOSE da UQAM e da Bloomberg School of Public Health da Johns Hopkins University. Cada indivíduo será identificado por um número.

Os pesquisadores se comprometem a fornecer os resultados individuais, mediante solicitação pelos sujeitos da pesquisa. Ao final do estudo, um encontro será organizado por todos os participantes a fim de divulgar os resultados globais e impessoais.

A informação obtida no estudo pode ser utilizada pelos responsáveis da pesquisa com total sigilo, à condição de que os elementos de natureza confidencial não sejam divulgados de maneira em que o sujeito da pesquisa possa ser reconhecido.

Os pesquisadores envolvidos nos estudos estarão à disposição para quaisquer esclarecimentos ao sujeito pesquisado, que poderá contactar particularmente a coordenação, conforme identificado acima.

CONSENTIMENTO LIVRE E ESCLARECIDO

Após ter sido devidamente informado sobre a pesquisa, você se sente perfeitamente esclarecido(a) sobre o conteúdo da mesma, assim como seus riscos e benefícios. È, de livre vontade do Sr.(a), aceita participar da pesquisa cooperando com a coleta de material biológico para exame e entrevista com questionário para coleta de dados adicionais.

Local: _____, Data ___/___/___

_____

Assinatura do Sujeito Pesquisado

QUESTIONÁRIO DE CONSUMO ALIMENTAR

1. Quantas vezes durante a última semana você se alimentou de peixe?
De maneira geral, qual porção de peixe você consume? _____ mg (Conforme o modelo)

| | **Número de refeições para cada dia** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ***Peixes*** | **Dia 1** | **Dia 2** | **Dia 3** | **Dia 4** | **Dia 5** | **Dia 6** | **Dia 7** | **Total** |
| Aracu | | | | | | | | |
| Barbado | | | | | | | | |
| Caratinga | | | | | | | | |
| Charuto / Flexeira | | | | | | | | |
| Curimatá | | | | | | | | |
| Curvina | | | | | | | | |
| Dentudo / Cangoia | | | | | | | | |
| Dourada | | | | | | | | |
| Filhote | | | | | | | | |
| Jaraqui | | | | | | | | |
| Mapará | | | | | | | | |
| Pacu | | | | | | | | |
| Pescada | | | | | | | | |
| Piranha | | | | | | | | |
| Pirarucu | | | | | | | | |
| Sarda / Apapá | | | | | | | | |
| Surubim | | | | | | | | |
| Tambaqui | | | | | | | | |
| Tucunaré | | | | | | | | |
| Ova de peixe<br>Qual peixe? | | | | | | | | |
| *Outros peixes* | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |

**2. Quantas vezes você comeu frutas durante a última semana?(conforme aos modelos de porções)**

| | **Número de frutas consumidas durante o dia** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ***Frutas do quital e colhidas no mato*** | **Dia 1** | **Dia 2** | **Dia 3** | **Dia 4** | **Dia 5** | **Dia 6** | **Dia 7** | **Total** |
| Banana | | | | | | | | |
| Laranja | | | | | | | | |
| Tangerina | | | | | | | | |
| Lima | | | | | | | | |
| Ingá | | | | | | | | |
| Agua de côco | | | | | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Popa de côco | | | | | | | | |
| Pupunha | | | | | | | | |
| Tucumã | | | | | | | | |
| Açai | | | | | | | | |
| Castanha-do-Pará | | | | | | | | |
| Leite de castanha-do-Pará | | | | | | | | |
| Castanha-de-sapucaia | | | | | | | | |
| Babaçu | | | | | | | | |
| Abacaba | | | | | | | | |
| Abacate | | | | | | | | |
| Goiaba | | | | | | | | |
| Jambo | | | | | | | | |
| Acerola | | | | | | | | |
| Suco de Maracuja | | | | | | | | |
| Suco de Cupuaçu | | | | | | | | |
| *Outras frutas* | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |

**3. Quantas vezes você comeu carne durante a última semana?**

De maneira geral, qual porção de carne você consume? _____mg (Conforme ao modelo)

| | **Número de refeições para cada dia** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ***Carnes*** | **Dia 1** | **Dia 2** | **Dia 3** | **Dia 4** | **Dia 5** | **Dia 6** | **Dia 7** | **Total** |
| Gado | | | | | | | | |
| Carne | | | | | | | | |
| Charque (jabá) | | | | | | | | |
| Miúdos, *Qual?* | | | | | | | | |
| Figado | | | | | | | | |
| Coração | | | | | | | | |
| Rim | | | | | | | | |
| *Outros?* | | | | | | | | |
| Frango | | | | | | | | |
| **Ovos de galinha** | | | | | | | | |
| Carne | | | | | | | | |
| Miúdos, *Qual?* | | | | | | | | |
| Figado | | | | | | | | |
| Coração | | | | | | | | |
| Rim | | | | | | | | |
| *Outros?* | | | | | | | | |
| Porco | | | | | | | | |
| Carne | | | | | | | | |
| Miúdos, *Qual?* | | | | | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Figado | | | | | | | | |
| Coração | | | | | | | | |
| Rim | | | | | | | | |
| *Outros?* | | | | | | | | |
| Carne de caça | | | | | | | | |
| Jabuti | | | | | | | | |
| Jacaré | | | | | | | | |
| Paca | | | | | | | | |
| Porco do mato | | | | | | | | |
| Tatu | | | | | | | | |
| Tracajá | | | | | | | | |
| Viado | | | | | | | | |
| Pato e outros aves<br>Qual? | | | | | | | | |
| | | | | | | | | |

**4. Quantas vezes você comeu verduras e condimentos durante a última semana? (portion?)**

| | **Número de refeições para cada dia** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ***Verduras e condimentos*** | **Dia 1** | **Dia 2** | **Dia 3** | **Dia 4** | **Dia 5** | **Dia 6** | **Dia 7** | **Total** |
| Couve | | | | | | | | |

**5. Quantas vezes você comeu laticínos durante a última semana? (modèle de portion)**

| | **Número de refeições para cada dia** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ***Laticínos*** | **Dia 1** | **Dia 2** | **Dia 3** | **Dia 4** | **Dia 5** | **Dia 6** | **Dia 7** | **Total** |
| Leite | | | | | | | | |
| Manteiga | | | | | | | | |
| Queijo | | | | | | | | |

## QUESTIONÁRIO DE HISTÓRIA MÉDICA E HISTÓRIA DE TRABALHO

IDENTIFICAÇÃO
Sexo : M ( ) F ( )
Idade : .....
Data de nascimento : .....
Grau de instrução : .....
Local de nascimento : .....
Residência : .....
Há quanto tempo reside nesta comunidade?:.....
De onde são os seus pais ? (estado e cidade)
Sua mãe:.....
Seu pai: .....

### SAÚDE GERAL

| Perguntas | sim | não | n.s. |
|---|---|---|---|
| 1. Você faz uso de algum remédio?<br>1.1. Qual remédio? .....<br>1.2. Por quê? ..... | | | |
| 2. Você tem alguma doença de nascência ou herdada?<br>2.1. Qual doença? ..... | | | |
| 3. Você é epiléptico ou tem crises convulsivas?<br>3.1. Faz uso de medicação?<br>3.2. Qual medicação?..... | | | |
| 4. Você sente fadiga fácil? | | | |
| 5. Você tem alguma doença nas juntas?<br>5.1. dores articulares ( ) artrite reumatóide ( ) outras:..... | | | |
| 6. Você sofre de perda de consciência? | | | |
| 7. Você tem ou já teve problema de fígado ou vesícula?<br>7.1. litiase ( ) cirrose ( ) hepatite ( ) outros :..... | | | |
| 8. Você já sofreu de poblema de pulmão? | | | |
| 9. Você apresenta dores nos quartos ou quadris? | | | |
| 10. Você tem ou já teve problemas de rins (inchaço, infecções)?<br>10.1. Qual(is)? ..... | | | |
| 11. Em comparação com seus familiares, você tem com mais frequência infecções, gripe ou resfriados? | | | |
| 12. Você dorme bem? | | | |
| 13. Você sofre de insônia? | | | |
| 14. A senhora está grávida? | | | |
| 15. A senhora teve filho recentemente? Quando?..... | | | |
| 16. A senhora está amamentando? | | | |

**SAÚDE CARDIOVASCULAR**

| Perguntas | sim | não | n.s. |
|---|---|---|---|
| 1. Você tem alguma doença do coração?<br>1.1. HAS ( ) ICC ( ) Qual? :..... | | | |
| 2. Você usa remédio contra doença de coração? | | | |
| 3. Você tem dor no peito? | | | |
| 4. Você se cansa sem fazer exercicio físico? | | | |
| 5. Você sofre de palpitações? | | | |
| 6. Você sofre de pressão alta? | | | |
| 7. Você usa remédio contra pressão alta? | | | |
| 8. Alguem da sua familia sofre de pressão alta? | | | |
| 9. Você sofre de diabete? | | | |
| 10. Você já teve derrame cerebral?<br>10.1. Quando?.....<br>10.2. Quais foram as consequências? ..... | | | |
| 11. Alguém da sua familia (pais, irmãos, etc.) morreu de infarto? | | | |

**DOENÇAS INFECTO-PARASITÁRIAS**

| Perguntas | sim | não | n.s. |
|---|---|---|---|
| Você apresenta parasita intestinal?<br>1.1. ascaris ( ) ancylostoma ( ) ameba ( ) solitária ( ) enterobius ( )<br>outros: ..... | | | |
| 2. Você tem refluxo gástrico? | | | |
| 3. Você tem caimbras abdominais? | | | |
| 4. Você vomita frequentemente? | | | |
| 5. Você precisa tomar antiácido? | | | |
| 6. Você tem diarréia frequentemente? | | | |

7. Você já teve malária? ( ) ( ) ( )

7.1. Quantas vezes? .....

| Benígna | | | | Malígna | | | |
|---|---|---|---|---|---|---|---|
| Ano | Quin. | Outro | Tipo | Ano | Quin. | Outro | Tipo |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |

8. Você já teve tuberculose? ( ) ( ) ( )

8.1. Quantas vezes?

1 vez ( ) Quando? .....

2 vezes ( ) Quando? .....

3 vezes ( ) Quando? .....

8.2. Qual foi o tratamento? .....

9. Você já teve febre amarela? ( ) ( ) ( )

**SAÚDE OCULAR**

| Perguntas | sim | não | n.s. |
|---|---|---|---|
| 1. Você vê mal de longe? | | | |
| 2. Você vê mal de perto? | | | |
| 3. Você vê em dobro (duplicado)? | | | |
| 4. Você tem dores nos olhos? | | | |
| 5. Você observa perda de campo de visão? | | | |
| 6. Você sofre ou já sofreu de: | | | |
| Catarata | | | |
| Blefarite | | | |
| Conjuntivite | | | |
| Carne crescida | | | |
| Pressão nos olhos | | | |
| Problema de retina | | | |
| Problema de cornea | | | |
| Olho cego | | | |
| Secura nos olhos | | | |
| Outros:..... ..... | | | |
| 7. Você tem a vista borrada? | | | |
| 8. Você utiliza colírio? | | | |
| 9. Você já foi operado dos olhos ou teve ferida grave? | | | |
| 10. Você usa oculos para correção da vista? Longe ( ) Perto ( ) | | | |

**INFORMAÇÕES ESPECÍFICAS DE NEUROLOGIA**

| Perguntas | S | N | n.s. |
|---|---|---|---|
| 1. Você apresenta distúrbios de memória e dificuldades para lembrar fatos importantes? | | | |
| 2. Você tem alguma dificuldade para identificar os objetos com o tato? | | | |
| 3. Você tem ou já teve algum traumatismo, queimadura, ferida ou perda de membros? | | | |
| 4. Já teve também fratura nas extremidades?<br>mão direita ( ) mão esquerda ( ) perna direita ( ) perna esquerda ( ) | | | |
| 5. Você tem formigamento?<br>5.1. mãos ( ) pés ( ) em torno da boca ( ) | | | |
| 6. Você tem fadiga nas pernas e nos braços? | | | |
| 7. Você sente dor nas pernas? | | | |
| 8. Você sente dor nos braços? | | | |
| 9. Você sente as mãos trêmulas? | | | |
| 10. Você tem espasmos ou caimbras nos músculos? | | | |
| 11. Você tem dificuldade para abotoar ou desabotoar a roupa? | | | |
| 12. Você precisa de ajuda para se levantar de uma cadeira? | | | |
| 13. Você tem dificuldade para andar? | | | |
| 14. Você tem perda de equilíbrio ou uma postura vertical instável? | | | |
| 15. Você sente dores de cabeça?<br>15.1. com frequência ( ) de vez em quando ( ) raramente ( ) | | | |
| 16. Você sente tonturas? | | | |

| | | | |
|---|---|---|---|
| 16.1. com freqüência ( ) de vez em quando ( ) raramente ( ) | | | |
| 17. Você tem problemas para se concentrar?<br>17.1. com frequência ( ) de vez em quando ( ) raramente ( ) | | | |
| 18. Você se sente deprimido?<br>18.1 com frequência ( ) de vez em quando ( ) raramente ( ) | | | |
| 19. Você tem dificuldade para identificar objetos na obscuridade? | | | |

**SAÚDE DAS UNHAS E DOS CABELOS**

| Perguntas | S | N | n.s. |
|---|---|---|---|
| 1.Você tem unhas fracas que quebram facilmente?<br>Mão ( )<br>Pé ( ) | | | |
| 2. Elas quebram no pé da unha?<br>Mão ( ) Quais dedos?.....<br>Pé ( ) Quais dedos?.....<br>2.1. Teve uma causa particular?.....<br>2.2 A unha cresceu normalmente ou mais grossa e rugosa? ..... | | | |
| 3. Seus cabelos são secos? | | | |
| 4. Seu couro cabeludo está irritado e coça? | | | |
| 5. Seus cabelos caem?<br>Nunca ( ) De vez em quando ( ) Frequentemente ( )<br>Pouco ( ) Mais ou menos ( ) Muito ( )<br>6.1. Com o quê você lava os cabelos?..... | | | |
| 7. Os pelos do seu corpo caem?<br>7.1. Com facilidade ( ) Com dificuldade ( ) | | | |

**SAÚDE DA PELE**

| Perguntas | S | N | n.s |
|---|---|---|---|
| 1. Você tem irritações ou erupções na pele? | | | |
| 2. Você tem irritações nas sobrancelhas? | | | |
| 3. Você tem manchas de cor amarelada ou avermelhada no lado exterior dos braços e pernas? | | | |
| 4. Você tem alguma descoloração na pele? | | | |
| 5. Você já teve hanseníase (lepra) ou outra doença de pele?<br>5.1. Qual?..... | | | |
| 6. Você precisa tomar remédios contra doenças de pele?<br>6.1 Qual? ..... | | | |

SAÚDE BUCAL

| Perguntas | sim | não | n.s. |
|---|---|---|---|
| 1. Você sente sabor de metal na boca? | | | |
| 2. Você sente (ou alguém observa) cheiro de alho na sua boca? | | | |
| 3. Você tem obturações?<br>Nenhumas ( ) Menos que 5 ( ) Mas que 5 ( ) | | | |

**INFORMAÇÕES RELATIVAS AO CONSUMO DE CIGARROS, BEBIDAS ALCOÓLICAS E ENTORPECENTES**

| Perguntas | sim | não | n.s. |
|---|---|---|---|
| 1. Você fuma cigarro ou cachimbo? ( ) ( )<br>Quantos por dia? .....<br>Há quanto tempo?..... | | | |
| 2. Você já fumou cigarro ou outras coisas? | | | |
| 3. Você bebe? | | | |
| 4. Você bebia antes?<br>4.1. Parou há quanto tempo? | | | |
| 5. Alguma vez você sentiu que deveria beber menos? "cut down" | | | |
| 6. Alguém já pediu para que não bebesse tanto ou criticou seu modo de beber ? "annoy" | | | |
| 7. Alguma vez você se arrependeu de ter bebido ? "guilty" | | | |
| 8. Tem acontecido de ter vontade de beber ao acordar, mesmo que seja para não tremer as mãos ? "eye opener" | | | |

De maneira geral, durante os fins de semana.....

| Freqüência | Cerveja | Cachaça | Outra(s) bebida(s):..... | Quantidade |
|---|---|---|---|---|
| Sábado | | | | |
| Domingo | | | | |
| Ocasião especial | | | | |
| Nunca | | | | |

Durante a semana ....

| Freqüência | Cerveja | Cachaça | Outra(s) bebida(s):..... | Quantidade |
|---|---|---|---|---|
| Todos os dias | | | | |
| Alguns dias na semana | | | | |
| Ocasião especial | | | | |
| Nunca | | | | |

| Perguntas | sim | não | n.s. |
|---|---|---|---|
| 1. Você consome droga?<br>Qual(is)?..... | | | |
| 2. Você já consumiu droga?<br>2.1. Qual(is)?..... | | | |

**INFORMAÇÕES RELATIVAS AO AMBIENTE DE TRABALHO**

| Perguntas | sim | não |
|---|---|---|
| 1. Você trabalha?<br>Há quanto tempo você trabalha (anos)? ..... | | |
| 2. Com qual dessas ocupações você se identifica? (pode ser mais que uma) | | |
| Agricultor | | |
| Pescador | | |
| Dona de casa | | |
| Professor | | |
| Funcionário(a) da escola | | |
| Estudante | | |
| Agente de saúde | | |
| Carpinteiro | | |
| Artesão, *tipo de artesanato*: | | |
| Comerciante | | |
| *Outras atividades*: | | |
| 3. Quais das atividades seguintes você pratica? | | |
| Você roça? | | |
| Você caça? | | |
| Você cria gado? | | |
| Você cria galinhas ou porco? | | |
| Você coleta de frutas na floresta? | | |
| Você pesca? ( ) ( ) | | |
| 4. Qual tipo de pesca você pratica? | | |
| Pescador comercial (vende diretamente para Itaituba) | | |
| Pescador comercial (isopor) | | |
| Pescador de subsistência (para consumo) | | |
| 5. Na sua casa, existe outro pescador? | | |

**INFORMAÇÕES RELATIVAS A EXPOSIÇÃO OCUPACIONAL**

| Perguntas | sim | não | n.s. |
|---|---|---|---|
| 1. Você trabalha com alguma substância química? | | | |
| 2. Você trabalhou com alguma substancia química?<br>2.1. Qual? Gasolina ( ) Agrotóxicos ( ) Óleos ( ) outras:.....<br>2.2. Com que frequência?<br>Diariamente ( ) Alguns dias na semana ( )<br>Algumas vezes por mês ( ) Alguns meses por ano ( )<br>2.3.Quando você parou de trabalhar com estas substâncias?..... | | | |
| 3. Durante este ultimo ano, você trabalhou no garimpo? | | | |
| 4. Você trabalha ou já trabalhou com mercúrio?<br>4.1. Qual é (era) a freqüência?<br>Diariamente( ) Alguns dias/semana( )Algumas vezes/mês ( )Alguns meses/ano ( )<br>4.2. Quando você foi em contato com mercúrio?<br>a)..... (ano:19____)<br>b)..... (ano:19____)<br>c)..... (ano:19____) | | | |

**MEDIDAS ANTROPOMÉTRICAS**

Altura: _____ cm

Peso: _____ kg

Cintura:_____ cm

Abdomen:_____ cm

**PRESSÃO ARTERIAL**

| | 1 Pressão sistolica (mmHg) | 1 Pressão diastolica **(mmHg)** | 2 Pressão sistolica **(mmHg)** | 2 Pressão diastolica **(mmHg)** | 3 Pressão sistolica **(mmHg)** | 3 Pressão diastolica **(mmHg)** |
|---|---|---|---|---|---|---|
| Braquial | | | | | | |
| Tornozelo (Ankle) | | | | | | |
| Frêqüencia cardiaca | | | | | | |

**Altura do coração na posição sentada: _____ cm**

**SINTOMAS DE TOXICIDADE AO SELÊNIO**

**AVALIAÇÃO DAS UNHAS E DOS CABELOS**

| Sintomas | sim | não |
|---|---|---|
| 1.Sua impressão geral das unhas?<br>Fragil ( ) Normal ( ) Forte ( ) | | |
| 2. Tem um espessamento simétrico (igual), estratificado e rugoso nas unas das mãos? | | |
| 3. Tem unhas das maos deformadas e frágeis (duras mas facilmente quebráveis)? | | |
| 4. Unhas quebrados no pé da raize da unha?<br>Mão ( ) Quais dedos?.....<br>Pé ( ) Quais dedos?..... | | |
| 5. Se tem novas unhas crecendo, como está a nova unha?<br>cresceu normalmente ( ) mais grossa e rugosa ( ) | | |
| 6. Tem uma area branca no pé da unha?<br>Mão ( ) Quais dedos?.....<br>Pé ( ) Quais dedos?..... | | |
| 7. Linhas verticais sobres as unhas?<br>Mão ( ) Pé ( ) | | |
| 8. Linhas horizontais sobre as unhas?<br>Mão ( ) Pé ( ) | | |
| 9. Escurecimento das unhas?<br>Mão ( ) Pé ( ) | | |
| 10. Discoloração branca-amarelada ou vermelhada das unhas? | | |
| 11. Cuticulas que crecem anormalmente? | | |
| 12. Cabelos secos e que quebram facilmente perto da raize? | | |
| 13. Cabelos não têm brilho? | | |
| 14. Cabelos têm pontas quebradas? | | |
| 15. Irritações do couro cabeludo? | | |
| 16. Mycoses no couro cabeludo? | | |
| 17. Piolhos? | | |
| 18. Cabelos caem? A pessoa tem...<br>Muito cabelos ( ) Normal ( ) Pouco cabelos ( ) Muito pouco, careca ( ) | | |
| 19. Pelos do corpo caidos, depilados? A pessoa tem...<br>Muito pelos ( ) Normal ( ) Pouco pelos ( ) | | |

**AVALIAÇAO DA PELE**

| Sintomas | sim | não |
|---|---|---|
| 1. Irritações de pele, area avermelhadas e inchada, com bolhas podendo apresentar erupção ou com descascamento? | | |
| Costa das mão | | |
| Costa dos pés | | |
| Exterior dos membros (braços, pernas) | | |
| Nuca | | |
| Sobrancelhas | | |
| 3. Pele amarelada ou vermelhada? | | |
| 4. Descoloração da pele? | | |

AVALIAÇAO BUCAL

| Perguntas | sim | não |
|---|---|---|
| 1. Dentição fraca? | | |
| 2. Cárie? | | |
| 3. Perdeu dentes?<br>3.1 Mas o menos quantos? ..... | | |
| 4. Linhas marrons sobre os dentes? | | |
| 5. Dentes são gastos? (erodidos) | | |
| 6. Escurecimento ou descoloração dos dentes? | | |
| 7. Cheiro de alho na boca? | | |

**TESTES VISUAIS**

Acuidade visual

| | | | | |
|---|---|---|---|---|
| **VISAO DISTANTE 6 m** | **Olho direito** | | | |
| | **Olho esquerdo** | | | |
| **VISAO PROXIMA 40 cm** | **Olho direito** | | | |
| | **Olho esquerdo** | | | |

REFRAÇãO

| | | |
|---|---|---|
| **VISAO DISTANTE 6 m** | **Olho direito** | |
| | **Olho esquerdo** | |
| **VISAO PROXIMA 40 cm** | **Olho direito** | |
| | **Olho esquerdo** | |

DomiNANÇA OCULAR

**Oll☐ direito** **Oll☐esquerdo**

saúde ocular

| | | **Medida (mmHg)** | **Hora** |
|---|---|---|---|
| **PRESSÃO INTRAOCULAR** | **Olho direito** | | |
| | **Olho esquerdo** | | |

| | | **Medida do ângulo maior** | | | |
|---|---|---|---|---|---|
| | | **1** | **2** | **3** | **4** |
| **ÂNGULO** | **Olho direito** | | | | |
| | **Olho esquerdo** | | | | |

Gotas

☐ Proparacaína 0.5% ☐ Fenilefrina 2.5% ☐ Tropicamida 1%

Segmento anterior

| | **Olho direito** | | **Olho esquerdo** | |
|---|---|---|---|---|
| NENHUM PROBLEMA | | | | |
| Carne crescida | | | | |
| Conjuntiva | | | | |
| Córnea | | | | |
| Cristalino | | | | |
| Outros | | | | |

Segmento posterior

| | **Olho direito** | | **Olho esquerdo** | |
|---|---|---|---|---|
| NENHUM PROBLEMA | | | | |
| Nervo óptico | | | | |
| Mácula | | | | |
| Periferia | | | | |
| Outros | | | | |

Diâmetro pupilar
OD _____mm OE _____mm

**SENSIBILIDADE AO CONTRASTE**

OLHO DIREITO

| | 1 | 2 | 3 |
|---|---|---|---|
| A | | | |
| B | | | |
| C | | | |
| D | | | |
| E | | | |

| | SC |
|---|---|
| A | |
| B | |
| C | |
| D | |
| E | |

**OLHO ESQUERDO**

| | 1 | 2 | 3 |
|---|---|---|---|
| A | | | |
| B | | | |
| C | | | |
| D | | | |
| E | | | |

| | SC |
|---|---|
| A | |
| B | |
| C | |
| D | |
| E | |

**TESTE DE CORES (LANTHONY)**

**SATURADA**

| Bino | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |

**NÃO SATURADA**

| OD | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |

| OE | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |

TESTES NEURO

TESTE SANTA ANA

MÃO DOMINANTE ? DIREITA ( ) ESQUERDA ( )

| PARA CADA TENTATIVA, 30 SEGUNDOS | | | |
|---|---|---|---|
| MÃO DIREITA | | MÃO ESQUERDA | |
| Tentativa | Numero | Tentativa | Numero |
| 1 | | 1 | |
| 2 | | 2 | |

TESTE DO DINAMÔMETRO

FORÇA MÁXIMA

| MÃO DIREITA | | MÃO ESQUERDA | |
|---|---|---|---|
| Tentativa | Força (Kg) | Tentativa | Força (Kg) |
| 1 | | 1 | |

FADIGA DO MÚSCULO

| MÃO DIREITA | | MÃO ESQUERDA | |
|---|---|---|---|
| Tentativa | Tempo (segundos) | Tentativa | Tempo (segundos) |
| 1 | | 1 | |

**TESTE GROOVED PEGBOARD**

PRIMEIRA TENTATIVA

| **MÃO DIREITA** | **MÃO ESQUERDA** |
|---|---|
| Tempo para completar o teste (segundos)<br>---------- | Tempo para completar o teste<br>---------- |
| Número de peões bem colocados<br>---------- | Número de peões bem colocados<br>---------- |
| Número de peões deixados<br>---------- | Número de peões deixados<br>---------- |

SEGUNDA TENTATIVA

| **MÃO DIREITA** | **MÃO ESQUERDA** |
|---|---|
| Tempo para completar o teste (segundos)<br>---------- | Tempo para completar o teste<br>---------- |
| Número de peões bem colocados<br>---------- | Número de peões bem colocados<br>---------- |
| Número de peões deixados<br>---------- | Número de peões deixados<br>---------- |

**TESTE DE DISCRIMINAÇÃO ENTRE DOIS PONTOS ESTÁTICOS**

| | ind d | aur d | ind e | aur e | halux d | halux e | Labio |
|---|---|---|---|---|---|---|---|
| 15 mm | | | | | | | |
| 14 mm | | | | | | | |
| 13 mm | | | | | | | |
| 12 mm | | | | | | | |
| 11 mm | | | | | | | |
| 10 mm | | | | | | | |
| 9 mm | | | | | | | |
| 8 mm | | | | | | | |
| 7 mm | | | | | | | |
| 6 mm | | | | | | | |
| 5 mm | | | | | | | |
| 4 mm | | | | | | | |
| 3 mm | | | | | | | |
| 2 mm | | | | | | | |
| 1 ponto | | | | | | | |

# APPENDICE C

## Questionnaire de fréquences alimentaires

FREQÜÊNCIAS DE CONSUMO ALIMENTAR E PRODUÇÃO AGRÍCOLA
**NOME DO ENTREVISTADOR:** _____
**DATA:** _____ **HORA:** _____

**Nome**:_____
**Comunidade**:_____
**ID participante**: _____

1. De onde são os seus pais ? (estado e cidade)
Sua mãe:_____
Seu pai: _____
2. Há quanto tempo reside neste terreno? _____ anos
3. Você tem uma terra para cultivar? Não ( )
Sim ( ) : Próprio ( ) Família ( ) Outros ( )
4. A sua casa fica perto da sua roça? Não ( ) Sim ( )
Quanto tempo até lá? _____ minutos / _____ km

| **5. Quais das atividades seguintes você pratica?** | **Não** | **Sim** |
|---|---|---|
| Você trabalha na roça da sua família? Area Plantada_____ | | |
| Você trabalha na roça de outras pessoas na comunidade? | | |
| Você tem gado? Area de pasto_____ A | | |
| Você cria galinhas? | | |
| Você cria porco? | | |
| Você tem frutas no seu quintal? | | |
| Você busca frutas no mato? | | |
| Você caça? | | |
| Você pesca? | | |
| Você tem um canteiro? | | |

| **6. Na sua roça, você utiliza.....** | **Não** | **Sim** | Quais são? |
|---|---|---|---|
| Agrotóxicos (veneno para pragas, insetos) ? | | | |
| Adubo orgânico (natural, esterco ou estrume) ? | | | |
| Fertilizante ou adubo químico (de caixa ou liquido)? | | | |

| **7. Com que freqüência você consome alimentos que.....** | Todos os dias | Alguns dias na semana | Alguns dias no mês | Nunca |
|---|---|---|---|---|
| Vêm da sua roça | | | | |
| Vêm da roça de outras pessoas na comunidade | | | | |
| Vêm da roça de outras comunidades<br>*Quais comunidades?*..... | | | | |
| São comprados na cidade<br>*Quais cidades?*..... | | | | |

| **8. Com que freqüência você consome....** | Todos os dias | Alguns dias na semana | Alguns dias no mês | Rara-mente Nunca | Sua roça? | Feira? | Mercado? |
|---|---|---|---|---|---|---|---|
| Arroz | | | | | | | |
| Mandioca | | | | | | | |
| Farinha d'água | | | | | | | |
| Farinha de tapioca | | | | | | | |
| Beiju | | | | | | | |
| Tucupí | | | | | | | |
| Macaxeira | | | | | | | |
| Milho inteiro (espiga) | | | | | | | |
| Farinha de milho (fuba, milharina) | | | | | | | |
| Feijão | | | | | | | |
| | Todos os dias | Alguns dias na semana | Alguns dias no mês | Rara-mente Nunca | Sua roça? | Feira? | Mercado? |
| Batata | | | | | | | |
| Batata doce | | | | | | | |
| Cará | | | | | | | |
| Inhame | | | | | | | |
| Pão | | | | | | | |
| Macarrão | | | | | | | |
| Biscoitos e bolachas | | | | | | | |
| Açucar | | | | | | | |
| *Outros alimentos da roça?* | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |

| **9. Com que freqüência você consome ....** | Todos os dias | Alguns dias na semana | Alguns dias no mês | Rara-mente Nunca | Você cria? | Feira? | Mercado? |
|---|---|---|---|---|---|---|---|
| Gado | | | | | | | |
| Carne | | | | | | | |
| Charque (jabá) | | | | | | | |
| Miúdos, *Qual?* | | | | | | | |
| Figado | | | | | | | |
| Coração | | | | | | | |
| Rim | | | | | | | |
| Bucho | | | | | | | |
| Tripa | | | | | | | |
| Língua | | | | | | | |
| *Outros?* | | | | | | | |
| Galinha Caipira | | | | | | | |
| **Ovos de galinha** | | | | | | | |
| Carne | | | | | | | |
| Miúdos | | | | | | | |
| Frango | | | | | | | |
| Ovos | | | | | | | |
| Carne | | | | | | | |
| Miudos | | | | | | | |
| Picote | | | | | | | |
| **Ovos de picote** | | | | | | | |
| Carne | | | | | | | |
| Miúdos | | | | | | | |
| Pato | | | | | | | |
| **Ovos de pata** | | | | | | | |
| Carne | | | | | | | |
| Miúdos | | | | | | | |
| Porco | | | | | | | |
| Carne | | | | | | | |
| Miúdos | | | | | | | |
| Figado | | | | | | | |
| Coração | | | | | | | |
| Rim | | | | | | | |
| Mocoto | | | | | | | |
| *Outros?* | | | | | | | |

| **10. Com que freqüência você consome carne de ...** | Todos os dias | Alguns dias na semana | Alguns dias no mês | Nunca |
|---|---|---|---|---|
| Capivara | | | | |
| Cutia | | | | |
| Tracajá | | | | |
| Jabuti | | | | |
| Jacaré | | | | |
| Paca | | | | |
| Porcos do mato (catitu, quexada) | | | | |
| Tatu | | | | |
| Viado | | | | |
| Aves, qual? | | | | |
| Outro tipo de caça? | | | | |

**11. De maneira geral, com que freqüência você consome peixes ?**
( ) Todos os dias ( ) Alguns dias na semana ( ) Alguns dias no mês ( ) Nunca

12. Os peixes que você come vêm de onde?
Da comunidade ( ), Onde são pescados?.....
Fora da comunidade ( ), De onde exatamente? .....

| **13. Na CHEIA, com que freqüência você consome ...** | Todos os dias | Alguns dias na semana | Alguns dias no mês | Nunca |
|---|---|---|---|---|
| HERBÍVOROS | | | | |
| **CARNÍVOROS** | | | | |
| Aracu | | | | |
| Barbado | | | | |
| Caratinga | | | | |
| Charuto / Flexeira | | | | |
| Curimatá | | | | |
| Curvina | | | | |
| Dentudo / Cangoia | | | | |
| Dourada | | | | |
| Filhote | | | | |
| Jaraqui | | | | |
| Mapará | | | | |
| Pacu | | | | |
| Pescada | | | | |
| Piranha | | | | |
| Pirarucu | | | | |
| Sarda / Apapá | | | | |
| Surubim | | | | |

| | | | | |
|---|---|---|---|---|
| Tambaqui | | | | |
| Tucunaré | | | | |
| Ovas de peixes<br>Qual peixe? | | | | |
| Outros peixes? | | | | |
| | | | | |
| | | | | |

**14. De maneira geral, você acha que seu consumo de frutas é:**

( ) Todos os dias ( ) Alguns dias na semana ( ) Alguns dias no mês ( ) Nunca

| **15. Na CHEIA, com que freqüência você consome** | Todos os dias | Alguns dias na semana | Alguns dias no mês | Raramente Nunca | Seu terreno? | Feira? | Mercado? |
|---|---|---|---|---|---|---|---|
| Banana | | | | | | | |
| Laranja | | | | | | | |
| Ingá | | | | | | | |
| Água de coco | | | | | | | |
| Popa de coco | | | | | | | |
| Coco ralado | | | | | | | |
| Mamão | | | | | | | |
| Tucumã | | | | | | | |
| Pupunha | | | | | | | |
| Açaí | | | | | | | |
| Caju | | | | | | | |
| Babaçu | | | | | | | |
| Bacaba | | | | | | | |
| Jambo | | | | | | | |
| Cacau | | | | | | | |
| Goiaba | | | | | | | |
| Manga | | | | | | | |
| Abacate | | | | | | | |
| Suco de maracujá | | | | | | | |
| Castanha-do-Pará | | | | | | | |
| Leite de castanha-do-Pará | | | | | | | |
| O leite mesmo | | | | | | | |
| Em suco | | | | | | | |
| Na comida | | | | | | | |
| *Derivados* | | | | | | | |
| Castanha-de-sapucaia | | | | | | | |

**16. Quantas castanheiras tem no seu terreno?_____**

| **17. Com que freqüência você consome ...** | Todos os dias | Alguns dias na semana | Alguns dias no mês | Rara-mente Nunca | Seu terreno? | Feira? | Mercado ? |
|---|---|---|---|---|---|---|---|
| Alho | | | | | | | |
| Alface | | | | | | | |
| Cebolinha | | | | | | | |
| Cebola | | | | | | | |
| Couve | | | | | | | |
| Jerimum | | | | | | | |
| Maxixe | | | | | | | |
| Pepino | | | | | | | |
| Pimentão | | | | | | | |
| Tomate | | | | | | | |
| *Outros alimentos da horta?* | | | | | | | |
| | | | | | | | |
| | | | | | | | |

| **18. Com que freqüência você consome...** | Todos os dias | Alguns dias na semana | Alguns dias no mês | Nunca | Sua família produz? | Feira? | Mercado? |
|---|---|---|---|---|---|---|---|
| Leite | | | | | | | |
| Manteiga | | | | | | | |
| Queijo | | | | | | | |
| Nata | | | | | | | |
| Doce de leite | | | | | | | |
| Coalhada | | | | | | | |
| Leite moça | | | | | | | |
| Creme de leite | | | | | | | |
| Yogourte | | | | | | | |

| **19.De onde vem a água que você usa para...** | Rio[1] | Poço a ceú aberto[2] | Poço artesiano[2] | Cacimba | Igarapé[1] | Grota |
|---|---|---|---|---|---|---|
| Beber | | | | | | |
| Cozinhar | | | | | | |
| Banhar-se | | | | | | |

[1] De qual rio / igarapé?

.....

[2] De qual poço (onde se encontra)? No terreno de quem?

.....

Você utiliza alguma forma de tratamento (pode ser mais que uma)?

Nada ( ) Coar ( ) Filtrar ( ) Cloro ( ) Ferver ( )