Université de Montréal

Évaluation des facteurs de modulation de l'exposition du consommateur de poissons et de mammifères marins au méthylmercure

Utilisation d'approches in vitro, in vivo et probabiliste

Par

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Thèse présentée en vue de l'obtention du grade de Philosophiae Doctor (Ph. D.) en sciences biologiques

Novembre, 2020

Université de Montréal

Département de sciences biologiques, Faculté des arts et des sciences

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Cette thèse intitulée

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Résumé

La chair de poisson et de mammifères marins constitue une source importante de protéines et de bons nutriments, tels que le sélénium (Se), la vitamine E et les acides gras polyinsaturés à longues chaînes. Cependant, la chair de ces animaux peut aussi bioaccumuler la forme organique de mercure (Hg), le méthylmercure (MeHg). Ce contaminant a fait l'objet de plusieurs études épidémiologiques, notamment en raison de sa neurotoxicité résultant d'une exposition *in utero*, étroitement reliée à l'ingestion de poissons fortement contaminés en MeHg. En réponse à ce risque toxicologique, les autorités sanitaires ont émis des lignes directrices quant à la consommation de poissons dans le but de protéger la population. Cependant, ces recommandations présentent certaines limites qui sont liées aux prémisses et aux omissions de l'équation déterministe utilisée pour évaluer l'exposition au MeHg par Santé Canada: (1) elles considèrent que 100% du Hg présent dans la chair de poisson est sous forme de MeHg, (2) elles supposent que le MeHg est distribué de façon homogène à l'intérieur de la chair de poisson, (3) elles prennent pour acquis que 100% du MeHg sera absorbé par le consommateur et (4) par l'utilisation d'une approche déterministe, elles omettent la potentielle incertitude et variabilité intra-populationnelle au niveau des données. L'objectif de cette thèse visait à explorer ces limites, afin de mieux comprendre l'exposition du consommateur au MeHg.

Nous avons tout d'abord évalué la distribution du MeHg, du Se (antagoniste du MeHg) et de l'arsenic (As : antagoniste du Se) à l'intérieur de l'appareil musculaire d'un même poisson, en fonction de sa composition en biomolécules (protéines et lipides). Nos résultats démontrent que la présence concomitante de muscles rouges et blancs induit un gradient important de biomolécules à l'intérieur de l'appareil musculaire d'un même individu, provoquant par le fait même une variation moyenne de 2.2 fois quant à la distribution des métal(loide)s, qui se distribuent en fonction de leurs affinités biochimiques. Ces résultats confirment que le MeHg peut se distribuer de façon hétérogène à l'intérieur du muscle de poisson, ce qui pourrait mener à une sous- ou surestimation de l'exposition au MeHg pour le consommateur, en fonction de la partie du poisson qui est consommée.

Par la suite, nous nous sommes attardés à l'hypothèse stipulant que 100% du MeHg est absorbé par le consommateur. Une des méthodes utilisées pour estimer la fraction de MeHg qui serait disponible à être absorbée par la paroi intestinale consiste à mesurer la bioaccessibilité, c'est-à-dire la fraction soluble de MeHg, à l'aide d'un modèle de digestion *in vitro*. Plusieurs études ayant utilisé cette approche observent que la cuisson de la chair de poisson diminue significativement la bioaccessibilité du MeHg, ce qui diminuerait sa potentielle absorption intestinale. Nous avons donc conduit une expérience *in vivo* en utilisant un modèle porcin et en parallèle, nous avons utilisé un modèle de digestion *in vitro*. Selon les profils sanguins porcins, la biodisponibilité orale du MeHg provenant de la chair de thon cuite n'est pas moins élevée que celle mesurée avec la chair de thon crue. En contraste, nous avons obtenu une bioaccessibilité de MeHg moins élevée avec la chair de thon cuite. Nos résultats démontrent que les modèles de digestion *in vitro* actuels ne sont pas suffisamment optimisés pour être utilisés directement dans les calculs d'exposition au MeHg, tels que proposés récemment dans la littérature.

Finalement, nous avons testé l'impact d'ajout de variables supplémentaires dans l'équation déterministe actuellement utilisée par Santé Canada pour évaluer l'exposition du consommateur au MeHg, en conduisant une évaluation probabiliste du risque. Notamment, nous avons considéré (1) la proportion de Hg méthylée par rapport à la quantité de Hg, (2) la bioaccessibilité et (3) l'augmentation de la concentration du MeHg suite à la cuisson de la chair de poisson, créée par la perte d'humidité. Nos résultats sont clairs : chaque ajout de variable indépendante augmente ou diminue significativement l'exposition calculée, soulignant la sensibilité de l'équation utilisée pour évaluer l'exposition au MeHg. Ceci suggère que de plus amples recherches devront être conduites avant d'effectuer une quelconque modification dans l'équation de l'exposition au MeHg, par souci de ne pas sous-estimer celle-ci.

Cette thèse illustre que les recommandations sont difficilement généralisables puisque la chair de poisson et de mammifères présente des propriétés différentes, en fonction de l'espèce animale considérée. Cette thèse démontre que l'équation déterministe utilisée par Santé Canada dans l'évaluation de l'exposition au MeHg devrait être mieux approfondie par la sphère scientifique, particulièrement dans le cas des mammifères marins.

Mots-clés : méthylmercure, sélénium, arsenic, poisson, type de muscle, bioaccessibilité, biodisponibilité orale, évaluation probabiliste du risque, nourriture traditionnelle

Abstract

Fish flesh and marine mammals is an important source of proteins and nutrients, such as selenium (Se), vitamin E and long chain polyunsaturated fatty acids. However, flesh of those animals may bioaccumulate the organic form of mercury (Hg), methylmercury (MeHg). This contaminant has been the subject of various epidemiological studies, namely because of its neurotoxicity through *in utero* exposure, closely related to highly MeHg contaminated fish consumption. In response to this toxicological risk, health authorities have set fish consumption guidelines in order to protect the population. Still, those guidelines present limits that are related to premises and omissions of the determinist equation used by Health Canada in order to assess the exposure to MeHg: (1) it considers that 100% of Hg in fish flesh is MeHg, (2) it supposes that MeHg is homogeneously distributed within fish flesh, (3) it takes for granted that 100% of MeHg will be absorbed by the consumer and (4) by using a determinist approach, they omit the potential uncertainty and intra-population variability in the data. The aim of this thesis was to address these limits, in order to better understand the exposure of MeHg for consumers.

We first assessed the distribution of MeHg, Se (MeHg antagonist) and arsenic (As, Se antagonist) within fish musculature, as a function of its biomolecule composition (proteins and lipids). Our results demonstrated that the concomitant presence of white and red muscle induces a large gradient of protein and lipid within the muscular apparatus of the same individual. This in turn causes on average a variation by 2.2-fold regarding MeHg, Se and As bioaccumulation, which are distributed according to their biochemical affinity. Those results confirmed that MeHg can distribute heterogeneously within fish muscle, which could lead to an under- or overestimation of MeHg exposure for consumers, as function of the part of the fish consumed.

Subsequently, we focused on the hypothesis stipulating that 100% of MeHg is absorbed by the consumer. A method used to assess the fraction of MeHg that would be available to be further absorbed by the gut wall consists of measuring the bioaccessibility, i.e. the soluble fraction of MeHg, using an *in vitro* digestion model. Several studies that assessed the bioaccessibility of cooked fish flesh observed a decreased of MeHg solubility, that would potentially diminish its intestinal absorption. However, those results have not been yet validated *in vivo*. To that end, we

conducted an *in vivo* experience using the pig model and, in parallel, we used an *in vitro* digestion model. According to the pig's blood profile, the oral bioavailability of MeHg from cooked tuna flesh is not less bioavailable than the MeHg from the raw tuna. Contrasting results have been found with the *in vitro* model, with a decrease of MeHg bioaccessibility observed when fish flesh is cooked. Our results demonstrated that *in vitro* digestion models are not optimized to be directly used in MeHg exposure calculus as recently proposed in the literature.

Finally, we tested the impact of adding variables to the deterministic equation currently used by Health Canada to assess consumer exposure to MeHg, by conducting a probabilistic risk assessment. We considered (1) the proportion of Hg that is MeHg, (2) the MeHg bioaccessibility and (3) the increased of MeHg level after the cooking of fish flesh due to moisture loss. Our results showed that each individual variable significantly increases or decreases the calculated exposure. Thereby, it highlights the sensitivity of the equation used to assess the exposure of MeHg. It strongly suggests that more research is needed to improve Hg exposure calculation to avoid underestimating the potential health risks of MeHg exposure.

This thesis presents important results regarding the exposure of MeHg through fish and marine mammals' consumption. This thesis shows that the recommendations are difficult to generalize since the flesh of fish and mammals has different properties, depending on the animal species considered. The conclusions of this thesis demonstrate that the premises and omissions of the deterministic equation used by Health Canada in the assessment of exposure to Hg should be better investigated by the scientific sphere, especially in the case of marine mammals.

Keywords: methylmercury, selenium, arsenic, fish, muscle type, bioaccessibility, oral bioavailability, probabilistic risk assessment, country food

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Liste des sigles et abréviations

Les caractères italiques proviennent de la langue anglaise

As : arsenic | a*rsenic*

AsB : arsénobétaine |*arsenobetaine*

AsI : arsenic inorganique

AsL : arsénolipide | *arsenolipid*

AsT : arsenic total

As^{5+} : *arsenate*

As^{3+} : *arsenite*

BW : *body weight*

C_{max} : *peak concentration*

CALA : *Canadian Association for Laboratory Accreditation*

CR : *consomption rate*

CQ : consommation quotidienne

CRM : *certified reference materials*

CTE : *central tendency exposure*

CV : *coefficient of variation*

DJA : dose journalière admissible

DMA : *dimethylarsenic acide*

d.w : *dry weight*

CVAFS : *cold-vapor fluorescence spectrometer*

GI : *gastrointestinal*

HBV_{Se} : *Selenium Health-Benefit-Value*

Hg : mercure | *mercury*

HgSe : séléniure de mercure | *mercury selenide*

HgT : mercure total

HQ : *hazard quotient*

iAs : *inorganic As*

ICP-MS/MS : *inductively coupled plasma-tandem mass spectrometry*

MeHg : méthylmercure | *methylmercury*

MeHg-Cys : complexe MeHg-cystéine | *MeHg-cysteine complex*

Métal(loïde)s : métaux et métalloïdes | *metals and metalloids*

MLF: *mass loss factor (unitless)*

MMA: *monomethylarsenic acid*

PBET *: physiologically based extraction test*

PDV: *portal drained viscera*

PC : poids corporel

pMeHg : *proportion of Hg that is MeHg (%)*

QD : quotient de dangerosité

RAF : *relative absorption factor (%)*

RBC : *red blood cell*

RMaE : *reasonable maximum exposure*

RSeH : sélénol | selenol

Se : sélénium | *selenium*

SeCys : sélénocystéine | *selenocysteine*

SelP *: selenoprotein P*

SeMet : sélénométhionine | *selenomethionine*

-SH : groupe sulfhydryle | *sulfhydryl group*

SD : *standard deviation*

SI : *supplementary information*

TDI : *tolerable daily intake*

T_{max} : *time to peak concentration*

TAs : *total arsenic*

THg : *total mercury*

UF : *uncertainty factor*

WB : *whole blood*

w.w. : *wet weight*

À moi-même qui a su poursuivre ses ambitions et foncer.

Remerciements

Cette section est *l o n g u e*. J'ai toujours dit que je me trouvais chanceuse d'être aussi bien entourée, que ce soit dans ma vie personnelle, qu'à l'école ou au labo. Sans cet entourage fait de béton, mais doux comme du coton, jamais cette histoire n'aurait été possible…

Merci à mes directeurs de thèse, Marc et Maikel. Malgré tout le temps que vous avez accordé à ma formation académique, ce que je retiens le plus de vous est votre soutien émotionnel lorsque cela allait bien, mais aussi lorsque cela allait vraiment moins bien. Je pense particulièrement à l'épisode de paralysie de Charlie. Vous avez été présents et vous m'avez donné le temps dont j'avais besoin sans jamais m'avoir reproché une baisse de productivité quelconque. Pour cela, je vous en serai éternellement reconnaissante. Concernant mon parcours académique, merci pour vos prodigieux conseils scientifiques et surtout, merci de m'avoir laissé cette belle liberté de réflexion et d'agissements. Pendant ma thèse, j'ai abordé les sujets et les hypothèses qui me tenaient à cœur et jamais rien ne m'a été imposé. C'est extrêmement gratifiant et motivant pour un étudiant d'explorer ses propres théories. Marc, merci pour tes « jokes » douteuses, ton humour spontané parfois involontaire et pour ta franchise. Maikel, merci pour ta fraîcheur, ton enthousiasme infini et ton calme apaisant.

Merci aux personnels de notre laboratoire incroyablement efficace. Dominic, même avec « d'la broue dans l'toupet », ta priorité #1 a toujours été d'aider les étudiants du labo. Merci pour tes précieux conseils techniques sans lesquels je n'aurais pas accompli le quart de ce que j'ai fait durant mon parcours. Merci de prendre le temps de jaser avec nous de ton week-end, de venir dîner avec nous, de t'informer de comment ça va…j'ai grandement apprécié te côtoyer.

Maria et Kathy, merci pour votre beau sourire quotidien, votre efficacité et votre réel désir de nous aider. Sans votre aide analytique, j'y serais encore pour longtemps.

Merci à mes deux stagiaires en or. Merci Alexandre pour ta minutie, ta sagesse et ton calme. Merci Noémie pour ta fraîcheur, ton regard pétillant, ton intelligence et ta maladresse. Merci à vous deux d'avoir été si autonomes et agréables à fréquenter. Vous avez allégé ma charge de travail de façon très significative ($p < 0.00005$).

Merci aux anciens et présents étudiants du labo. Mélissa, Gwyneth, Catherine, Kawina et Marie-Christine, vous avez été un super modèle de femmes scientifiques à caractère fort, intelligentes et inspirantes. Merci. Jérémy, merci pour ton écoute et ta présence rassurante. Maxime, merci pour tes prodigieux conseils scientifiques et pour ton authenticité. Tes yeux rieurs, tes « jokes » de « pipes » et de « flipage de pneus » vont me manquer. Kimberley et Lise, mes BFFs du labo Amyot. Le *trio Amyotique parfait*. Merci pour votre support quotidien, nos discussions, nos tomates, votre écoute, votre compréhension, votre belle âme, votre sincère gentillesse et votre amitié.

Merci aux membres de l'Usine CrossFit Terrebonne et à ceux de l'Escadron - Unité Athlétique. Merci à mon ancien coach Gab et à ma partenaire d'entrainement Pascale. Ces multiples heures passées à suer et à tout donner en votre compagnie m'ont grandement aidé à gérer mes émotions et à faire le vide dans ma tête parfois trop pleine. Gab, merci pour tes « moves » de danse et Pascale, t'as mal où? Merci à mon nouveau coach Mathieu et à ma partenaire d'entrainement Roxanne. Mathieu, tu es toujours à l'écoute de nos besoins, tu sais autant nous faire rire avec tes « jokes de papa » que de nous surpasser physiquement et mentalement. Merci pour ton support virtuel pendant les 2 vagues de la pandémie. Roxanne, ma jumelle cosmique, merci pour ton support émotionnel durant mon dernier « stretch » de thèse. Ton écoute et ta compréhension ont fait la différence dans mon quotidien. Merci aussi pour ta révision grammaticale fort appréciée!

Merci à mes amies et à ma famille. Merci à mes amies Stéphanie, Ariane et Myriam que je néglige depuis mon entrée à l'université, il y a déjà 10-11 ans de cela. Merci pour votre soutien et votre compréhension de « j'ai pas l'temps, faut qu'j'étudie/j'travail ». Merci à mon amie biologiste/agricultrice/maman extraordinaire Mélody pour son support ***q u o t i d i e n*** depuis Sept-Îles, sans qui mon BAC n'aurait pas été la même histoire. Maman, Billy, papa et Chantal (j'ai la chance d'avoir beaucoup de parents), merci pour vos encouragements infatigables, votre compréhension et vos accommodements par rapport à mon horaire souvent chargé. Vous avez toujours fait passer mes études avant tout et cela a simplifié les choses pour moi. Maman et papa, merci de m'avoir enseigné la persévérance, merci d'avoir eu confiance en moi comme si j'étais l'bon Dieu et merci d'avoir respecté et encouragé mon cheminement scolaire. « En autant que tu

fais c'que t'aimes, c'est ça l'important ». Billy, merci pour ton calme olympien et ton amour inconditionnel. Chantal, merci pour ta curiosité et ta douceur. Merci à mon petit frère Alex pour son soutien émotionnel, sa curiosité et sa douceur acquise de sa maman. Merci à mon grand frère Kevin, sans qui je n'aurais pas acquis le dixième de mes compétences dans et sans qui mon dernier chapitre de thèse n'aurait probablement jamais existé. Merci pour tes conseils statistiques instantanés par message texte à toutes les heures de la journée, merci pour tes conseils hebdomadaires « de me calmer les nerfs » et de me rappeler à quel point « je suis capable, belle et intelligente ». Je t'aime.

Merci à mon conjoint Marc-André pour tout. Pratiquement 10 ans de vie commune. 10 ans durant lesquels j'ai été sur les bancs d'école sans relâche. 10 ans à m'encourager, malgré ton désir incommensurable de fonder une famille. 10 ans à m'aimer assez pour me laisser vivre mes rêves et d'atteindre mes objectifs. Merci pour ta patience, merci pour toutes les fois où tu m'as pris dans tes (gros) bras lorsque je n'en pouvais plus, merci pour toute la confiance que tu avais en moi quand moi-même je m'y croyais plus capable. Merci pour ta douceur, merci pour ton regard empathique, je t'aime. Merci à mes bébés (canins) Casca et Charlie. Mes partners de télétravail quotidien. Oui, vous m'avez demandé beaucoup de temps alors que je n'en avais même pas pour moi-même…mais vous m'avez redonné au centuple! Votre amour est tellement simple, sincère et inconditionnel. Avec votre joie contagieuse, vous me rappelez chaque jour de vivre dans le moment présent, de ne rien prendre trop au sérieux et d'apprécier chaque petit plaisir de la vie.

Mise à jour : Alors que je m'apprête à effectuer le dépôt final de cette thèse, voilà que j'en suis à 11 semaines de grossesse. Une grossesse prématurée, certes, mais ô combien bien accueillie. La voici notre famille, mon amour.

Introduction générale

La chair de poisson et de mammifères marins constitue une source importante de protéines alimentaires (Blanchet et al., 2000; FAO/WHO, 2010). Malgré les nutriments essentiels à la santé humaine que leur consommation apporte (acides gras polyinsaturés à longues chaînes, sélénium (Se), vitamines, etc.) (Blanchet et al., 2000; Myers et al., 2007), leur chair peut s'avérer à être contaminée par divers composés métalliques tels que le mercure (Hg) (Cizdziel et al., 2001; Lemire et al., 2015). De façon générale, on retrouve l'espèce chimique méthylée à de très grandes proportions dans la chair de poisson, soit au-delà de 80 % du mercure total (HgT : le Hg sous toutes ces espèces chimiques) (Magalhães et al., 2007). Cette proportion varie grandement chez les mammifères marins, en fonction de l'espèce et de l'organe considéré (Lemes et al., 2011; Lemire et al., 2015; Wagemann et al., 1998). Le MeHg possède des propriétés qui favorisent sa bioaccumulation et sa bioamplification tout au long de la chaîne alimentaire (Chen et al., 2009). Ce dernier phénomène se traduit par une augmentation de la teneur en MeHg de proies en prédateurs (Chen et al., 2009). Ainsi, la chair des prédateurs en haut de la chaîne alimentaire peut atteindre des niveaux de contamination en MeHg importantes.

Le phénomène de bioaccumulation s'est montré problématique pour la santé humaine à la fin des années 1950 à Minamata, au Japon. Cet évènement a été causé par le rejet d'eaux usées industrielles contenant du MeHg directement dans la baie de Minamata (Clarkson, 2002). Les poissons et les fruits de mer présents dans la baie ont bioaccumulé le MeHg jusqu'à des niveaux inquiétants, allant jusqu'à 35 ppm (Harada, 1995). À titre comparatif, la norme canadienne pour la vente commerciale de poissons est de 0.5 ppm de HgT (Health Canada, 2007b). Jusqu'à 2200 résidents de la baie ayant consommé ces poissons ont démontré des dommages neurologiques, tels que le manque de coordination, la constriction du champ visuel et un engourdissement aux extrémités des membres (Harada, 1995). Quant à elle, la mortalité s'élève à 1000 cas (Harada, 1995).

En réponse à son omniprésence dans les écosystèmes aquatiques et à sa toxicité, les autorités ont émis des recommandations de consommation concernant les espèces de poissons

fortement contaminées en MeHg (> 0.5 ppm), telles que celles mentionnées dans le *Guide alimentaire canadien* (Santé Canada, 2019b). Le Hg est d'ailleurs le seul élément considéré lors de la conception de ces recommandations (Santé Canada, 2007). Bien qu'elles soient essentielles, les recommandations présentent certaines limites qui sont liées aux prémisses et aux omissions de l'équation déterministe utilisée pour évaluer l'exposition au MeHg par Santé Canada. L'équation déterministe utilisée présentement par Santé Canada pour estimer l'exposition (*EXP*) au MeHg est:

$$EXP = \frac{CQ \times [HgT]}{PC}$$

où *CQ* représente la consommation quotidienne (mg en poids humide de chair de poisson par jour), *[HgT]* est la concentration de HgT de la chair de poisson (mg × kg^{-1} poids humide) et *PC* correspond au poids corporel du consommateur (kg).

La première limite soulevée est reliée au fait que l'exposition *(EXP)* est calculée en fonction de la teneur en HgT (Santé Canada, 2004) et non en fonction de la concentration en MeHg, surestimant l'exposition du consommateur à ce dernier. À ceci nous ajouterions que les recommandations de consommation de poissons omettent la potentielle hétérogénéité au niveau de la bioaccumulation du MeHg au sein d'un même poisson, tel qu'observé avec le HgT dans la littérature (Bosch et al., 2016; Cizdziel et al., 2001; Piraino & Taylor, 2009), ce qui pourrait modifier la réelle exposition du consommateur au MeHg. Le **Chapitre 1** de cette thèse s'intéresse donc à mieux comprendre le patron de bioaccumulation du MeHg à l'intérieur de la musculature d'un même poisson. Cette étude a été bonifiée par l'ajout de l'évaluation du Se et de l'arsenic (As) puisque le MeHg est connu pour sa grande affinité de liaison chimique envers le Se (Khan & Wang, 2009), tout comme l'As qui se lie aussi au Se (Zeng et al., 2005). En fait, Le Se est un antagoniste connu du MeHg pouvant atténuer la toxicité de ce dernier (Peterson et al., 2009). Cependant, l'As inorganique (AsI) est un antagoniste du Se qui pourrait limiter les effets protecteurs du Se envers le MeHg (Zeng et al., 2005). Sans directement évaluer leur potentielle interaction, nous avons étudié leur co-variation tout en considérant la composition en biomolécules (protéines et lipides) de la chair de poisson.

Une troisième limite en ce qui a trait à l'équation déterministe utilisée présentement par Santé Canada pour estimer l'exposition au MeHg est liée à la prémisse considérant que 100% du MeHg présent dans la chair de poisson sera absorbé par le consommateur. Cette prémisse est basée, entres autres, sur deux études datant des années 1970 où des humains ont été exposés à du nitrate de MeHg ($MeHgNO_3$) (Aberg et al., 1969; Miettinen et al., 1971). Ils ont effectivement observé un taux d'absorption très élevé (entre 95 et 100%). Ces résultats sont supportés par plusieurs études basées sur l'utilisation de MeHg radioisotopique utilisant des modèles mammifères, où l'exposition orale au MeHg a mené à de hauts taux d'absorption (> 80%) (Berlin et al., 1975; Bergman et al., 1972; Hollins et al., 1975; Reuhl & Pounds, 1981). Cependant, aucune de ces expériences n'a utilisé le MeHg naturellement bioaccumulé dans la chair de poisson. En réalité, le MeHg s'insère généralement dans l'appareil musculaire du poisson en créant des liens avec les groupes sulfhydryle (-SH) (Harris et al., 2003). Il est acceptable de penser qu'une molécule de MeHg en solution « libre » sera plus facilement absorbée qu'une molécule de MeHg imbriquée dans la chair de poisson. Effectivement, pour que le MeHg soit relâché du muscle de poisson, la matrice doit être digérée pour que le MeHg se trouve sous forme soluble dans les fluides gastro-intestinaux, alors que le MeHg en solution est probablement initialement sous forme dissoute et soluble. Ainsi, le MeHg naturellement bioaccumulé dans la chair de poisson est possiblement moins bien absorbé au niveau de la paroi intestinale du consommateur qu'une forme de MeHg en solution. Au meilleur de notre connaissance, une seule étude a estimé la biodisponibilité orale du HgT provenant de la chair de poisson. Celle-ci a utilisé la souris comme modèle et a mesuré une biodisponibilité orale variant de 38 à 99% (Li & Wang, 2019). Aucune littérature ayant estimé la biodisponibilité orale du MeHg provenant de la chair de poisson n'a été répertoriée.

Une façon d'estimer l'absorption du MeHg par l'humain est de procéder à l'évaluation de la *bioaccessibilité*, qui représente l*a fraction d'un élément qui se trouve sous forme soluble dans les fluides gastro-intestinaux* (Fig. 1, schématisée en gris) (Nordberg et al., 2009). La bioaccessibilité n'est pas à confondre avec la *biodisponibilité orale* qui se définit comme étant la *fraction d'un élément ingérée qui atteint la circulation sanguine* (Fig. 1, schématisée en rouge) (Shen, 2001).

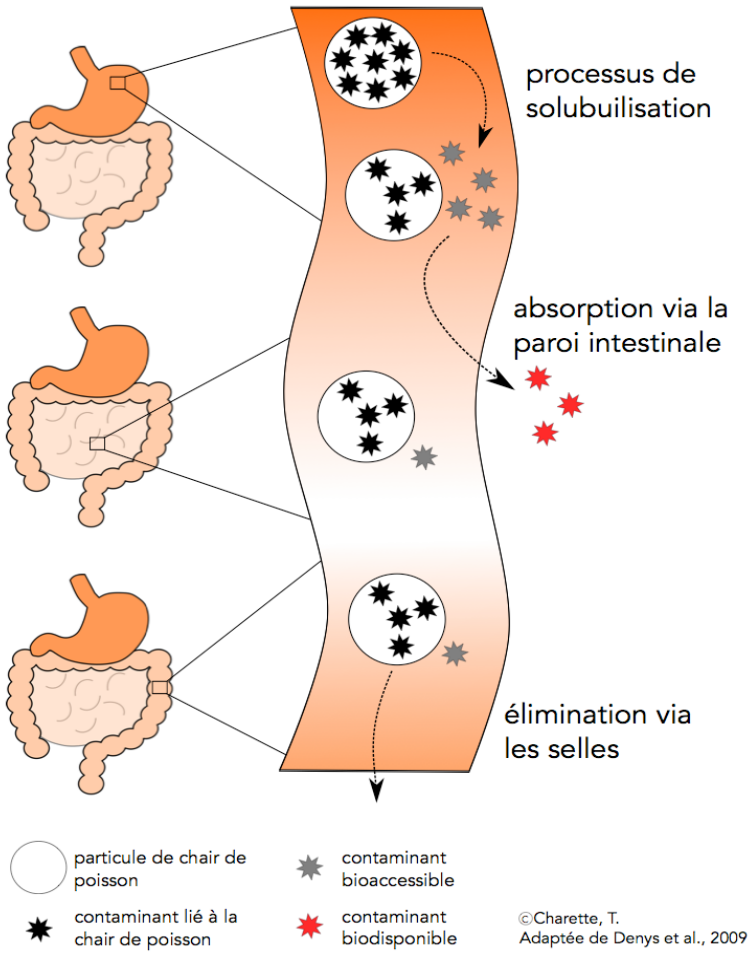

Figure 1. Schématisation de la bioaccessibilité et de la biodisponibilité orale.

Plusieurs études ont démontré que la cuisson de la chair de poisson réduit considérablement la bioaccessibilité (Fig. 1, schématisée en gris) du HgT et du MeHg (Alves et al., 2018; Girard et al., 2017; Ouédraogo & Amyot, 2011), suggérant qu'un traitement thermique diminuerait la *biodisponibilité orale* ou l'absorption intestinale du Hg (Fig. 1, schématisée en rouge). Bien que ces résultats soient intéressants, ils ont été produits en laboratoire et requièrent une validation *in vivo*. Le **Chapitre 2** de cette présente thèse compare donc l'influence de la cuisson de la chair de thon sur la bioaccessibilité et la biodisponibilité orale du MeHg chez le modèle porcin.

Finalement, une dernière limite soulevée concernant les recommandations de consommation de poissons se trouve au niveau de la nature déterministe de l'équation utilisée par Santé Canada pour estimer l'exposition du consommateur au MeHg. Celle-ci omet la considération de la variation et l'incertitude par rapport à divers paramètres utilisés dans l'équation *EXP*, telle que la concentration de HgT dans la chair de poisson qui varie d'un individu à l'autre ou le PC du consommateur qui varie à l'intérieur d'une même population, par exemple. Une variation au niveau de ces variables influencerait directement le calcul d'exposition au MeHg.

En plus, pour le moment, l'équation déterministe *EXP* présentée ci-haut omet d'inclure l'impact de la cuisson sur la teneur en MeHg, la proportion de Hg qui est méthylée et la proportion du MeHg qui est absorbée par le consommateur. Au **Chapitre 3** de cette thèse, un modèle d'évaluation probabiliste du risque incluant ces paramètres novateurs sera présenté afin d'estimer l'exposition du consommateur au MeHg.

Le texte suivant abordera la littérature pertinente en lien avec les trois chapitres de cette thèse. Dans le cadre du **Chapitre 1**, nous aborderons les différents types de muscles retrouvés chez les poissons et présenterons très brièvement les facteurs influençant la bioaccumulation du MeHg, du Se et de l'As au sein de l'appareil musculaire des poissons. Par la suite, nous exposerons divers facteurs modulant l'exposition du consommateur au MeHg, ainsi que des méthodes permettant d'estimer cette exposition, en lien avec le **Chapitre 2** et le **Chapitre 3**.

Les principaux processus associés à la bioaccumulation

En écotoxicologie, la *bioaccumulation* se définit comme étant [traduction] « l'augmentation progressive de la quantité d'une substance dans un organisme ou une partie de l'organisme qui se produit parce que l'apport provenant de toutes les sources et de toutes les routes possibles excède la capacité de l'organisme à éliminer la substance de son corps » (Nordberg et al., 2009). Ainsi, la bioaccumulation comprend plusieurs processus : l'ingestion, l'absorption, la distribution interne et l'élimination (Newman, 2003).

Dans le contexte de cette thèse, la route d'exposition qui nous intéresse est l'ingestion orale. Une fois ingéré, le MeHg est habituellement bien absorbé au niveau des entérocytes (Chapman & Chan, 2000). Par la suite, il atteint la circulation sanguine, qui constitue son principal moyen de transport afin d'être distribué vers d'autres tissus et organes (European Food Safety Authority, 2012). Une fois dans le compartiment sanguin, le MeHg possède deux destins : soit il est bioaccumulé au sein des tissus de l'organisme, soit il est éliminé par un mécanisme de détoxication qui habituellement procède *via* la déméthylation du MeHg (National Research Council, 2000a). Il existe deux mécanismes de déméthylation bien étudiés, soit la déméthylation hépatique impliquant le Se ou encore, l'implication du microbiote au niveau du gros intestin (Guo et al., 2018; Khan & Wang, 2009). Une fois déméthylée, la forme inorganique de Hg est habituellement éliminée *via* les fèces (Clarkson, 2002). Si le taux d'élimination est plus faible que le taux d'absorption, il se produit alors une bioaccumulation.

La bioaccumulation musculaire chez le poisson

Chez le poisson, l'étude de la bioaccumulation des métaux interorgane est bien documentée, comparativement à celle intraorgane (Bosch et al., 2016; Maury-Brachet et al., 2006; O'Bryhim et al., 2017; Oliveira et al., 1996). L'appareil musculaire du poisson constitue le principal site de storage pour la bioaccumulation en MeHg (Bloom, 1992; Peng et al., 2016). La variation du contenu en biomolécules entre les types de muscles (blanc, rose et rouge) pourrait influencer la bioaccumulation des métaux et des métalloïdes (métal(loïde)s) en fonction de leurs affinités physico-chimiques (Greer-Walker & Pull, 1975; Johnston et al., 1975; Jones & Sidell, 1982).

Les différents types de muscles d'un poisson téléostéen

Cette thèse se concentre particulièrement sur les poissons téléostéens, puisqu'ils sont largement plus consommés que les poissons cartilagineux (Santé Canada, 2007). De façon générale, le myotome des téléostéens est composé de 2 types de muscles distincts : les muscles rouges et les muscles blancs (Johnston et al., 1975) (Fig. 2). Sous la peau, les muscles rouges

longent la ligne latérale du poisson, partant de la tête jusqu'au bout du pédoncule caudal. Comparativement aux muscles blancs, ils possèdent une haute concentration en mitochondries et lipides, sont très vascularisés et correspondent aux muscles lents à métabolisme aérobique. De leur côté, les muscles blancs sont situés au niveau de la musculature axiale, ainsi que sous les muscles rouges au niveau de la ligne latérale. Ils possèdent moins de lipides, leurs fibres sont de plus gros diamètres que celles des muscles rouges et correspondent aux muscles rapides à métabolisme anaérobique (Greer-Walker & Pull, 1975; Johnston et al., 1975; Jones & Sidell, 1982). Chez plusieurs espèces de poissons, un troisième type de muscle s'ajoute à la musculature. Il s'agit des muscles appelés *intermédiaires* ou *roses* et sont localisés au niveau de la ligne latérale, entre les muscles rouges et blancs (Fig. 2). Ce troisième type de muscle possède un métabolisme aérobique et est utilisé lors de la nage rapide (Kiessling et al., 2006).

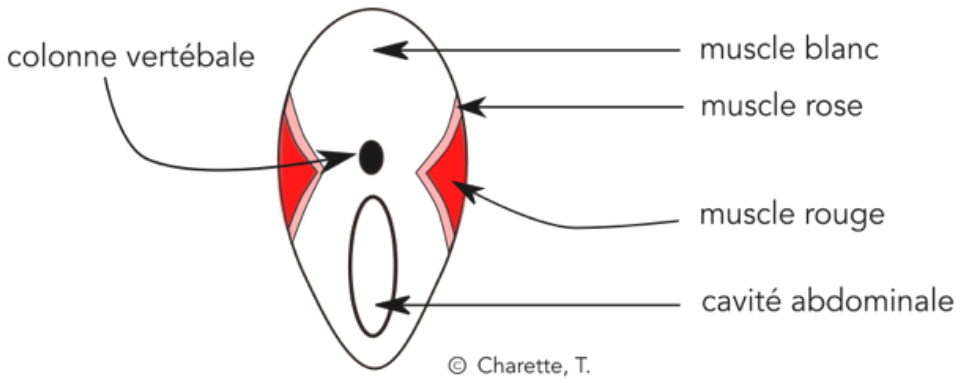

Figure 2. Schématisation de la coupe transversale d'un poisson téléostéen présentant l'emplacement des muscles blancs, roses et rouges.

Mieux comprendre les mécanismes reliés à la bioaccumulation musculaire du MeHg en fonction du type de muscle est pertinent dans le contexte où au Canada, la consommation de poissons est limitée uniquement en raison de la contamination de la chair de poisson en Hg (Santé Canada, 2007). D'autres contaminants environnementaux sont également monitorés, mais leur teneur dans la chair de poisson n'est habituellement pas considérée comme étant problématique (Santé Canada, 2019a).

De plus en plus, le Se est additionné aux études portant sur le MeHg, en raison de la grande affinité de liaison entre ce dernier et les biomolécules contenant un groupe sélénol (RSeH, où R représente un radical fonctionnel allant de la famille des acides aminés jusqu'aux protéines) (Wang et al., 2012). Les affinités de liaisons chimiques peuvent dicter la mobilité du MeHg lorsqu'il est ingéré. Par exemple, en se liant à la sélénocystéine (SeCys), le complexe MeHg-SeCys peut soit entrer dans le cycle de synthèse protéinique ou être dégradé sous forme de complexe insoluble de séléniure de Hg (HgSe) (Ralston & Raymond, 2010). L'As est également un élément complémentaire à considérer puisque sous ses formes inorganiques, il peut se lier au Se (Zeng et al., 2005).

La bioaccumulation du méthylmercure

Le Hg est émis naturellement dans la nature, par des éruptions volcaniques ou par l'érosion de la roche mère par exemple. Cependant, sa présence est majoritairement causée par des activités anthropiques, telles que la fusion des métaux, les usines chloro-alkali ou la combustion de charbon (Pacyna et al., 2006). Afin de diminuer les émissions de Hg dans l'environnement, la Convention de Minamata est entrée en vigueur en 2017 (Programme des Nations Unies pour l'Environnement, 2019). Résultant de cette convention et en se basant sur les cycles du Hg, Chen et al. (2016) anticipe que les niveaux de Hg dans la chair de poisson vivant en haute mer diminueront d'ici quelques années ou même décennies, alors que ce délai pourrait s'allonger en termes de siècles pour les poissons vivants en milieu côtier.

Une fois relâché dans l'environnement, le Hg peut ensuite se faire méthyler en milieu aquatique *via* des processus abiotiques et biotiques (Paranjape & Hall, 2017). Il s'introduit par la suite dans les chaînes alimentaires aquatiques, depuis la matrice abiotique (eau et sédiments) vers les organismes à la base de la chaîne trophique, tels que les algues (Fitzgerald et al., 2007). Ensuite, par son ingestion, le MeHg est bioaccumulé au sein des tissus de plusieurs organismes, incluant le poisson (Redmayne et al., 2000). Ainsi, dans les réseaux trophiques aquatiques, le MeHg bioaccumulé par les organismes de bas niveau trophique est transféré *via* prédation vers les organismes de haut niveau trophique, où la concentration de MeHg est bioamplifiée à chaque

palier de ce réseau (Chen et al., 2009). La principale voie d'entrée du MeHg chez le poisson se produit *via* l'alimentation (de Pinho et al., 2002). Plusieurs facteurs biologiques peuvent influencer la bioaccumulation du MeHg dans la chair de poisson tels que l'espèce, les habitudes alimentaires et l'âge du poisson (Polak-juszczak, 2015).

Chez le poisson, le MeHg serait transporté au-delà de la barrière intestinale en créant des complexes avec la cystéine (MeHg-Cys) naturellement présente dans les fluides intestinaux (Leaner & Mason, 2004). Ensuite, toujours complexé à la cystéine, il est transporté vers le tissu musculaire, où la cystéine s'y déplace habituellement à des fins de synthèse protéinique (Amlund et al., 2007). Ainsi, une fois ingéré, le MeHg serait bioaccumulé préférentiellement au niveau des tissus musculaires (Mieiro et al., 2009). Plus précisément, une étude a observé que la majorité du Hg [étendue 50-98.6%] se trouvait au sein de la fraction des protéines solubles (Nong et al., 2020). Dans cette expérience, ils ont mesuré dans la chair de thon que 38% du Hg était lié à la protéine bêta actine, celle-ci étant constituée, entres autres, de 5 résidus de cystéine (Nong et al., 2020). La cystéine est un acide aminé soufré et le MeHg possède une forte affinité de liaison envers les -SH (Leaner & Mason, 2004). Cette affinité de liaison chimique s'est aussi transcrite dans les résultats de deux études ayant répertorié, au niveau du muscle de poisson (*Colossoma macropomum* et *Semaprochilodus spp*), l'hémoglobine comme étant un potentiel biomarqueur de Hg, en raison de sa composition en résidus de cystéine au niveau de sa chaîne β (Bittarello et al., 2019; Vieira et al., 2018).

En consommant la chair de poisson, l'humain est majoritairement exposé aux complexes MeHg-Cys puisqu'il s'agit de l'espèce chimique de MeHg la plus abondante dans le muscle de poisson (Harris et al., 2003). Suite à son incorporation au sein de la matrice musculaire, le MeHg est difficilement éliminé par l'organisme. Effectivement, son temps de résidence au sein du muscle est long; des études effectuées sur la morue (*Gadus morhua* L.) et le brochet (*Esox lucius*) ont respectivement mesuré une demi-vie d'environ une et deux années (Amlund et al., 2007; Lockhart et al., 1972). Ce taux de résidence aussi élevé explique bien la relation positive observée entre la teneur en MeHg et l'âge du poisson (Glass et al., 2001).

La bioaccumulation du sélénium

Le Se est un métalloïde qui provient à la fois de sources naturelles (éruptions volcaniques et érosion de la croûte terrestre) et anthropiques, majoritairement liées à la combustion (charbon, d'huile, de bois, etc.) et l'utilisation de produits agricoles (Mosher & Duce, 1987; Nriagu, 1989; Nriagu & Pacyna, 1988). Le Se est fondamental à la survie des organismes, mais devient rapidement toxique à haute concentration : il oscille constamment entre le statut d'élément essentiel et toxique (Mason, 2013).

À la base de la chaîne alimentaire, le Se inorganique est assimilé et converti en espèces organiques, telles que la SeCys et la sélénométhionine (SeMet), par les microorganismes et les végétaux (Mason, 2013). Par la suite, possédant un taux d'absorption estimé à 100%, les formes organiques de Se peuvent monter les paliers de la chaîne alimentaire (Taylor et al., 2009). Au sein de l'appareil musculaire du poisson, on retrouve notamment la SeMet (Cabañero et al., 2005), la SeCys (Jagtap et al., 2016), ainsi que la sélénonéine qui a été principalement identifiée au niveau des muscles striés de thon rouge (Yamashita et al., 2011). Également, de nombreuses formes de sélénoprotéines peuvent s'y retrouver (Wang et al., 2018). La forme inorganique de Se est parfois détectée, mais en faible quantité (Cappon & Smith, 1982).

Le transfert trophique du Hg est influencé par la concentration du Se dans l'environnement (Ralston & Raymond, 2010). Les concentrations de ce métalloïde varient géographiquement, soit en fonction de l'abondance naturelle ou en raison des activités anthropiques (Mason, 2013). En cooccurrence dans le milieu intracellulaire, le Hg et le Se peuvent former le complexe HgSe soupçonné d'être non-biodisponible pour le transfert trophique. De cette façon, dans un environnement riche en Se, la bioaccumulation du Se serait accentuée et la bioamplification du Hg diminuerait (Ralston & Raymond, 2010) (Fig. 3).

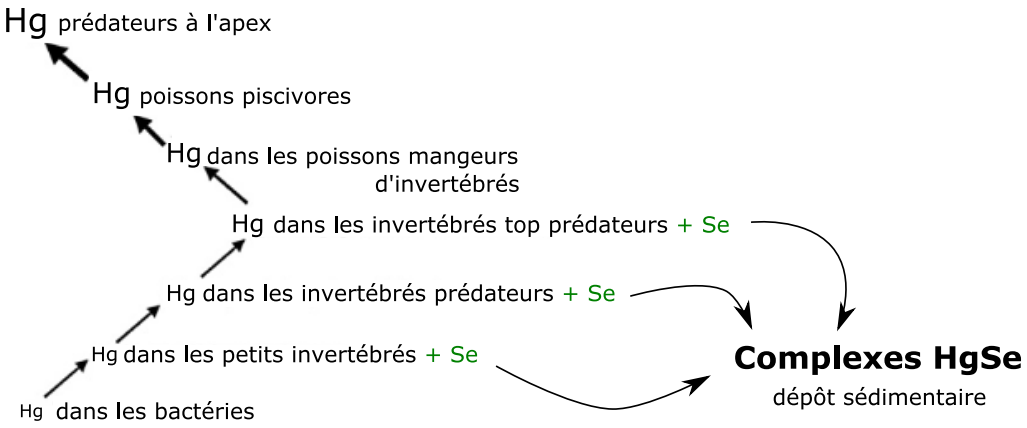

Figure 3. Bioaccumulation du Hg dans un environnement riche en Se. La disponibilité du Hg pour les organismes est proportionnelle à la formation de complexes insolubles HgSe, qui sont retirés dans les sédiments plutôt que bioaccumulés. Adaptée de Ralston & Raymond (2010).

Cependant, à ce jour, l'effet du Se sur la bioaccumulation et la bioamplification du Hg dans les écosystèmes aquatiques n'est pas tout à fait démystifié (Gerson et al., 2020). Alors que certaines études ne décèlent aucun impact du Se sur la concentration du Hg dans les organismes aquatiques, et vice versa, d'autres mentionnent que la prise en charge du Hg dépend de la spéciation du Se dans la colonne d'eau (Pelletier, 1986; Wang et al., 2004). Deux études expérimentales à l'échelle de l'écosystème ont évalué l'effet de l'ajout de Se dans le milieu lacustre sur la bioaccumulation du Hg dans les organismes aquatiques. Une étude a eu lieu aux États-Unis où le Se a été ajouté à différentes concentrations (1, 10 et 100 µg/L), tandis que l'autre étude s'est déroulée en Suisse, où de plus faibles concentrations ont été utilisées (0.4−5 µg/L) (Paulsson & Lundbergh, 1989; Turner & Rudd, 1983). Ces deux expériences ont monitoré une diminution de la teneur en Hg dans les organismes de haut niveau trophique, alors qu'aucun impact de l'ajout du Se n'a été observé pour les organismes de bas niveau trophique, tels que les invertébrés (Paulsson & Lundbergh, 1989, 1991; Turner & Rudd, 1983).

Plusieurs questions fondamentales en ce qui a trait à comment le Se module la bioaccumulation et la bioamplification du Hg dans les écosystèmes aquatiques restent à élucider (Gerson et al., 2020). Cependant, il existe une littérature débattant le fait que ce n'est pas la

concentration de MeHg présente dans un tissu qui est critique, mais bien le ratio molaire Se:Hg qu'on y retrouve (Raymond & Ralston, 2004). Ceci dit, les poissons de grandes tailles habituellement plus contaminés en MeHg que ceux de petite taille semblent être moins bien protégés par l'effet antagoniste du Se, en ce qui a trait à la toxicité du MeHg (Burger & Gochfeld, 2013), tout comme leur consommateur. Ralston (2008) suggère qu'un ratio molaire Se:Hg supérieur à 1 pourrait être suffisant pour contrer les effets néfastes du MeHg au niveau du neurodéveloppement. De leur côté, l'équipe de Burger & Gochfeld (2012) reste prudente en déclarant que le ratio molaire pouvant avoir un effet protecteur n'est pas encore établi par la sphère scientifique. La figure 4 illustre les potentiels scénarios possibles, dans le cas où il est assumé qu'une mole de Se interagit avec une mole de Hg (Khan & Wang, 2009).

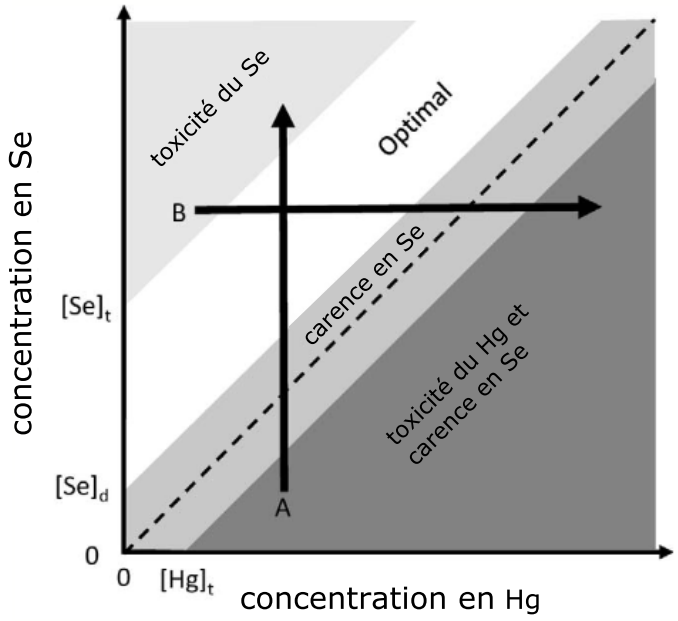

Figure 4. Effets biologiques potentiels suivant une exposition à différentes concentrations de Hg et de Se. $[Hg]_t$: seuil de toxicité du Hg; $[Se]_d$: seuil de carence en Se; $[Se]_t$: seuil de toxicité par rapport au Se. Flèche A : à une [Hg] spécifique, une augmentation de la [Se] va diminuer la toxicité du Hg et combler les besoins en Se, pour finalement atteindre $[Se]_t$. Flèche B : à une [Se] spécifique, une augmentation dans la [Hg] va tendre à diminuer la toxicité reliée au Se, pour ensuite créer une carence en Se. La région blanche illustre le scénario optimal. La ligne pointillée représente un ratio molaire Hg/Se 1 :1, où la quantité de Se n'est pas suffisante pour combler les besoins métaboliques. Adaptée de Khan et Wang (2009).

La bioaccumulation de l'arsenic

Tout comme le Se, l'As est un métalloïde constituant de la croûte terrestre (Singh et al., 2007). Dans la chair de poisson, l'As se retrouve majoritairement sous forme organique incluant l'arsénolipide (AsL) et l'arsénobétaine (AsB) (Dembitsky & Levitsky, 2004; Mandal & Suzuki, 2002; Shen et al., 2013). En plus faible quantité, mais parfois détecté, l'AsI peut se lier aux cystéines protéiniques, pouvant mener à une modification de la conformation de la protéine lui faisant perdre sa fonction (Shen et al., 2013). Bien que chez les mammifères, le Se ait été répertorié comme étant un antagoniste de l'As *via* la formation de divers complexes insolubles (Gailer 2007 et 2009), il semblerait que chez le poisson, la sélénométhionine pourrait potentialiser la toxicité de l'As^{3+} (Jamwal et al., 2019).

Alors que les mécanismes de bioaccumulation du MeHg, du Se et de l'As chez le poisson sont bien documentés, il existe un manque d'information concernant les facteurs influençant la bioaccumulation de ces éléments au sein des différents types de muscles d'un même poisson (Bosch et al., 2016), lesquels présentent une composition en biomolécules différentes (Moody & Cassens, 1968). Connaître la distribution de ces métal(loide)s dans la chair de poisson est d'intérêt puisque des interactions ont été observées entre ceux-ci, comme la formation de complexe HgSe ou encore Hg-Se-As, proposé par Korbas et son équipe (2008).

Méthylmercure : Exposition de l'humain *via* l'alimentation

En écotoxicologie, l'*exposition* se définit comme étant [traduction] « la concentration, la quantité ou l'intensité d'un agent physique, chimique ou environnemental qui atteint une population cible, un organisme, un organe, un tissu ou une cellule, généralement communiqué en terme numérique de concentration, de durée et de fréquence » (Nordberg et al., 2009). De façon générale, l'humain est principalement exposé au MeHg *via* la consommation de poissons prédateurs hautement contaminés en MeHg (European Food Safety Authority, 2012) et par la consommation de certains mammifères marins, tels que la viande de béluga (Lemire et al., 2015).

Telle que mentionnée dans la section précédente *La bioaccumulation musculaire chez le poisson*, la chair de poisson contient habituellement, de façon simultanée, du MeHg, du Se et de l'As. Certaines études ont démontré que la toxicité du MeHg était directement reliée à la consommation de poissons, résultant en des troubles neurologiques et cardiaques (Castoldi et al., 2001; Salonen et al., 1995). En se liant aux résidus de Se au sein des sélénoprotéines, le MeHg inhibe la régénération de sélénoprotéines *de novo* et tend à créer un état de carence intracellulaire en Se, menant à un dérèglement de l'homéostasie de l'état redox (Ralston et al., 2008). La principale voie de toxicité neurologique du MeHg proviendrait d'ailleurs de l'induction d'un stress oxydatif important (Farina et al., 2003). Cependant, en déméthylant le MeHg, le Se est capable de diminuer la toxicité de ce dernier (Khan & Wang, 2009; Manceau et al., 2021), faisant du Se un antagoniste du MeHg. Or, puisque le MeHg tend à créer une carence en Se, mais que celui-ci est essentiel à la détoxication du MeHg, l'idéal est d'avoir une quantité de moles de Se qui est supérieure à celle du MeHg (Fig. 4). Une supplémentation alimentaire en Se s'est d'ailleurs démontrée efficace afin de réapprovisionner les réserves en Se, provoquant la restauration de l'activité des sélénoenzymes cibles, ainsi que la restauration de l'environnement redox intracellulaire (Branco et al., 2012; Carvalho et al., 2011; Meinerz et al., 2011). Pour sa part, l'AsI est connu pour sa toxicité, sa cancérogénicité ainsi que sa capacité à interférer avec le cycle du Se (Singh et al., 2007; Zeng et al., 2005). En contraste, la forme organique d'As possède une toxicité moins inquiétante en raison de sa rapidité d'excrétion hors du corps de l'humain *via* l'urine (Singh et al., 2007). Heureusement, la chair de poisson contient habituellement 90% de l'As sous forme organique (Mandal & Suzuki, 2002).

Malgré leur interrelation indéniable, aucune étude n'a considéré ces trois éléments simultanément lors du processus de digestion humaine. Ci-dessous, nous discuterons des différentes façons dont l'humain peut diminuer son exposition au principal élément métallique de cette thèse, soit le MeHg. Également, quelques méthodes utilisées à des fins d'évaluation de l'exposition de l'humain à ce contaminant seront abordées.

Facteurs modulant la préexposition de l'humain au méthylmercure

Dans ce contexte-ci, les facteurs modulant la « préexposition » fait référence à tous les facteurs qui précèdent la réelle exposition, c'est-à-dire, le moment où le MeHg est ingéré.

Le choix de la proie consommée

Tout d'abord, l'exposition de l'humain au MeHg varie en fonction de la proie choisie. Effectivement, il est connu que les concentrations de MeHg seront plus élevées chez une espèce prédatrice, de plus grande taille et plus âgée (Polak-juszczak, 2015). En plus, l'environnement dans lequel se trouve le poisson peut aussi avoir de grand impact sur la contamination de sa chair (Ralston & Raymond, 2010). Il devient donc très complexe d'émettre des recommandations de consommation de poissons qui sont générales. Pour ces raisons, au Canada, les *guides de pêche sportive* sont émis individuellement par chaque province et territoire et contiennent des recommandations précises pour chaque espèce de poisson pêché (portion et fréquence de consommation), en fonction de leur taille et de leur emplacement géographique (Santé Canada, 2013).

En ce qui a trait aux poissons commerciaux, les limites de consommation sont émises par Santé Canada et ne concernent que les poissons hautement contaminés, tel que thon frais ou congelé, le requin, l'espadon, le marlin, l'hoplostète orange et l'escolier. Pour ces derniers, Santé Canada propose la consommation maximale de 150 g hebdomadaires pour la population générale, de 150 g mensuellement concernant les femmes enceintes, de 125 g pour les enfants âgés entre 5 et 11 ans et de 75 g mensuellement pour les enfants de 1 à 4 ans. Ces limites de consommation ne s'appliquent pas au thon en conserve, qui possède des recommandations particulières. Santé Canada stipule que le *thon blanc* (aussi appelé thon *germon*) contient des concentrations en MeHg beaucoup plus élevées que celles retrouvées chez le *thon pâle* (souvent composé de plusieurs espèces de thon : *listao, à nageoires jaunes* et *mignon*). Les limites de consommation de thon en conserve suivantes ne concernent uniquement que le thon *blanc* : par semaine, 300 g pour les femmes enceintes, 150 g pour les enfants âgés entre 5 et 11 ans et 75 g

pour les enfants de 1 à 4 ans (Santé Canada, 2019b). Aucune limite de consommation n'est destinée à la population générale.

Les traitements culinaires

L'action de cuire des aliments est un acte culinaire depuis très longtemps effectué. Effectivement, la cuisson est pratiquée depuis plus de 250 000 ans et aurait contribué au développement physique et mental de l'humain, de par sa propriété à faciliter l'extraction énergétique provenant des aliments cuits, comparativement à ceux-ci consommés à l'état cru (Carmody & Wrangham, 2009). De nos jours, la chair de poisson est généralement consommée cuite, afin d'optimiser le goût, la digestibilité des nutriments, ainsi que la sécurité du consommateur en inactivant la croissance de la plupart des micro-organismes (Sobral et al., 2018).

Une littérature grandissante étudie l'effet de la cuisson (bouilli, frit, au four, grillé) de la chair de poisson sur sa concentration en contaminants. Pour le moment, aucun consensus n'a été établi en ce qui a trait au MeHg. Pour toutes méthodes de cuisson confondues, certaines études démontrent une augmentation de la concentration de HgT et de MeHg (Afonso et al., 2015; Burger et al., 2003; Costa et al., 2013, 2016; Maulvault et al., 2011), d'autres observent une diminution de ceux-ci (Liao et al., 2019, 2020; Mieiro et al., 2016) et finalement Anacleto et al. (2020) obtiennent des résultats ambivalents. En ce qui concerne l'augmentation de la concentration suite à un traitement culinaire, les auteurs suggèrent que celle-ci proviendrait de la perte d'humidité de la chair de poisson, réduisant la masse totale, ce qui ferait augmenter par ricochet la concentration de MeHg (Afonso et al., 2015; Costa et al., 2013, 2016). D'un autre point de vue, la diminution de la concentration en MeHg observée suite à la cuisson de la chair pourrait s'expliquer par deux hypothèses : il y aurait le phénomène de relâchement de ce métal dans le jus de cuisson, ainsi que la volatilisation de celui-ci stimulée par de hautes températures (Liao et al., 2019; Mieiro et al., 2016). Finalement, il est intéressant de noter que l'équipe de Schmidt n'a observé aucun effet de la cuisson sur la spéciation du Hg dans la chair de poisson (Schmidt et al., 2015).

Facteurs modulant l'exposition de l'humain au méthylmercure et méthodes d'évaluations associées

Une fois que le poisson possiblement contaminé en MeHg et que le type de méthode culinaire sont choisis, plusieurs facteurs peuvent encore faire varier l'exposition du consommateur au MeHg. Effectivement, le MeHg étant fermement lié aux protéines (Harris et al., 2003; Nong et al., 2020), la chair de poisson ingérée par l'humain doit subir d'importants processus digestifs afin que celui-ci soit relâché de l'appareil musculaire pour se retrouver sous forme soluble/bioaccessible dans les fluides gastro-intestinaux (Van de Wiele et al., 2007). Ensuite, la fraction de MeHg soluble peut être internalisée par les entérocytes et rejoindre la circulation sanguine. Cependant, entre le lumen intestinal et le compartiment sanguin, plusieurs facteurs peuvent intervenir et limiter la capacité du MeHg à atteindre ce dernier tissu, tels qu'abordé ci-bas.

La solubilisation du méthylmercure dans le tractus digestif

Puisque l'évaluation de la bioaccessibilité requiert l'accès direct aux fluides gastro-intestinaux à des fins d'analyses, il est éthiquement difficile d'effectuer ce genre d'expérience sur un modèle vivant (Cardoso et al., 2014). L'étude de la bioaccessibilité en laboratoire se résume à la réplication des processus digestifs humains, le plus représentativement possible. Pour ce faire, l'environnement physico-chimique distinct de la bouche, de l'estomac, de l'intestin grêle et du gros intestin est répliqué. Le premier et le dernier compartiment digestif tout juste énoncés sont rarement inclus dans le modèle, puisque l'effet de la salive sur la solubilisation des contaminants a été documenté comme étant limité, tandis que l'environnement microbiotique du gros intestin est difficile à reproduire (Denys et al., 2009; Laird et al., 2009; Oomen et al., 2002). À ce jour, il existe plusieurs modèles de digestion *in vitro* différents, présentant chacun leurs particularités (Denys et al., 2009). Par exemple, au **Chapitre 2** de cette thèse nous avons utilisé le modèle *Physiologically Based Extraction Test* (PBET) qui exclut la phase orale et celle du côlon. Les pH suggérés pour cette méthode sont de 2.5 pour l'environnement stomacal et de 7 pour l'intestin grêle. De son côté, le modèle *Simulator of the Human Intestinal Microbial Ecosystem* (SHIME)

inclut l'action du microbiote au niveau du côlon et propose un pH stomacal de 5.2 et de 6.5 pour l'intestin grêle (Denys et al., 2009). Ces différences observées entre les modèles de digestion *in vitro* mènent à une grande variabilité de résultats en ce qui a trait à la bioaccessiblité du Hg dans la chair de poisson (Afonso et al., 2015; Cabanero et al., 2004; Ouédraogo & Amyot, 2011; Torres-Escribano et al., 2010; Wang et al., 2013), rendant pressante une validation *in vivo* de ces résultats.

Deux éléments majeurs ont été répertoriés pour leur efficacité à réduire la solubilisation du MeHg provenant de la chair de poisson : tout traitement thermique confondu (bouillir, frire, griller) et la co-consommation (simultanément boire ou manger d'autres aliments en combinaison avec du poisson). Effectivement, plusieurs études ont démontré une moins grande bioaccessibilité du HgT et du MeHg après la cuisson de la chair de poisson (Alves et al., 2018; Girard et al., 2017; He & Wang, 2011; Ouédraogo & Amyot, 2011; Torres-Escribano et al., 2010). Pour le moment, la principale hypothèse derrière ce phénomène serait que la chaleur (> 100°C) induit une agrégation de protéines (Fig. 5), réduisant l'efficacité enzymatique de la pepsine, limitant donc la digestibilité des protéines et la solubilisation du MeHg qui y est liée. Cependant, l'hypothèse d'une moins grande digestibilité résultant d'un traitement thermique n'est pas concordante avec les résultats d'expériences *in vivo* (Bax et al., 2013; Oberli et al., 2015), pointant encore une fois vers le besoin d'une validation *in vivo* quant aux modèles de digestion *in vitro* évaluant la bioaccessibilité du MeHg.

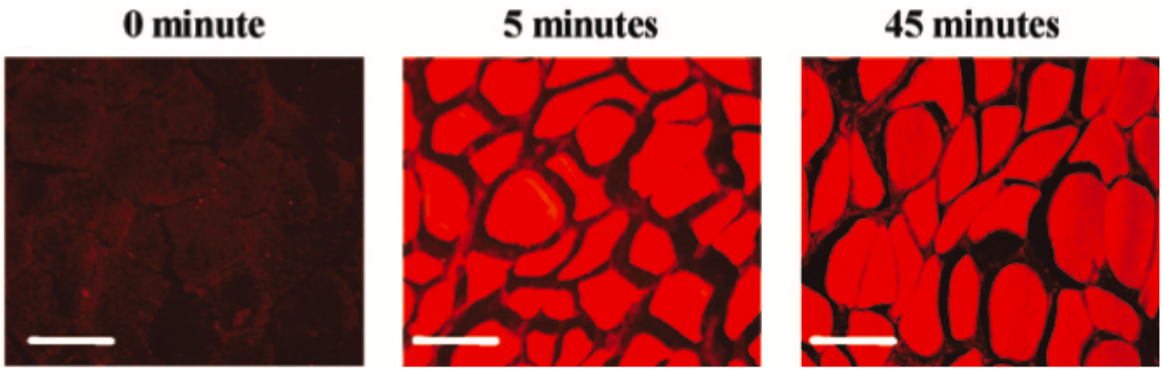

Figure 5. Effet de la cuisson à 100 °C sur l'agrégation des protéines myofibrillaires (*M. Rectus abdominis*) bovines mesurée par microscopie à fluorescence avec le colorant *Nile Red* (échelle blanche = 100 µm) (Santé-Lhoutellier et al., 2008).

En complément, la co-consommation d'aliments riches en phytoéléments, tels que le café noir, le thé, des polyphénols purifiés (Girard et al., 2017; He & Wang, 2011; Ouédraogo & Amyot, 2011), des protéines de soya, ainsi que du son d'avoine et de blé (Shim et al., 2009) ont démontré en laboratoire leur capacité à diminuer la solubilisation du HgT et du MeHg. Les polyphénols sont généralement des métabolites végétaux (Bravo, 1998) qui sont connus comme ayant des propriétés chélatrices envers les métaux (Chew et al., 2008; Ragan et al., 1979) soupçonnées de limiter la bioaccessibilité au MeHg. Jadán Piedra et son équipe (2016) ont élargi l'hypothèse des phytoéléments et ont testé l'effet de plusieurs composés sur la solubilisation du MeHg en solution. Ils ont sélectionné des composés d'origine alimentaire ayant des propriétés chélatrices envers le MeHg ou d'autres cations. Ils ont observé une diminution de bioaccessibilité allant jusqu'à 75% suite à l'ajout de pectine, d'acide tannique, de lignine et de dérivés cellulosiques.

Certaines études *in vitro* ont même testé la combinaison de la cuisson et de la co-consommation sur la bioaccessibilité du MeHg. Dans l'une de celles-ci, l'impact de la cuisson et de phytoéléments provenant de breuvage (thé et café) a démontré une diminution atteignant 99% de la bioaccessibilité par rapport au contrôle (poisson cru) (Girard et al., 2017). Une seconde étude a voulu augmenter la représentativité d'un repas de poissons copieux en testant la solubilité du HgT d'un ragoût de thon, avec diverses combinaisons de riz à la tomate et de légumes (oignons, pommes de terre et brocolis) en accompagnement, tous ajoutés simultanément à la digestion *in vitro*. Chaque combinaison de repas est composée à 50% (poids total du repas) de chair de thon et 50% d'accompagnement. Ils ont obtenu des résultats intéressants : en accord avec la littérature, ils ont mesuré une diminution de la bioaccessibilité suite à la cuisson de la chair de thon lorsque celle-ci a été évaluée isolément. Cependant, ils ont noté que l'ajout de riz et de légumes augmentait significativement la solubilisation du HgT (variant de 21.8 à 31.2% selon la combinaison d'accompagnement), comparativement au ragoût sans accompagnement (13.5%) (Marmelo et al., 2020). Ils ont du mal à expliquer ces résultats, mais proposent cependant que les ingrédients ajoutés au ragoût de thon aient eu un effet facilitateur sur l'activité digestive, favorisant la solubilisation du HgT.

Ces études *in vitro* sont utiles pour mieux comprendre certains processus digestifs ciblés, mais les résultats ne devraient pas être extrapolés directement à la digestion humaine. Il existe

pour le moment un manque de concordance entre les études *in vitro* et *in vivo*. Une étude récente portant sur l'effet de la co-exposition du MeHg et du thé du Labrador (*Rhododendron tomentosum*) a été conduite chez le rat (Pelletier et al., 2019). Le phénomène de solubilisation du MeHg limité par la co-exposition aux phytoéléments n'a pas été observé. Effectivement, après 20 jours de co-exposition par le biais de biscuits contenant les deux éléments à l'étude, aucune différence de concentration en MeHg n'a été mesurée dans le sang, le foie, les reins et les fèces, entre le groupe contrôle et celui co-exposé (Pelletier et al., 2019). Dans le cas où la solubilité du MeHg aurait été limitée par la co-exposition de polyphénols, cela aurait par le fait même diminué l'absorption intestinale de ce contaminant et on aurait pu observer une différence de bioaccumulation de MeHg entre le groupe exposé et contrôle. Une seconde étude a été conduite avec une cohorte de 50 humains volontaires, ayant mangé 2 repas de poissons par jour, pour 3 jours, avec ou sans thé à boire (Canuel et al., 2006). Deux prises sanguines ont été effectuées : avant l'ingestion de poisson et à la fin des trois jours. Leurs résultats indiquent que la cohorte n'ayant pas été exposée au thé aurait métabolisé environ 100% du MeHg présent dans la chair de poisson, tandis que la cohorte co-exposée au poisson et au thé aurait métabolisé plus de 140% du MeHg. Les auteurs proposent que l'exposition au thé ait accéléré le cycle entéro-hépatique, se traduisant par une augmentation de la concentration de MeHg dans le sang de façon temporaire (Canuel et al., 2006).

Interaction entre le méthylmercure et la paroi intestinale

Une fois que le MeHg se trouve sous forme soluble dans les fluides intestinaux, il peut théoriquement se faire absorber par la paroi intestinale et atteindre la circulation sanguine. Cependant, il existe de multiples facteurs limitant le déplacement du MeHg jusqu'à ce tissu. Tout d'abord, une fois relâché par la matrice de poisson dans le tractus digestif, il est possible que le MeHg interagisse avec son environnement et forme des complexes avec d'autres constituants provenant de la diète ou du processus de digestion lui-même. Par exemple, Huang et al. (2013) propose la formation du complexe HgSe insoluble dans le lumen intestinal de l'esturgeon blanc pour expliquer la diminution de MeHg et de Se sanguin observée suite à la co-admistration de

SeMet et de MeHg. Une seconde recherche ayant comme sujet une communauté amazonienne consommatrice de poissons a observé un potentiel lien négatif entre la consommation de fruits et la concentration sanguine de HgT (Passos et al., 2007). Les auteurs ont séparé la communauté en deux cohortes selon leur consommation de fruits. Pour chaque poisson ingéré, une augmentation de 9.8 ug/L de HgT sanguin a été mesurée chez les faibles consommateurs de fruits, comparativement à une hausse 3.3 ug/L de HgT pour les forts consommateurs de fruits. Les auteurs ont émis l'hypothèse que les fibres solubles ainsi que d'autres nutriments prébiotiques provenant des fruits auraient agi sur le microbiote en favorisant la déméthylation du MeHg. Les microbes intestinaux sont notamment connus pour leur capacité à déméthyler le MeHg en brisant le lien carbone-mercure, tel que proposé par Clarkson (2002). Une récente étude *in vitro* suggère que le taux de déméthylation par le microbiote humain varie en fonction de la structure de la communauté bactérienne, qui elle, dépend de la composition de la diète ingérée (Guo et al., 2018).

Ensuite, dans le cas où le MeHg rejoint la barrière intestinale sous forme bioaccessible, c'est-à-dire soluble, son absorption pourrait être réduite par une compétition avec d'autres métaux ou nutriments utilisant le même transporteur membranaire (Jadan-Piedra et al., 2017). Effectivement, dans les fluides gastro-intestinaux, le MeHg peut se trouver sous forme complexée à la cystéine qui elle, provient soit de la matrice de poisson ou des produits de dégradation de la muqueuse intestinale (Dahm & Jones, 1994; Harris et al., 2003). Le complexe MeHg-Cys est ensuite absorbé *via* un transporteur d'acides aminés neutres (Bridges & Zalups, 2005), utilisant le même mécanisme d'absorption que la majorité des acides aminés neutres (Broer, 2008). Ainsi, l'absorption épithéliale du complexe MeHg-Cys pourrait subir une compétition par rapport à l'utilisation des transporteurs d'acides aminés, ce qui pourrait limiter son absorption intestinale.

Une fois absorbé par les entérocytes, le MeHg peut soit traverser la membrane basolatérale de la cellule et rejoindre la circulation sanguine ou rester stocké à l'intérieur de celle-ci. Le cas échéant, le MeHg serait éliminé *via* les fèces lors du renouvellement de l'épithélium intestinal qui se produit tous les quelques jours (Smith & Morton, 2010).

L'évaluation de l'absorption intestinale peut être effectuée en exposant la fraction soluble de MeHg provenant d'une digestion *in vitro* à un modèle cellulaire monocouche composé d'entérocytes (cellules d'absorptions constituant 90% de la paroi intestinale chez l'humain (Smith & Morton, 2010)) (Fig. 6). Les modèles cellulaires permettent de mesurer la fraction d'un élément qui ne traverse pas la paroi et qui reste dans le compartiment apical (Fig. 6), la fraction qui reste trappée au niveau entérocytaire et la fraction qui la traverse entièrement et qui se rend dans le compartiment basal (Fig. 6) (Siedlikowski et al., 2016b). L'équipe de Calatayud et al. (2012) a exposé la fraction soluble de HgT provenant de la chair d'Espadon (*Xiphias gladius*) digérée à un modèle cellulaire *Caco-2* (composé à 100% d'une monocouche d'entérocytes) et a mesuré un faible transport au-delà de la membrane cellulaire (<14%), comparativement au taux de rétention cellulaire élevé (49-69%). Dans cette étude, ils ont observé un lien inverse entre la concentration de HgT à laquelle la culture cellulaire a été exposée et la quantité de HgT qui a traversé la monocouche d'entérocytes. Finalement, elle n'a observé aucun lien entre le temps d'exposition et les résultats ci-haut présentés. À notre connaissance, l'effet de la cuisson et de la co-ingestion sur l'absorption du HgT et du MeHg en utilisant le modèle cellulaire *Caco-2* n'a pas été exploré.

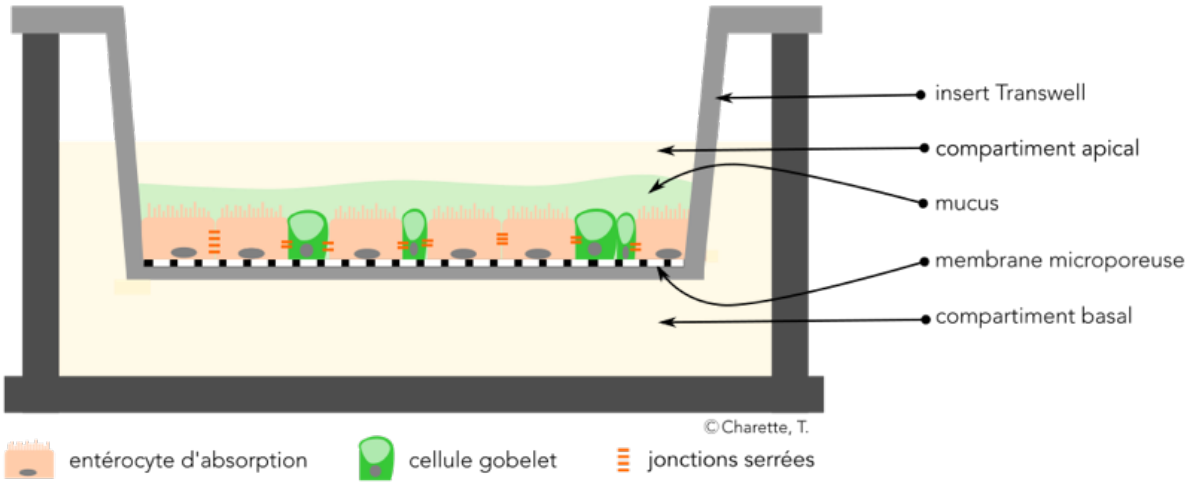

Figure 6. Schématisation d'un modèle cellulaire monocouche en co-culture composé d'entérocytes d'absorption et de cellules gobelets.

Un dernier facteur pouvant modifier l'absorption du MeHg est le mucus intestinal, connu pour jouer un grand rôle de défense contre les composés xénobiotiques, tels que les contaminants (Gillois et al., 2018) et pourrait même constituer une barrière pour la diffusion de ceux-ci vers la paroi intestinale (Calatayud et al., 2012). Vazquez et ses collaborateurs (2013) ont d'ailleurs observé ce phénomène en laboratoire, en exposant une solution standard de MeHg à une co-culture cellulaire contenant à la fois des cellules d'absorption (entérocytes) et sécrétrice de mucus (cellules gobelets).

La biodisponibilité orale du méthylmercure

La *biodisponibilité orale* peut se définir comme étant la fraction d'un élément ingérée qui atteint la circulation sanguine (Shen, 2001), tandis que l'*absorption systémique* constitue [traduction] « l'absorption dans le sang et transport *via* le sang d'une substance vers un ou plusieurs organes » (Nordberg et al., 2009). Le sang est composé de deux compartiments généraux : les érythrocytes et la fraction liquide correspondant au sérum et au plasma. Le sérum et le plasma sanguin ont une composition similaire, excepté que le sérum ne contient pas la majorité des facteurs de coagulation sanguine (Pietrowska et al., 2019). Une fois rendu dans le sang, le MeHg se partitionne entre la fraction liquide (sérum et plasma) et les érythrocytes, en fonction de ses affinités de liaisons. Bien que l'affinité de liaison du MeHg envers les groupes -SeH domine par rapport aux groupes –SH, l'abondance supérieure des groupes -SH permet de surmonter les contraintes thermodynamiques rendant possible l'échange de ligand MeHg-SeH vers MeHg-SH (Nogara et al., 2019). Ainsi, au sein du tissu sanguin, le MeHg forme des complexes majoritairement avec des biomolécules riches en groupes -SH, telles que l'hémoglobine, l'albumine, la cystéine et le glutathion (Naganuma & Imura, 1979; Naganuma et al., 1980). L'équipe d'Ancora et al. (2002) a observé un lien positif entre la concentration de MeHg et de -SH dans le sang de cétacé (*Tursiops truncatus*). Les auteurs suggèrent que cela serait le résultat d'une distribution asymétrique de groupes -SH entre le plasma et les érythrocytes, ces derniers étant 30 fois plus riches en groupes -SH.

Chez l'humain, plus de 90% du MeHg est partitionné au niveau des érythrocytes. Connaître la distribution du MeHg entre les compartiments sanguins est pertinent, puisque seul le MeHg se trouvant dans la fraction liquide est biodisponible pour les tissus extravasculaires (Thomas & Smith, 1982). Lorsqu'un élément est internalisé par les érythrocytes, son transfert vers les tissus extravasculaires se fait lentement et en plusieurs étapes : des protéines cytoplasmiques vers les protéines membranaires de l'érythrocyte, vers un ligand potentiel de la fraction liquide sanguine, pour finalement lier potentiellement un tissu cible (Oliveira et al., 1999) (Fig. 7). Quelques études suggèrent que les érythrocytes constituent un tampon transitoire. Effectivement, la formation de complexes insolubles HgSe a été observée *in vitro* dans le sang de cétacé (Ancora et al., 2002), tandis que l'équipe de Korbas et al. (2008) propose la formation du complexe MeHg-Se-As [$(GS)_2AsSeHgCH_3$] chez les mammifères, en se basant sur les résultats d'une étude *in vitro* utilisant du lysat érythrocytaire de lapin blanc de Nouvelle-Zélande.

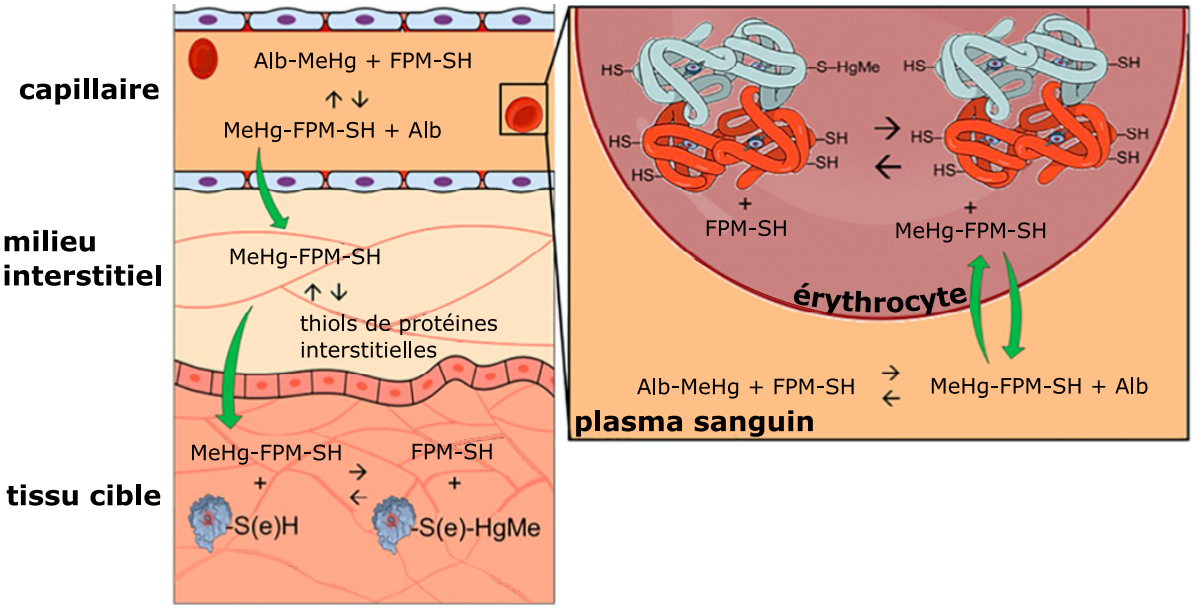

Figure 7. Distribution du MeHg dans le sang. Dans le plasma sanguin, le MeHg va lier essentiellement l'albumine (Alb) et les thiols de faibles poids moléculaires (FPM-SH). Au niveau de l'érythrocyte, le MeHg se liera aux molécules d'hémoglobines riches en -SH. Adaptée de Nogara et al. (2019).

Finalement, la sélénonéine est une espèce chimique de Se d'intérêt en raison de ses propriétés déméthylatrices de MeHg (Yamashita et al., 2013). La sélénonéine a d'ailleurs été répertoriée au niveau des érythrocytes de l'humain notamment (Achouba et al., 2019), et s'y accumulerait préférentiellement en utilisant les transporteurs OCTN1 (Gründemann et al., 2005; Yamashita et al., 2013).

Chez l'humain, la concentration sanguine de HgT dans le sang est souvent utilisée comme proxy de MeHg et corrèle avec la consommation de poissons (Mahaffey et al., 2004; Sanzo et al., 2001; Schober et al., 2003). À l'aide de modèle *in vivo*, il est possible d'utiliser la concentration de Hg sanguine pour estimer la biodisponibilité orale de cet élément en comparant la dose oralement administrée à ce qui se retrouve dans le sang. À ce jour, les études portant sur l'absorption systémique du MeHg *in vivo* comportent plusieurs limitations. Tout d'abord, ces expériences sont conduites en utilisant des modèles rongeurs (Berntssen et al., 2004; Landry et al., 1979; Norseth & Clarkson, 1971). Cependant, la physiologie digestive des rongeurs est loin d'être similaire à celle de l'humain, comparativement à celle du porc, qui constitue un substitut de choix (Sciascia et al., 2016). En plus, ces études sont majoritairement réalisées avec une matrice alimentaire quelconque à laquelle du sel de MeHg (CH_3HgCl) a été rajouté, ce qui s'avère à être plus ou moins représentatif du MeHg bioaccumulé naturellement dans la chair de poisson. Finalement, l'absorption systémique du MeHg par l'animal est souvent évaluée par prises sanguines au niveau de la carotide ou d'un membre appendiculaire, ce qui constitue une approximation peu précise par rapport à la quantité de MeHg qui est réellement absorbée par la paroi intestinale.

Avoir des valeurs de biodisponibilité orale de MeHg fiables et représentatives de la réalité humaine est essentiel pour mieux caractériser le risque relié à la consommation de poissons. Pour l'instant, Santé Canada possède trop peu d'informations à ce sujet et se doit d'appliquer le principe de précaution en émettant des recommandations de consommation de poissons conservatrices, en utilisant les prémisses que 100% du Hg dans la chair de poisson est méthylé et que 100% de celui-ci sera absorbé par le consommateur (Santé Canada, 2007).

Évaluation du risque au mercure

Au Canada, les consignes de consommation de poissons et de fruits de la mer utilisent une *dose journalière admissible (DJA)* basée sur le contenu en HgT pour calculer la portion et la fréquence de consommation permise, pour chaque sous-groupe de la population en fonction de leur vulnérabilité au MeHg (Santé Canada, 2007). La DJA correspond à la dose maximale (mg × kg^{-1} poids corporel par jour) de MeHg qui peut être ingérée tous les jours pendant toute une vie sans risque de porter atteinte à la santé humaine (Rice et al., 2000).

Les équations utilisées présentement par Santé Canada pour estimer l'exposition (*EXP*) au MeHg et son quotient de dangerosité (*QD*) sont :

$$EXP = \frac{CQ \times [HgT]}{PC}$$

$$QD = \frac{EXP\ (mg/kg\ par\ jour)}{DJA\ (mg/kg\ par\ jour)}$$

où *CQ* représente la consommation quotidienne (mg en poids humide de chair de poisson par jour), *[HgT]* est la concentration de HgT (proxy utilisé pour le MeHg) de la chair de poisson (mg × kg^{-1} poids humide) et *PC* correspond au poids corporel (kg). Le *QD* est le ratio entre l'exposition mesurée et la *DJA*. En se basant sur l'exposition au MeHg, un QD > 1 pourrait impliquer un risque pour la santé des individus exposés, tandis qu'un QD ≤ 1 devrait n'être relié à aucun danger.

L'utilisation de l'équation *EXP* pour évaluer l'exposition du consommateur au MeHg implique que 100% du Hg présent dans la chair de poisson est méthylé et que 100% de celui-ci est absorbé. Ces suppositions surestiment l'exposition réelle du consommateur par rapport au MeHg et en plus, ils omettent la potentielle variabilité intrapopulationnelle en ce qui a trait au PC. L'approche employée par Santé Canada pour évaluer l'exposition au MeHg est de type *déterministe*, signifiant qu'ils utilisent une valeur estimée ponctuelle pour chaque variable de l'équation *EXP*. Dans ce cas-ci, pour atteindre la certitude que le risque n'est pas sous-estimé, le choix des valeurs se fait de façon très conservatrice pour simuler le pire scénario (Hinck et al., 2009; Santé Canada, 2007). En comparaison, une approche d'évaluation *probabiliste* utilise une distribution de probabilités pour définir une ou plusieurs variables, afin de considérer la

potentielle variabilité et l'incertitude des valeurs sélectionnées, résultant en un intervalle et une fréquence d'exposition hypothétique (U.S. EPA, 1997) (Fig. 8).

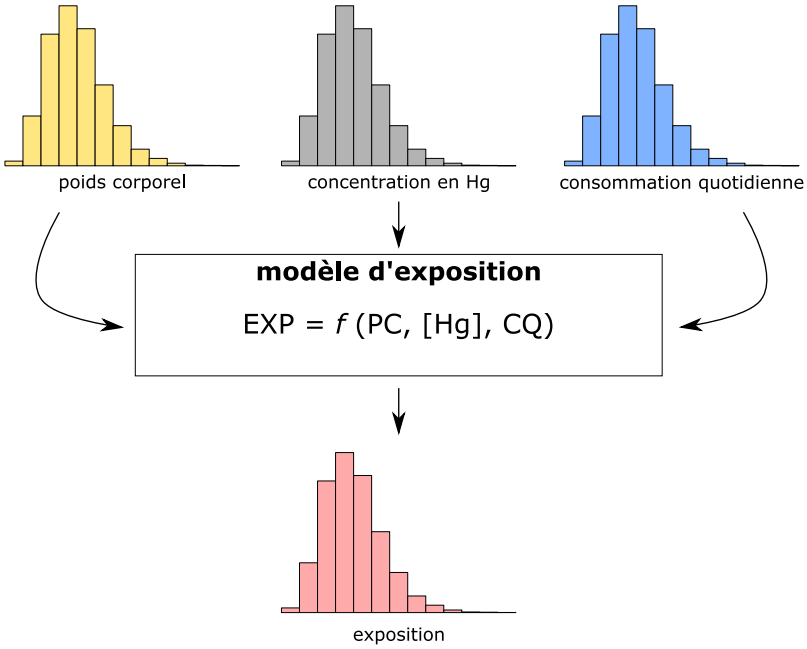

Figure 8. Représentation conceptuelle de l'analyse probabiliste de l'exposition. Inspiré de Santé Canada (2010).

Cadre conceptuel et objectifs généraux de la thèse

Pour l'être humain, la principale source d'exposition au MeHg se fait *via* la consommation de poissons et de mammifères marins, lorsque ces derniers sont hautement contaminés en MeHg (Lemire et al., 2015; National Research Council, 2000b). Un des enjeux importants en contexte autochtone réside dans le fait que les mammifères marins peuvent faire partie intégrante de l'alimentation de subsistance de ces communautés (Council of Canadian Academies, 2014). Par des processus de bioaccumulation et de bioamplification, les animaux à l'apex de la chaîne trophique peuvent devenir hautement contaminés en MeHg et représenter un potentiel risque d'effets néfastes sur la santé des consommateurs (Chen et al., 2009; Redmayne et al., 2000). En

réponse à cette problématique, Santé Canada a émis des lignes directrices quant à la consommation de poissons afin de protéger la population. Ces recommandations de consommation de poissons sont directement liées à l'équation déterministe utilisée par Santé Canada pour évaluer l'exposition du consommateur au MeHg.

Cependant, les recommandations présentent certaines limites qui sont liées aux prémisses et aux omissions de l'équation déterministe utilisée par Santé Canada pour évaluer l'exposition au MeHg. Ces prémisses et omissions ont été l'objet de motivation de cette thèse, telles que schématisées par la figure 9. Celle-ci est articulée en deux objectifs principaux : le premier est d'évaluer les facteurs de modulation de l'exposition au MeHg présent dans la chair de poisson et le deuxième est d'évaluer l'impact d'ajouts de variables dans l'équation d'exposition au MeHg qu'utilise présentement Santé Canada.

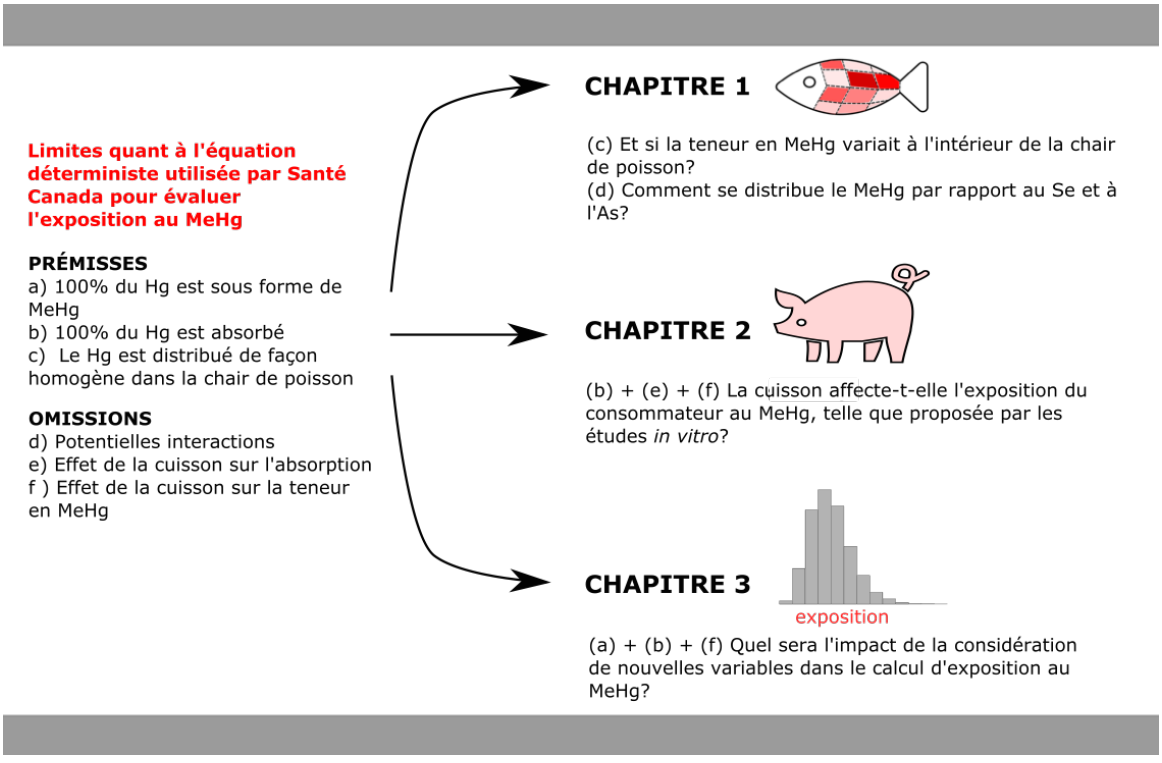

Figure 9. Cadre conceptuel de la thèse, présentant les thèmes des chapitres.

Tout d'abord, une des limites que nous avons explorées repose sur la prémisse où il est pris pour acquis que le MeHg se distribue de façon homogène à l'intérieur de l'ensemble de l'appareil musculaire du poisson. Ainsi, au **Chapitre 1** de cette thèse nous avons évalué la distribution du MeHg, du Se et de l'As dans la chair de bar rayé (*Morone saxatilis*) et de brochet (*Esox lucius*). Ces deux espèces de poissons téléostéens ont été sélectionnées en raison de leur musculature différente : le bar rayé possède une importante couche de muscle rouge au niveau de la ligne latérale, tandis que chez le brochet, ces muscles sont pratiquement imperceptibles à l'œil nu. Sachant que le MeHg se lie préférentiellement aux protéines (Leaner & Mason, 2004) et que les muscles rouges sont plus riches en lipides (Greer-Walker & Pull, 1975), nous nous attendions à une bioaccumulation en MeHg moins importante au niveau de ces muscles.

Une deuxième limite par rapport à l'équation déterministe utilisée par Santé Canada pour évaluer l'exposition au MeHg est celle considérant que 100% du MeHg ingéré sera absorbé par le consommateur. Plusieurs études ayant utilisé des modèles de digestion *in vitro* proposent que la cuisson de la chair de poisson diminue la bioaccessibilité du HgT et du MeHg, et donc, diminue l'exposition du consommateur par rapport à ce contaminant (Alves et al., 2018; Girard et al., 2017; Ouédraogo & Amyot, 2011). Cependant, l'effet de ce phénomène n'a pas été évalué *in vivo*. Le **Chapitre 2** de cette thèse s'attarde précisément à évaluer l'effet de la cuisson de la chair de poisson sur la bioaccessibilité *in vitro* et la biodisponibilité orale du MeHg chez le porc. L'utilisation du modèle porcin dans ce contexte est pertinente dans la mesure où il possède un système digestif similaire à l'humain (Rees et al., 2009).

Finalement, l'équation déterministe utilisée par Santé Canada pour évaluer l'exposition au MeHg chez les consommateurs est très conservatrice et a pour but de protéger la population à risque. Pour l'instant, les recommandations de consommation sont basées sur les prémisses que 100% du Hg dans la chair de l'animal se trouve sous forme de MeHg, que 100% de ce MeHg sera absorbé par le consommateur et que la cuisson n'aura aucun impact sur les concentrations de MeHg se trouvant dans la chair de l'animal. Ces prémisses peuvent mener à une sous- ou surestimation de l'exposition au MeHg. Le **Chapitre 3** de cette thèse s'inscrit au sein de cette problématique. À l'aide de modélisations statistiques probabilistes et de données de la

littérature, nous avons évalué l'impact de l'ajouts de nouvelles variables (pMeHg, RAF et MLF) dans l'équation d'exposition au MeHg qu'utilise présentement Santé Canada.

Les résultats des **Chapitres 1, 2 et 3** sont présentés sous forme d'articles scientifiques. Un article scientifique a été rédigé pour chaque chapitre. Chacun de ces articles a été évalué par les pairs et a été publié dans une revue scientifique. Cette thèse se termine en présentant une conclusion générale contenant de l'information sur ses contributions à la littérature scientifique actuelle. Finalement, quelques pistes de recherches futures sont présentées.

Chapitre 1 : Ménage à trois : de quelle façon cohabitent le méthylmercure, le sélénium et l'arsenic au sein de l'appareil musculaire du bar rayé et du brochet?

Tranche de vie #1 : Ma routine

Pour la rédaction de cette thèse, je me suis infligé une discipline *quasi* militaire :

5h30 : levé

6h00 : marche avec mes chiens

6h30 : me préparer à la rédaction

7h00 à midi : rédaction

13h à 15h : tâches moins cérébrales

16h : gym

20h : couché

Repeat × plusieurs mois

Mapping metal (Hg, As, Se), lipid and protein levels within fish muscular system in two fish species (*Morone saxatilis* and *Esox lucius*)

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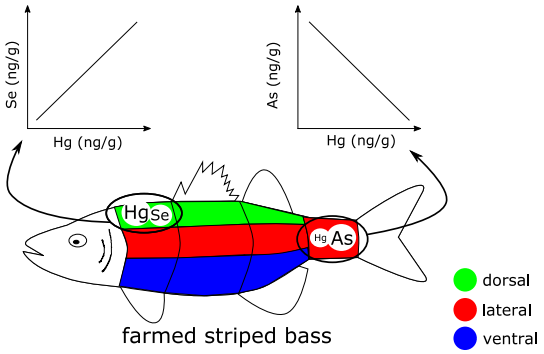
Published in Chemosphere, 265: 129036.

DOI: 10.1016/j.chemosphere.2020.129036

Minor edits to the published text have been made here, following suggestions by this thesis' reviewers.

Abstract

Current guidelines tend to limit fish consumption based on mercury (Hg) or monomethylmercury (MeHg) content in fish flesh, without considering the presence of antagonist chemical elements that could modulate Hg toxicity once absorbed by the consumer. However, it is difficult to assess the potential for antagonistic interactions of these elements since their covariation within muscle tissues is poorly known. Here we present the first study simultaneously mapping multiple metal(oid)s (Hg, As and Se), lipids and proteins in fish fillets in order to assess the magnitude of intra-organ variability of metals and the potential for antagonistic interactions. We mapped two fish species (*Morone saxatilis* and *Esox lucius*) with contrasting muscular structure with respect to the presence of white, intermediate and red muscles. In individual Striped Bass muscle tissues, metals varied on average by 2.2-fold. Methylmercury and selenium covaried strongly and were related to protein content as assessed by % N; arsenic was inversely related to these elements and was associated with the lipid fraction of the muscle. In Pike, no such relationships were found because the content in proteins and lipids were less variable. Arsenic speciation revealed that arsenobetaine and arsenolipids were the only As species in those fish species, whereas the more toxic inorganic As species (As^{3+}) was under the limit of detection. Arsenobetaine was related to % N, whereas arsenolipids covaried with % lipids. Elemental associations found with muscle lipids and proteins could help explain changes in bioaccumulation patterns within and between individuals with potential implications on fish toxicology, biomonitoring and human consumption guidelines.



Graphical abstract

Key words: methylmercury, metalloids, protein, lipid, muscle type, fish

Introduction

Fish flesh consumption constitutes an important source of proteins and other nutrients (long chain polyunsaturated fatty acids, proteins, selenium (Se) and vitamins) for billions of persons worldwide (FAO/WHO, 2010). However, it may also be contaminated by pollutants, such as monomethylmercury (MeHg) (Myers et al., 2007) well known for its toxicity to the nervous and cardiovascular systems (Castoldi et al., 2001). As a result, health authorities have developed fish consumption guidelines and, in Canada, mercury (Hg) is the main metal usually considered for setting safe consumption levels (Health Canada, 2007a). These recommendations are based on the assumptions that intra-individual Hg concentrations within fish muscle barely vary. Furthermore, those guidelines omit the possible interactions of MeHg with Se, a well-known antagonist of MeHg, which could decrease MeHg toxicity (Peterson et al., 2009), and the antagonism of Se is inorganic arsenic (iAs), which could limit the protective effect of Se towards MeHg (Zeng et al., 2005). Research addressing these issues would lead to better tools to ensure fish consumption guidelines are adequately set. Further, a better understanding of the intra-organ variability of metals and their potential interactions will help predict adverse effects of metals in fish. Indeed, at high levels, MeHg has been found to cause several health damages in fish, including the impairment of growth, survival and reproduction (Wiener & Spry, 1996). However, if Se distribution co-vary with it of MeHg, health damages could be reduced (Peterson et al., 2009).

The muscular system of all teleost fish is composed of two main muscle types, *red* and *white* (Gill et al., 1989). Red muscles are located under the lateral line just under the skin, from the head to the tail (Fig. 1). Compared to white muscles, they possess an elevated content in mitochondria and lipids, are highly vascularized and correspond to slow muscles with aerobic metabolism (Greer-Walker & Pull, 1975; Johnston et al., 1975; Jones & Sidell, 1982). White muscles form the axial musculature, and are responsible for fast bursts and possess an anaerobic metabolism (Greer-Walker & Pull, 1975; Johnston et al., 1975; Jones & Sidell, 1982). While red muscles are only distributed on the surface, white muscles are found at the surface or at depth. A third muscle type occurs in several fish species, called *intermediate* or *pink* (Kiessling et al.,

2006). These intermediate muscles are distributed between the red and white muscles (Fig. 1) and they possess intermediate properties: they are aerobic and are used for fast burst (Kiessling et al., 2006). Muscle types have different protein and lipid contents which could affect the intra-organ bioaccumulation of metal(loid)s (Johnston et al., 1975). Little information is available concerning the relative bioaccumulation of MeHg, Se and arsenic (As) between these three types of muscle (Bosch et al., 2016).

Methylmercury is known to bioaccumulate in fish flesh in the protein fraction, mostly by forming complexes with the amino acid cysteine (MeHg-Cys), as a result of its high affinity for thiol groups (Amlund et al., 2007; Harris et al., 2003; Leaner & Mason, 2004). However, MeHg binding affinity for Se is even higher than for thiol groups (Khan & Wang, 2009; Sugiura et al., 1978). Inorganic As also has strong binding affinity for cysteine, but As in fish muscle can also be found as different organo-species, including arsenolipids (AsL) and arsenobetaine (AsB); As could therefore be found in the protein or lipid fraction of fish flesh (Dembitsky & Levitsky, 2004; Shen et al., 2013). Selenium in fish flesh can occur as a selenoprotein distributed in the protein fraction (Peterson et al., 2009), as well as selenomethionine (Cabañero et al., 2005) and the newly found selenoneine (Yamashita et al., 2011).

Whereas inter-organ THg and MeHg bioaccumulation is well understood (Leaner & Mason, 2002, 2004; O'Bryhim et al., 2017), variation of Hg levels within a fish organ (i.e. skeletal muscle) is less documented (Gremillion et al., 2005; Piraino & Taylor, 2009), and the influence of muscle type and depth has only been assessed once (Cizdziel et al., 2001). Further, to our knowledge no study has explored the within-organ covariation of Hg, Se and As, in addition to the speciation of the latter. The within-organ distribution of those metal(loid)s are of interest since strong chemical interactions are known to occur, such as the formation of mercury selenide (HgSe) or even the proposed Hg-Se-As complex described by Korbas et al. (2008).

The aim of this study was to determine whether MeHg, Se and As levels varied within muscle tissues of fish. We investigated the bioaccumulation patterns of these metals in relation to proteins (estimated by nitrogen content) and lipids. We also assessed the influence of muscle depth and muscle types on this bioaccumulation. We chose Striped Bass (*Morone saxatilis*) and

Northern Pike (*Esox lucius*) as model species because they have contrasting muscle structure. Striped Bass, a migratory fish, has a thick layer of red muscles at the lateral line in addition to pink and white muscles, whereas Northern Pike, an ambush predator, possess very little pink and red muscles (Bone, 1966). We predicted that there would be less intra-organ variability in Pike compared to Bass, because only one type of muscle is present in Pike. We anticipated that MeHg and Se would co-vary and follow protein distribution, whereas As would be more associated with lipids.

Materials and methods

Sampling of fish muscle tissues

Seven farmed Striped Bass of similar size (CV = 4.6%) and weight (CV = 6.7%) were purchased, gutted and eviscerated in a local fish shop (Poissonnerie La Mer, Montréal, Québec) (Table S1). Using a 10 mm stainless-steel biopsy punch (Robbins Instruments, Inc.), we biopsied 10 areas (3 dorsally, 4 laterally and 3 ventrally) (Fig.1) of the left-sided musculature where the skin had been previously removed. For each biopsy punch, we then separated the top section (first 5 mm under the skin for the dorsal and ventral zone) from the deeper section. In the case of the lateral line where red muscles were found, the top section consisted of red muscles, intermediate section contained pink muscles and the bottom section was made of white muscles (Fig. 1A). Fish muscles were sampled the same day of the purchase or were kept at -20 °C until further analysis. To ease the analysis, we distinguished four muscle types in Striped Bass based on their location and not by their histochemical properties: (1) *white axial muscle* including muscle from dorsal and ventral zones, (2) *red muscle* found under the skin along the lateral line, (3) intermediate muscle distributed between the red and white muscles, and (4) *white lateral muscle* found below intermediate muscles along the lateral line (Fig. 1B-C).

In the St. Maurice River (Canada, Quebec), 7 Pikes of similar size (CV = 7.6 %) and weight (CV = 18.5%) (Table S1) were caught with a fishing line, gutted, eviscerated, filleted left sided musculature) and subdivided in 10 sampling zones (Fig.1) on the day of their capture. The skin

was removed from the biopsies. We then divided each biopsy core in two even sections (surface and deep muscle), prior to freezing in liquid nitrogen. Back at the University of Montréal (Canada, Quebec) three weeks later, the samples were transferred and kept at -20 °C until further analysis. Before analysis, all samples were freeze-dried for a minimum of 48 hours (Freezone6, Labconco).

Metal(loid) analyses in muscles (Striped Bass and Pike #1 to #6)

THg was measured in biopsies using a direct mercury analyzer (DMA 80, Milestone Inc.), by atomic absorption spectrophotometry. Certified reference materials (CRM), TORT-2 (lobster hepatopancreas, National Research Council, Canada), DORM-2, DORM-3 (fish protein, National Research Council, Canada) and marine sediment (PACS-2) were used for quality control. Average recoveries were 105 ± 3% for TORT-2 (*n* = 42), 96 ± 3% (*n* = 34) for DORM-2, 96 ± 11% (*n* = 16) for DORM-3, and 99 ± 5% for PACS-2 (*n* = 6). Quality control was also monitored every 10 samples, where measurement uncertainty was estimated performing duplicate analyses (mean coefficient of variation (CV) of 6 ± 5% and 3 ± 3% for Striped Bass and Pike, respectively). Detection limit for THg was 0.05 ng/kg.

For MeHg analyses, freeze-dried muscle samples (0.01 - 0.02 g dry weight (d.w.)) were digested overnight in 5 mL by 4M HNO_3 (Fisher Scientific, ACS-pur). Samples were then analyzed with a gas chromatograph coupled with a cold-vapor fluorescence spectrometer (GC-CVAFS) (Tekran 2700, Tekran Instruments Corporation). TORT-2 and DORM-3 were used for quality control, and average recoveries were 103 ± 5% (*n* = 42) for TORT-2 and 89 ± 8% for DORM-3 (*n* = 9). For control quality, duplicates were performed in order to monitor the measurement uncertainty, for which we obtained a mean CV of 5 ± 2% and 2 ± 2%, for Striped Bass and Pike, respectively. Detection limit for MeHg was 0.01 ng/kg.

For Se and total As (TAs), freeze-dried samples were digested in HCl (OmniTrace Ultra, EMD) and ultrapure HNO_3 (5%) (OmniTrace Ultra™, MilliporeSigma) for 3 hours in a pressure steam sterilizer (#50X 25-quart electric sterilizer, ALL AMERICAN). The digestion was completed with 250 μL ultra-pure OPTIMA grade H_2O_2; then, ultra-pure MilliQ water (>18.2 MΩ cm^{-1}) (EMD

Millipore) was added to reach a total volume of 10 mL. Samples were analyzed by inductively coupled plasma-tandem mass spectrometry (ICP-MS/MS 8900 Triple Quadrupole, Agilent). TORT-2, DORM-2 and DORM-3 were used for quality control, and recovery average for all metals and all CRM ranged from 92 to 98% (*n* = 17). We performed duplicates for the measurement uncertainty for which we obtained a mean CV of 5 ± 5% and 9 ± 8%, for Striped Bass and Pike, respectively. Analyses met the criteria of a Canadian Association for Laboratory Accreditation (CALA) intercalibration exercise. Detection limits (ng/kg) for TAs was 0.71 and 0.49 for Se.

Arsenic speciation in muscles (Striped Bass and Pike #7)

As speciation was performed as described in Taleshi et al. (2010) and on a single individual per fish species (Striped Bass and Pike #7). In the case of Striped Bass, we biopsied the left and right side of musculature, and the left side of Pike. First, fractionation was accomplished on grounded lyophilized fish flesh using chloroform : methanol solution (2:1) (>99.8 and 99.9%, Fisher Scientific). The resulting solution was filtered (Machery-Nagel, MN 85/70, 45 mm filter papers). The filtrate was evaporated to dryness to form an oily phase, from which a second extraction was performed with chloroform : methanol : ultra-pure MilliQ water solution (2:1:1). The chloroform phase was evaporated and the oily substance containing fat-soluble As species were digested according to the Quebec (Canada) ministry protocol *Ma.200-Mét 1.2.* and analyzed by ICP-MS/MS. The aqueous phase holding water-soluble As species was further injected in High-Performance Liquid Chromatography and analyzed by ICP-MS/MS. Quality control was performed with TORT-2 for AsT (recovery of 101 ± 0.04%, *n* = 2) and DORM-4 for AsB (recovery of 105 ± 0.01%, *n* = 2). Analyses met the criteria of a Canadian Association for Laboratory Accreditation (CALA) intercalibration exercise. Detection limits (µg/g) for As species was: 0.02 (AsL), 0.01 (AsB), 0.012 (dimethylarsenic acide; DMA), 0.008 (monomethylarsenic acid; MMA), 0.012 (arsenite; As^{3+}) and 0.01 (arsenate; As^{5+}).

Biochemical parameters in muscles

For the elemental analysis of total nitrogen (% N), fish flesh was lyophilized (Freezone6, Labconco) for 48 hours and dry samples were weighed into tin capsules and combusted by oxygen at 1800 °C. This procedure was done at G.G. Hatch Stable Isotope Laboratory – University of Ottawa (Canada) and GRIL Laboratory – University of Montréal (Canada) for logistic reason. Interlaboratory calibration was done with 20 samples and resulting regression slope (r^2 = 0.94) did not differ from 1 (ANCOVA, p > 0.05) indicating that both laboratories gave similar results. Total lipids, expressed here as proportion of the mass balance (lipid concentration reported as % lipids), were quantified by gravimetry using a method adapted from Folch et al., 1956) (see supplementary information (SI) for detailed protocol) and we confirmed the efficiency of this method by obtaining 91.7 ± 5.4% of total lipids from seal fat (n = 3) (data not shown). Moisture content (%) in fish flesh was quantified by subtracting sample d.w. from wet weight (w.w.) after drying.

Statistics and data handling

Every fish was considered as an experimental unit, and for each species we had 6 replicates (n = 6). All data are presented in dry weight and as arithmetic means (± standard deviation, SD). Normality of data and residuals of linear regressions were tested with the Shapiro test, and homoscedasticity of residuals were examined by visual inspection of residual plots. To relate metal(loid) content with matrix components of fish flesh, we applied a linear regression and excluded data with a Cook's distance superior to 4/n (Bollen & Jackman, 1985). Nonparametric Kendall test was used when the data set were not normally distributed. Model II linear regression was used to explore MeHg, Se and As covariation, since those elements are subject to stochastic fluctuations and there are no dependent/independent variables. We used a mixed linear model with « fish individual » as random effect in order to pool samples of fish of same species together. Tukey test as post *hoc* procedure was used to compare metal(loid)s and flesh component (% N and % lipids) between muscle depth, zone and type. Significance level was

$\alpha < 0.05$. Statistical analyzes were performed with R software (R Development Core Team) and graphics have been done with *ggpplot2* packages in addition to *khroma* package which used a color-blind friendly palette.

Results

Intra-individual variability of MeHg, Se and As muscle

We mapped the distribution of MeHg, Se and As in fillets of Striped Bass and Northern Pike by taking ten biopsy punches per individual (*n* = 6 individuals) (Fig. 1). The proportions of MeHg to THg were high (as expected) and stable within fish muscles with 91 ± 8% (*n* = 128) and 92 ± 6% (*n* = 120) for the Striped Bass and the Pike, respectively; we therefore chose to focus on MeHg, which is the species of concern for human consumption of fish.

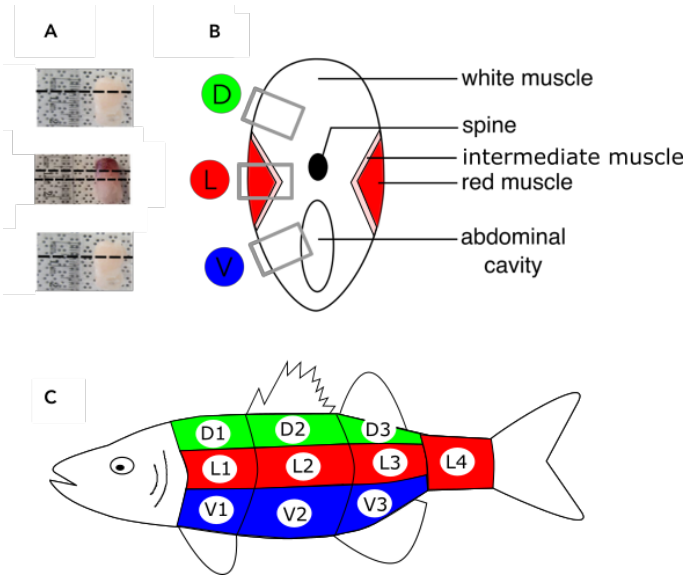


Figure 1. Distribution of white, intermediate and red muscles in most teleost fish and distribution of sampling locations. A- Photography of sampling muscle taken with a biopsy punch in Striped Bass. B- Cross section illustrating the position of white, intermediate and red muscles. D: dorsal; L: lateral; V: ventral; gray rectangles: approximate location of muscles sampling taken using the biopsy punch. C- Ten sampling areas for Striped Bass and Northern Pike.

Fish consumption guidelines are based on wet weight Hg levels, whereas our study focused on dry weight levels in order to avoid the potential influence of moisture variation between muscle type on metal(loid)s bioaccumulation. Therefore, Table S2 presents ratio between metal(loid)s dry and weight wet, as function of muscle type (Table S2).

In Striped Bass (*n* = 6 individuals), biopsies taken within a given fillet varied on average by a factor of 2.2 ± 0.5, 2.0 ± 0.4 and 2.5 ± 0.6, for MeHg, Se and As, respectively (calculated using the ratio between the maximum and minimum concentrations; Table S3; Fig. 2). In contrast, biopsies from Northern Pike (*n* = 6 individuals) displayed less variations for MeHg, Se, and As, with factors of 1.3 ± 0.1, 1.2 ± 0.1 and 1.6 ± 0.1. Pikes were on average 35 times more concentrated in MeHg than Striped Bass but had 2 times less Se and 11 times less As (Table S3).

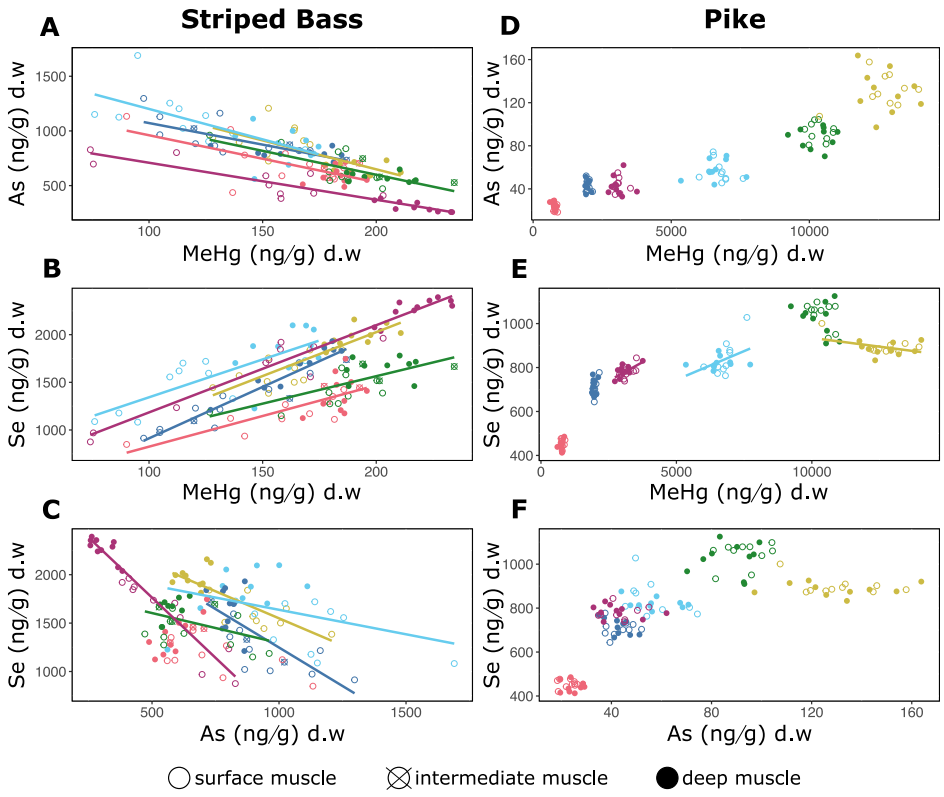


Figure 2. Relationships between MeHg, Se and As (ng/g d.w) in Striped Bass (A, B, C) and Pike flesh (D, E, F). Each individual (*n* = 6) had 10 sampling areas, subdivided in surface (open symbols), intermediate (Striped Bass, crossed symbols) and deep muscle (closed symbols), yielding 20-23 samples per fish. Only significant regressions are shown.

In order to better understand the intramuscular As distribution, we further investigated As speciation. In Striped Bass and Pike, we detected AsB and AsL, whereas the following As species were under the limit of detection: MMA, DMA, As^{3+} and As^{5+}. Mean mass balance (AsB+AsL/TAs) was 97.3 ± 8.5% (range [79% to 109%], *n* = 14). Figure 3 presents the proportion (%) of AsB and AsL compared to TAs, between various muscle zones and depth. The impact of muscle depth was assessed only for the Bass, because it seemed to influence As distribution according to figure 2 (with surface muscle (open symbols) more contaminated than deep muscle (closed symbols)), unlike Pike. In Pike flesh, we measured a weak variation of As species between the different muscle zones. Indeed, AsL levels varied by a factor of 1.8 (range [0.015 to 0.028 µg/g]). In contrast, AsL varied with a factor of 3.6 (range [0.23 to 0.83 µg/g]) within Bass flesh, with highest levels found in areas rich in red muscles (Fig. 3, red color).

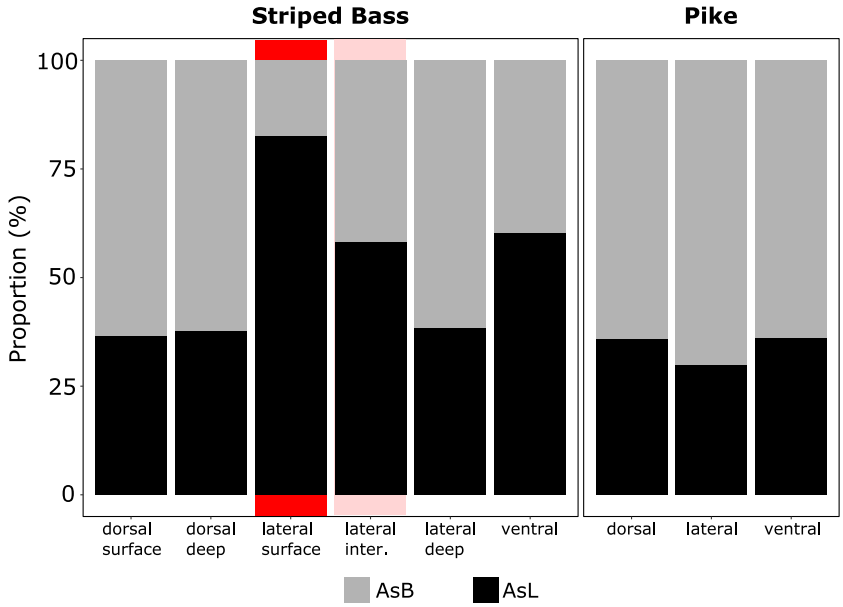


Figure 3. Proportion (%) of arsenic species compared to arsenic total in Striped Bass #7 (1 Striped Bass recto and verso, *n* = 2) and Pike #7 flesh (1 Pike recto only, *n* = 1), as function of muscle zone, type and depth. One value coming from red muscle of Bass was excluded (outlier). Red color shows red muscles and pink color identifies the intermediate ones. All other As species were under the detection limits.

Relationships between metal concentrations in fish muscle

We explored the covariation of MeHg, As and Se in biopsies for each species. In Striped Bass, strong positive relationships were found between MeHg and Se (n = 6 individuals) (r^2 from 0.45 to 0.94; $p < 0.05$; Fig. 2B), whereas negative ones were observed between MeHg and As (n = 6 individuals) (r^2 from 0.43 to 0.86; $p < 0.05$; Fig. 2A), and between As and Se (n = 5 individuals) (r^2 from 0.15 to 0.93; $p < 0.05$; Fig. 2C). Regression slopes were similar between individuals for the MeHg/Se relationship, but differed in other cases, notably for the As/Se pair (slope range: -2.19 to -24.18) (Fig. 2B).

For Northern Pike, no statistically significant trends were observed within individuals when As was concerned. Weak and contrasting relationships between MeHg and Se (n = 3 individuals) (r^2 from 0.02 to 0.3; $p < 0.05$; Fig. 2E) were found, compared to Bass. However, individuals that were more contaminated with one metal tended to be more contaminated with all metals, and Se seemed to reach a plateau around 1000 ng/g (Fig. 2 D-F).

Relationships between protein, lipid and metal concentrations

The observed intramuscular relationships between metals in Bass fillets (but not in Pike) could be linked to changes in muscle composition. We therefore explored the link between changes in protein and lipid contents and changes in metal levels within fillets.

In Striped Bass, there was a strong and positive association between changes in % N (proxy of protein levels) within a fillet, and the concentrations of MeHg (n = 6 individuals) (r^2 from 0.55 to 0.91, $p < 0.05$) and Se (n = 6 individuals) (r^2 from 0.49 to 0.90, $p < 0.05$) (Fig. 4 A and B), and a negative correlation with lipids for both metals (MeHg (n = 5 individuals): r^2 from 0.34 to 0.77; Se (n = 6 individuals): r^2 from 0.33 to 0.88, $p < 0.05$) (Fig. S1). In contrast, As was strongly associated with lipids (n = 6 individuals) (r^2 from 0.43 to 0.76, $p < 0.05$) (Fig. 4 C), but not with % N. Deep muscles (Fig. 4, closed symbols) were richer in MeHg, Se, % N and poorer in As and lipids (for each Bass, $p < 0.05$).

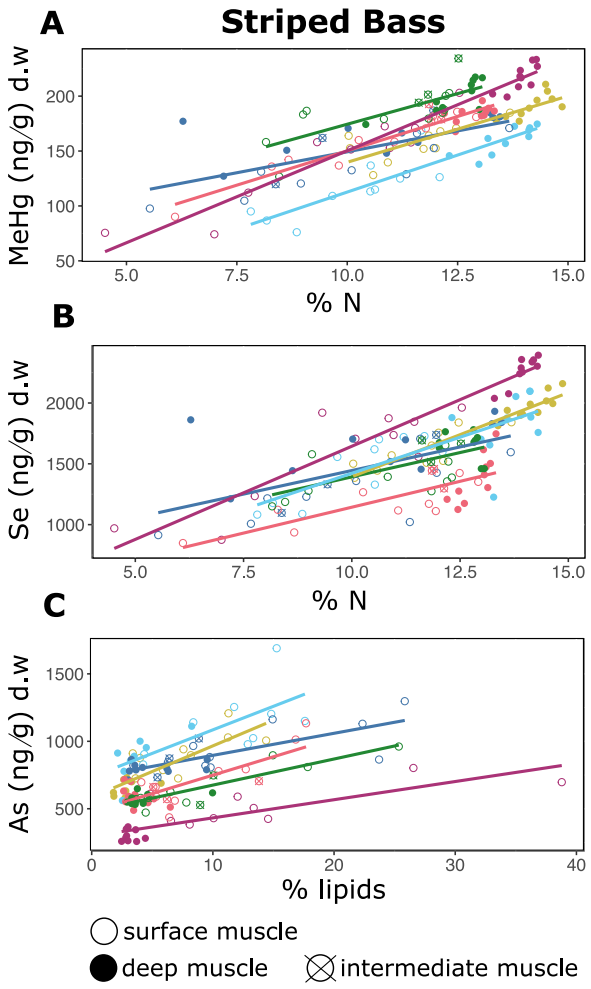


Figure 4. Relationships between metals and muscle composition in Striped Bass. Each individual (n = 6) had 10 sampling areas, subdivided in surface (open symbols), intermediate (crossed symbols) and deep muscle (closed symbols), yielding 20-23 samples per fish. Only significant regressions are shown.

In Northern Pike, we measured a weak negative correlation for only one individual, with slopes different from zero (ANCOVA, $p < 0.05$) between MeHg and lipids ($r^2 = 0.23$, $p < 0.05$) and between Se and lipids ($r^2 = 0.28$, $p < 0.05$) (Fig. 5). In contrast, MeHg ($r^2 = 0.32$) and Se ($r^2 = 0.30$ and 0.36, $p < 0.05$) were positively correlated to % N (Fig. S2). Unlike Striped Bass, no relationship was observed between the distribution of metal(loid)s and flesh component (% N, % lipid) as function of muscle depth (Fig. 5, Fig S2, open and closed symbols homogeneously distributed).

These observations are likely related to the very narrow range of values found for proteins and lipids in this fish species. Indeed, whereas % N and % lipids for Striped Bass varied from 4.5 to 14.9% and from 1.8 to 38.8%, respectively, those for Northern Pike only ranged from 13.1 to 14.8% and from 1.2 to 3.8%.

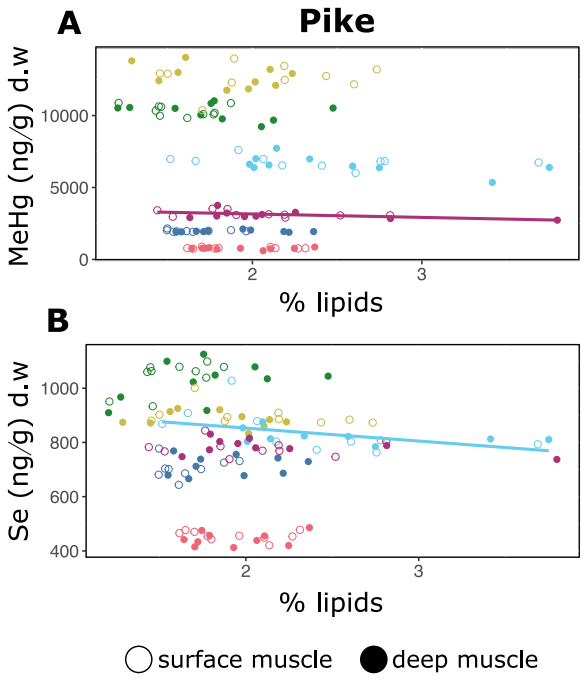


Figure 5. Relationships between metals and lipids content in Pike. Each individual (n = 6) had 10 sampling areas, subdivided in surface (open symbols) and deep muscle (closed symbols), yielding 20 samples per fish. Only significant regressions are shown.

Since red muscles are fattier (Greer-Walker & Pull, 1975) and they contain a greater proportion of AsL (Fig. 3), we explored the covariation of lipids and AsL. We obtained a strong positive regression (Fig. S3, r^2 = 0.91 and $p < 0.05$). Further, we measured a positive relationship between AsB and % N (Fig. S3, r^2 = 0.72 and $p < 0.05$).

Metal accumulation in red versus white muscles

The large intra-organ variation in % N, lipids and metals in Striped Bass compared to Northern Pike may be linked to the presence of three muscle types, red, intermediate and white, in the former, and of only one muscle type (white muscles) in the latter. We therefore investigated average levels of lipids, % N and metals in Striped Bass red, intermediate and white muscles, without considering muscle depth (Fig. S4).

Red muscles had lower protein, MeHg and Se content than white and intermediate muscles (Fig. S4 A-C) but had higher lipids and As content (Fig. S4 D-E). Indeed, compared to white muscles, red muscles had approximately 19%, 19% and 15% less protein, MeHg and Se (calculated as follows: 1 – [average level of the element in red muscle / average of the element in white muscle] * 100), and 234% and 126% more lipids and As (calculated as follows: [average level of the element in red muscle / average of the element in white muscle] * 100). No difference was denoted between white and intermediate muscle (Fig. S4). Furthermore, red muscle contained 265% more AsL and 57% less AsB than white muscle (Fig. 3).

We further compared protein, lipid and metal concentrations at different depths along the biopsy cores (i.e., surface samples vs. deep samples for dorsal and ventral zones; and the addition of intermediate muscles at the lateral zone) taken at different locations across the Bass fillet.

We distinguished between the dorsal, lateral and ventral areas. Surface samples from the lateral areas were the only ones containing red muscles (red boxplots on Fig. 6). When compared to average values obtained from all biopsy samples (dotted line on Fig. 6), surface samples from the lateral line were the ones with the lowest MeHg, Se and % N mean concentrations, and the highest As and lipid levels (Fig. 6, left panels). According to a mixed linear model, deep white muscles were higher in MeHg, Se, and % N, and lower in As and lipids, regardless of their position on the fish (Fig. 6, right panels). Muscle depth at the lateral line showed a gradient, with MeHg levels gradually increasing with depth, as for Se and % N. The inverse pattern can be observed for % lipids, and to some extent for As. No statistical difference was measured between muscle zones at a given depth, for any component in Fig. 6.

Muscle depth did not have the same effect in Pike flesh as can be seen in Fig. 2 and Fig. 5, where no distinct distribution pattern was observed between surface (open symbols) and deep muscle (closed symbols).

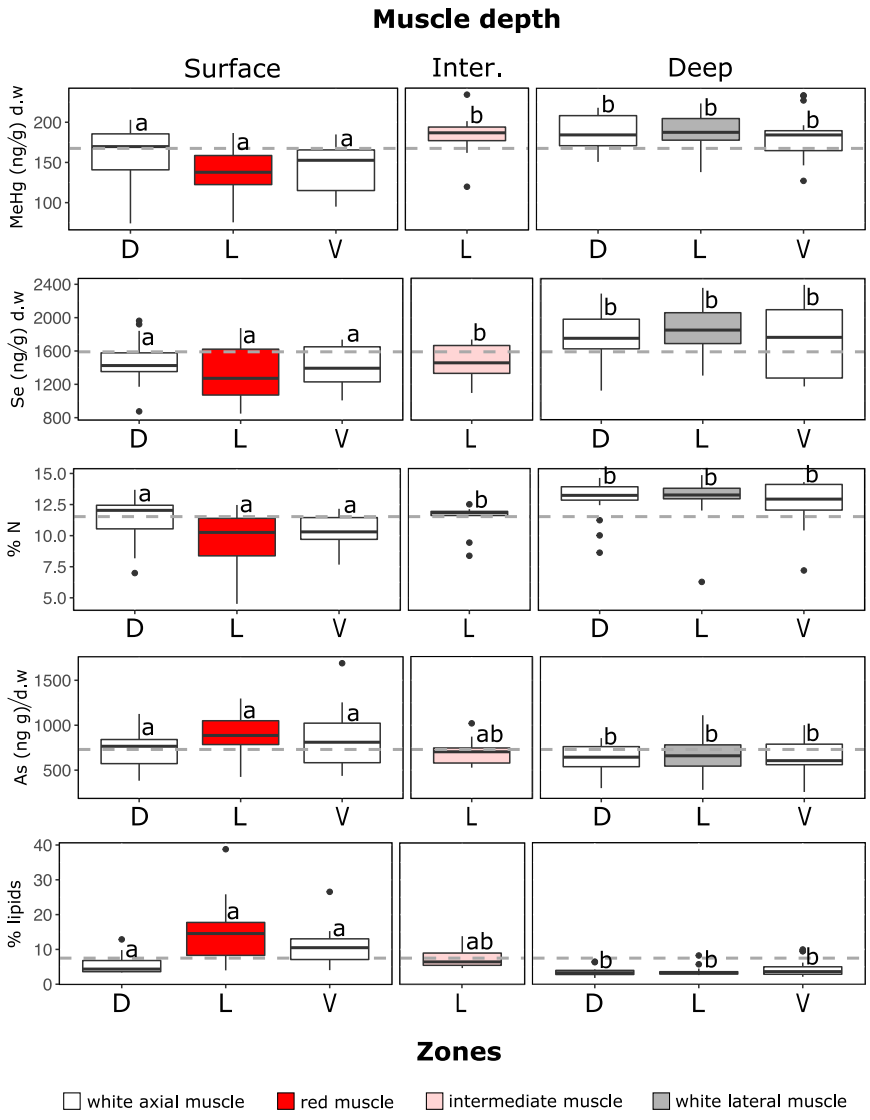


Figure 6. Distribution of metal(loid)s, % N and % lipids in Striped Bass (*n* = 6) muscle depending of muscle types and depth. Dotted lines represent the mean when all biopsies of the 6 Bass are considered (given 20 to 23 samples per fish). Zone D (dorsal, *n* = 18 per boxplot), L (lateral, *n* = 24 for red and white lateral muscle; *n* = 9 for intermediate muscle) and V (ventral, *n* = 18). Mixed linear model was applied with « fish individual » as random effect, with Tukey test as post *hoc* procedure. Letters are used to indicate a significative difference ($p < 0.05$) between muscle depth. In a given muscle depth, muscle zones were not different.

Discussion

Intra-individual protein and lipid distribution may influence metal(loid)s bioaccumulation

Our study establishes that metals can vary up to threefold within a single muscular system. This variability may be species-dependent and is likely related to changes in protein and lipid contents associated with different muscle types and depth.

It is known that ecological behavior of fish can influence its muscular system development (Gill et al., 1989). Striped Bass is a migratory and active predatory fish; this behavior is linked to an accentuated development of red muscles. In contrast, Pike is a sedentary ambush predator and, consequently, poorly equipped with red muscles (Gill et al., 1989; Walter III & Austin, 2001). Possessing three muscle types which differ in their biochemical composition (Johnston et al., 1975) may have repercussion on the variation of % N and % lipids within the fillet. Indeed, the Bass presented a maximum: minimum ratio of 3.3 for % N and 21.6 for % lipids, in contrast to 1.1 and 3.2 for the same components in the Pike. This variation range of % N and % lipids in the Bass may explain its wider concentration range of metal(loid)s in comparison of the Pike flesh, as a result of different chemical affinity of these metal(loid)s towards different components of muscle types.

Literature assessing metal(loid)s distribution within a fish fillet is scarce. Studies on Rainbow Trout (*Oncorhynchus mykiss*), and migratory Long-finned Eel (*Anguilla dieffenbachia*) and Bluefin Tuna (*Thunnus thunnus*) have reported some intra-individual variations for Hg (min:max ranging from 1.3 to 1.7) (Katz, 2002; Kim, 1995; Redmayne et al., 2000). These species are known to contain red muscles near the lateral lines (Ellerby et al., 2001; Lokmanl et al., 2003). However, these ranges of Hg variation are substantially less than for the Striped Bass (min:max of 1.6 to 3.1). This may be due to different species-dependent accumulation patterns, or to differences in methods. Indeed, compared to our study, previous reports have used less biopsy numbers (6 to 7 compared to 10 per individual), and have not taken into account muscle type and

depth. For the Rainbow Trout study, outer layers of flesh were shaved away, likely removing some red muscles (Kim, 1995).

MeHg and Se bioaccumulation is strongly related to protein distribution between muscle type and depth in Striped Bass

The Bass muscle presents a heterogenous distribution of protein content (assessed by % N) between red and white muscles, as well as a function of muscle depth (Fig. 6 and Fig. S4). This flesh composition pattern could explain the intra-individual bioaccumulation of MeHg and Se according to their chemical binding affinities.

MeHg binding affinity is high for Se, thiols (sulfur group) and nitrogen compounds ($SeH > SH >> Se\text{-}Se > NH_2$) (Khan & Wang, 2009; Sugiura et al., 1978). Hence, MeHg and Se are likely associated with protein content (Peterson et al., 2009), as suggested by our results (Fig. 2 B, Fig. 4 A-B and Fig. S4 A-B). A recent study evaluated the distribution of contaminants in Tuna flesh, coming from different countries. They observed that Hg was mostly found in the soluble proteins fraction [range 50-98.6%] and that the remaining Hg was located in the cellular debris fraction (Nong et al., 2020). In the same study, beta actin was identified as a novel Hg-binding protein with up to 37.6% of all Hg within fish flesh bound to it. Authors explained this observation by the 5 cysteine residues (sulfur-rich) contain in the amino acid sequence of beta actin (Nong et al., 2020).

The positive association of MeHg and Se with proteins could explain the inverse link between those metal(loid)s and the lipid fraction in Bass muscles (Fig. S1). Similar observations were made by Balshaw et al. (2008) who noticed an intra-individual negative correlation between THg and total lipids in Southern Bluefin Tuna (*Thunnus maccoyii*) white edible muscular tissue cuts (akami, chu-toto and o-toro), whereas Ando et al. (2016) found no correlation between THg and lipids content in Bluefin Tuna (*Thunnus orientalis*).

With respect to Se, no study has yet investigated the intramuscular distribution of this element in fish. At the inter-individual level, Marval-Leon et al. (2014) measured total Se content in the edible portion of twenty fish and shellfish species purchased in Spanish markets and found

a non-significant positive correlation between Se content and total protein, whereas they observed a negative correlation between the metalloid and total lipid content.

When muscle type is considered, the distribution of % N, MeHg and Se becomes clear: white muscles of Bass contained significatively more % N, as well as MeHg and Se (Fig. S4 A-B-C), than red muscles. Opposing results were found by Bosch et al. (2016) in Yellowfin Tuna (*Thunnus albacores*): white muscles contained less THg compared to the red ones (no significant difference was measured for MeHg). In heterotherm fish like Yellowfin Tuna, red muscles are distributed at the surface, like the non-heterotherm fish, in addition to an internal loin of red muscles along the spine, associated with large specialized blood vessels (Katz, 2002). The internal red muscles are fattier than the white ones (George, 1975; Mukundan et al., 1979) but the considerable blood supply could explain the higher THg level in large tunas, despite their biochemical composition.

We also assessed the influence of muscle depth on metal bioaccumulation patterns and denoted a clear distribution: deep muscles from the 3 different zones (dorsal, lateral and ventral) contained more MeHg, Se, % N (Fig. 6). To our knowledge, no other study has simultaneously evaluated the effect of muscle depth on MeHg, Se and As bioaccumulation in fish flesh. Cizdziel et al. (2001) assessed the influence of muscle depth on THg bioaccumulation in wild Striped Bass (*Roccus saxitilis*), Largemouth Bass (*Micropterus salmoides*) and Tilapia (*Oreochromis aureus*) at the dorsal and lateral line areas. They found a higher THg concentration in deep muscles located at the lateral line for Striped Bass and Largemouth Bass ($p < 0.05$) but not at the dorsal level (Cizdziel et al., 2001). This lack of depth patterns in the dorsal area contrasts with our results and may be related to different sampling strategies. We used 0 – 5 mm and 5 – 10 mm for surface and deeper muscle respectively, and we divided red muscles from the white ones at the lateral level, no matter the depth. In comparison, Cizdziel et al. (2001) used 0 – 3 mm and 3 – 6 mm as muscle depth sampling, and no mention was made in relation to red muscle management.

As bioaccumulation may be related to its speciation

Since we observed a positive correlation between As and lipids (Fig. 4 C) in Bass flesh, we further investigated the distribution of lipid content as a function of muscle type. We measured 2.3 times more lipids in red muscles compared to white ones (Fig. S4 E). Similar ratios in favor of red muscles have been reported, for instance, in Antarctic fish species: 4.4 in *Catostomus commersoni* (*n* = 2), 1.9 in *Dissostichus mawson* (*n* = 2), 3.7 in *Pagothenia borchgrevink* (*n* = 4) and 3.8 in *Trematomus bernacchi* (*n* = 3) (Clarke et al., 1984; Mai & Kinsella, 1979). Heterogeneous intra-organ total lipid content was also observed in white edible muscular tissue cuts (akami, chu-toto and o-toro) of Southern Bluefin Tuna (*Thunnus maccoyii*), with a ratio of 6.6 (*n* = 6) (Balshaw et al., 2008). This considerable intra-individual variation of lipids may have driven the bioaccumulation of metal(loid)s according to their chemical affinities.

With respect to As bioaccumulation patterns, we must consider As chemical species present in fish muscle. This metalloid can be found in organic or inorganic forms, this latter being in lesser proportion and varying among studies (from <2% to 30% of total As) (de Rosemond et al., 2008; Leufroy et al., 2011; Shah et al., 2010). In fact, no inorganic As was found in our study for both fish species, which agrees with the result of Kirby & Maher (2002) and Šlejkovec et al. (2004) for fish muscle from freshwater species.

Organic As forms include AsB, arsenosugars, arsenocholine, and methylated arsenicals; more recently, AsL have also been detected (de Rosemond et al., 2008; European Food Safety Authority, 2009; Leufroy et al., 2011; Shah et al., 2010; Taylor et al., 2016). In the studies of Kirby and Maher (2002) and Šlejkovec et al. (2004), ranges of AsB proportion of total As varied from 0.4 to 100%, whereas we obtained a range of 17.6 to 70.2%. Moreover, some studies have reported that AsL may be a dominant As species in fish flesh, representing 42% in Tuna (*Thunnus sp.*) and 62% in Herring (*Clupea harengus*) of total As (Lischka et al., 2013; Taleshi et al., 2010), which agree with proportion found in Bass flesh (Fig. 3, range [36 to 82%]).

Since we obtained a positive correlation between AsL and % lipids, as well as between AsB and % N (Fig. S3), As species properties might therefore explain the general As distribution patterns in the Bass muscle. To our knowledge, no other study investigated the intra-organ

distribution of As species, which seems to be of interest for fish species with different muscle type.

Since we did not find any inorganic As in Striped Bass and Pike flesh, it is unlikely that As plays a significant role in the interactions between Se and MeHg in those fish species. Studies on the potential molecular interaction (MeHg-Se and Se-As) should consider metal(loid)s speciation, since each species possess its own chemical affinities. Ideally, chemical interactions between these elements should be confirmed with emerging metallomics tools (Shi & Chance, 2008).

Conclusion

We found that fish species with considerable amount of red muscle display a wide range of metal(loid)s distribution within a single fish musculature. Indeed, in Striped Bass red muscle, we found 19% and 15% less MeHg and Se, while it contains 265% more AsL than white muscle. In both fish species, we did not find any toxic inorganic As species (As^{3+}) (Mandal & Suzuki, 2002).

From a toxicological point of view, the wide range of metal(loid)s distribution implies that one individual could have some muscle areas where the metal concentration exceeds toxicity reference values and other areas where the concentration is safe for fish health. In the context of a tissue residue-based approach study, this could lead to a misinterpretation of the results: for instance, metal concentrations measured in fish individuals could be below the toxic threshold, while being affected in reality.

Another implication resides in fish biomonitoring toward human consumption guidelines. For Hg, World Health Organization (WHO) fixed the acceptable contamination level at 500 ng/g w.w. in fish flesh to preserve human health (WHO, 1990). Depending on the sampling area within a fish fillet, the assessment of risk for human health may differ. Some monitoring organizations pool fillets of many specimens to create a single aliquot, thereby avoiding any issues linked to intramuscular variation. However, in other cases, monitoring is done by randomly selecting muscles parts, by always selecting the same muscle part, or by using biopsies. Our results suggest

that these latter practices could lead to guidelines that either limit fish consumption and ingestion of beneficial nutrients or under protect the consumer from the harmful effect of MeHg.

Further, risk assessments of MeHg exposure to humans through food consumption has benefited from the rapid development of *in vitro* digestion models which evaluate MeHg bioaccessibility (Bradley et al., 2017). Bioaccessibility can be defined as the fraction of a compound that is released from fish flesh components (mainly composed of proteins, lipids and water content (Venugopal & Shahidi, 1996)), by the action of digestive processes (Van de Wiele et al., 2007). However, a large variability is observed in inter-laboratory bioaccessibility results (Afonso et al., 2015; Cabanero et al., 2004; Ouédraogo & Amyot, 2011; Torres-Escribano et al., 2010; Wang et al., 2013) and even within a single laboratory working on a single species (Girard et al., 2017). Since bioaccessibility is closely linked to fish flesh composition (Fernández-García et al., 2009) it is likely that the variability of bioaccessibility for THg observed in literature is partly caused by a variation at the level of fish flesh composition. Indeed, when metal(loid)s bioaccessibility is assessed, there is currently no guideline regarding the muscle part used for the *in vitro* digestion. To limit metal(loid)s bioaccessibility variability, we propose that muscle type and muscle region used for this purpose should be standardized, or at least properly reported. These studies should systematically include the analysis of flesh components to better understand metal(loid)s bioaccumulation in fish musculature and its behavior during human digestion. A better understanding of MeHg solubility as a function of the composition of fish flesh could be a key information in human risk assessment. For instance, Peng et al. (2017) proposed that the weak MeHg bioaccessibility observed with the crayfish (*Procambarus clarkii*) muscle tissue was related to its high content in cysteine and arginine, leading to a strong binding between MeHg and proteins, diminishing the solubility of MeHg in gastrointestinal fluids. Thus, further studies should be undertaken to test such assumptions and, if confirmed, risk assessment could consider cysteine and arginine content when assessing mercury exposure.

Acknowledgements

The author thanks Dominic Bélanger, Alexandre Arscott-Gauvin and Noémie Lacombe for assistance in the laboratory. Daniel Borcard, Miguel Chagnon and Kevin Charette were consulted for statistical advice. Research was funded through the Canada Research Chair program (MA), a NSERC Discovery grant (MA, MR), a CRD NSERC/Hydro-Quebec grant (MA) and an NSERC CREATE Ecolac stipend to TC.

Supplementary information

Supplementary method

Protocol for gravimetry

Briefly, 10 mL of a chloroform : methanol solution (2:1) (>99.8 and 99.9%, Fisher Scientific) were added to 0.5 g of fresh fish flesh in a Falcon tube. The samples were homogenized with a homogenizer (Polytron Type PT 10 OD, Brinkmann Instruments Inc., Ontario, Canada). Tubes were left overnight under the fumehood. 2 mL of ultra-pure MilliQ water (>18.2 MΩ cm^{-1}) were added to each tube; the tubes were then centrifuged (3000 g $\times$ 20 minutes, 20 °C). The upper supernatant and the pellet were discarded, while the inferior supernatant was transferred in pre-weighted aluminum vessels and 2 mL of methanol was added to each vessel. Samples were air-dried under the fumehood overnight. Percent lipid content was quantified with the following equation:

$$lipid\ content = \frac{mass\ of\ vessel\ with\ sample\ (g) - masse\ of\ empty\ vessel\ (g)}{mass\ of\ fresh\ flesh\ homogenized} \times 100$$

Supplementary tables

Table S1. Details of Striped Bass (*Morone Saxatilis*) and Pike (*Esox Lucius*) used for analysis. Data is expressed as mean (SD) (*n* = 7).

	Striped bass (#1 to #7)	**Pike (#1 to #7)**
Origin	Aquaculture from South America	Saint-Maurice river from Canada
Storage condition	Kept at -20 °C	Flash freeze, then kept at -20 °C
Body weight (g)	1060.7 (71.5)	1047.9 (194.3)
CV (%) [min-max]	6.7% [902.5-1102.6]	18.5% [728.3-1352.2]
Standard length (cm)	34.8 (1.6)	56.1 (4.3)
CV (%) [min-max]	4.6% [32.1-37.1]	7.6% [49.1-59.9]

Table S2. Mean (SD) ratio[†] between wet and dry weight concentration for MeHg, Se and As, as function of fish species and muscle type.

Fish species	Muscle type	MeHg	Se	As
	White	4.01 (0.7)	4.01 (0.9)	3.86 (1.3)
Striped Bass	Intermediate	3.96 (0.7)	3.94 (0.6)	3.76 (0.8)
	Red	3.28 (0.7)	3.30 (0.8)	2.98 (0.7)
Pike	White	5.11 (3.8)	4.94 (1.2)	5.03 (2.9)

[†] was calculated as follows: mean $[M]_{\text{weight wet}}$ / mean $[M]_{\text{dry weight}}$

Table S3. Mean (SD) MeHg, Se and As bioaccumulation in Striped Bass (*Morone Saxatilis*) (*n* = 6) and Pike (*Esox Lucius*) flesh (*n* = 6). Between 20-23 muscle areas were analyzed per individual. Range refers to [min] and [max] within an individual. CV for coefficient of variation (%).

	Striped bass			Pike		
MeHg bioaccumulation (ng/g) d.w						
fish ID	**mean concentration (SD)**	**CV (%)**	**mean CV (SD)**	**mean concentration (SD)**	**CV (%)**	**mean CV (SD)**
p7	174.28 (21.84)	12.53		1975.37 (68.86)	3.49	
p8	137.54 (30.62)	22.26		777.43 (59.40)	7.64	
p9	180.00 (48.69)	27.05	17.72 (6.11)	10307.99 (469.70)	4.56	6.37 (1.90)
p10	148.83 (27.83)	18.70		12694.77 (858.06)	6.76	
p11	170.76 (24.45)	14.32		6684.40 (511.30)	7.65	
p12	192.01 (21.96)	11.44		3131.99 (254.98)	8.14	
Se bioaccumulation (ng/g) d.w						
fish ID	**mean concentration (SD)**	**CV (%)**	**mean CV (SD)**	**mean concentration (SD)**	**CV (%)**	**mean CV (SD)**
p7	1789.51 (233.90)	13.07		711.29 (35.17)	4.94	
p8	1641.32 (316.57)	19.29		450.03 (21.90)	4.87	
p9	1916.33 (458.79)	23.94	17.88 (4.67)	1034.52 (63.97)	6.18	5.09 (1.36)
p10	1439.45 (316.07)	21.96		891.82 (33.53)	3.76	
p11	1290.21 (213.29)	16.53		832.16 (59.28)	7.12	
p12	1522.99 (190.55)	12.51		782.33 (28.56)	3.65	
As bioaccumulation (ng/g) d.w						
fish ID	**mean concentration (SD)**	**CV (%)**	**mean CV (SD)**	**mean concentration (SD)**	**CV (%)**	**mean CV (SD)**
p7	784.97 (168.20)	21.43		43.07 (4.86)	11.28	
p8	992.24 (242.28)	24.42		23.85 (3.43)	14.37	
p9	438.46 (177.82)	40.56	24.70 (8.30)	90.40 (9.26)	10.24	13.86 (2.94)
p10	879.34 (144.67)	16.45		130.87 (17.19)	13.13	
p11	656.27 (159.65)	24.33		56.77 (9.16)	16.13	
p12	630.78 (132.41)	21.00		43.09 (7.77)	18.03	
MeHg distribution - ratio [max]/[min]						
fish ID	**range [min;max]**	**[max]/[min]**	**mean [max/min] (SD)**	**range [min;max]**	**[max]/[min]**	**mean [max/min] (SD)**
p7	128.08 - 210.80	1.6		1886.38 - 2152.36	1.1	
p8	76.07 - 174.61	2.3		606.16 - 886.84	1.5	
p9	74.18 - 233.41	3.1	2.2 (0.5)	9225.38 - 11020.30	1.2	1.3 (0.1)
p10	97.65 - 186.88	1.9		10370.58 - 14039.06	1.4	
p11	90.14- 195.85	2.2		5349.27 - 7723.61	1.9	
p12	126.68 - 324.34	1.8		2728.48 - 3758.19	1.7	
Se distribution - ratio [max]/[min]						
fish ID	**range [min;max]**	**[max]/[min]**	**mean [max/min] (SD)**	**range [min;max]**	**[max]/[min]**	**mean [max/min] (SD)**
p7	1385.04 - 2159.34	1.6		643.74 - 777.35	1.2	
p8	1082.20 - 2097.96	1.9		412.37 - 485.66	1.2	
p9	875.22 - 2393.16	2.7	2.0 (0.4)	909.66 - 1125.18	1.2	1.2 (0.1)
p10	913.82 - 1931.50	2.1		832.79 - 1000.78	1.1	
p11	848.54 - 1746.82	2.1		763.05 - 1027.92	1.2	
p12	1150.42 - 1780.55	1.5		737.35 - 843.94	1.1	
As distribution - ratio [max]/[min]						
fish ID	**range [min;max]**	**[max]/[min]**	**mean [max/min] (SD)**	**range [min;max]**	**[max]/[min]**	**mean [max/min] (SD)**
p7	580.40 - 1207.57	2.1		34.89 - 52.05	1.5	
p8	560.74 - 1689.91	3		18.40 - 29.22	1.6	
p9	257.32 - 828.02	3.2	2.5 (0.6)	70.24 - 104.36	1.5	1.6 (0.1)
p10	724.75 - 1297.36	1.8		97.17 - 163.80	1.7	
p11	436.45 - 1133.52	2.6		43.96 - 74.36	1.7	
p12	471.78 - 961.27	2.0		32.83 - 62.02	1.9	

Supplementary figures

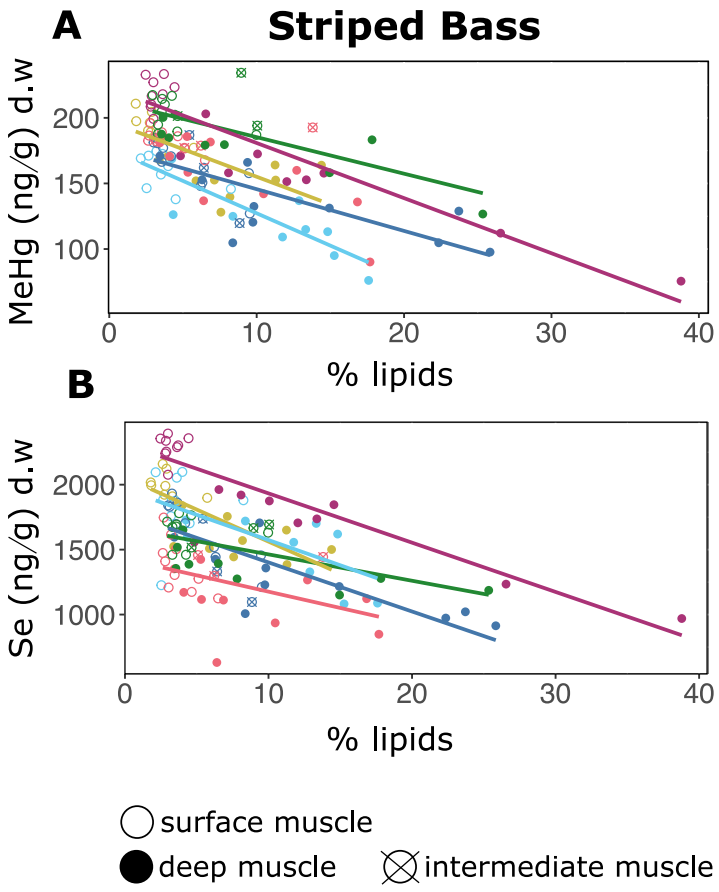


Figure S1. Relationships between metals and lipids in Striped Bass. Each individual (*n* = 6) had 10 sampling areas, subdivided in surface (open symbols), intermediate (crossed symbols) and deep muscle (closed symbols), yielding 20-23 samples per fish. Only significant regressions are shown.

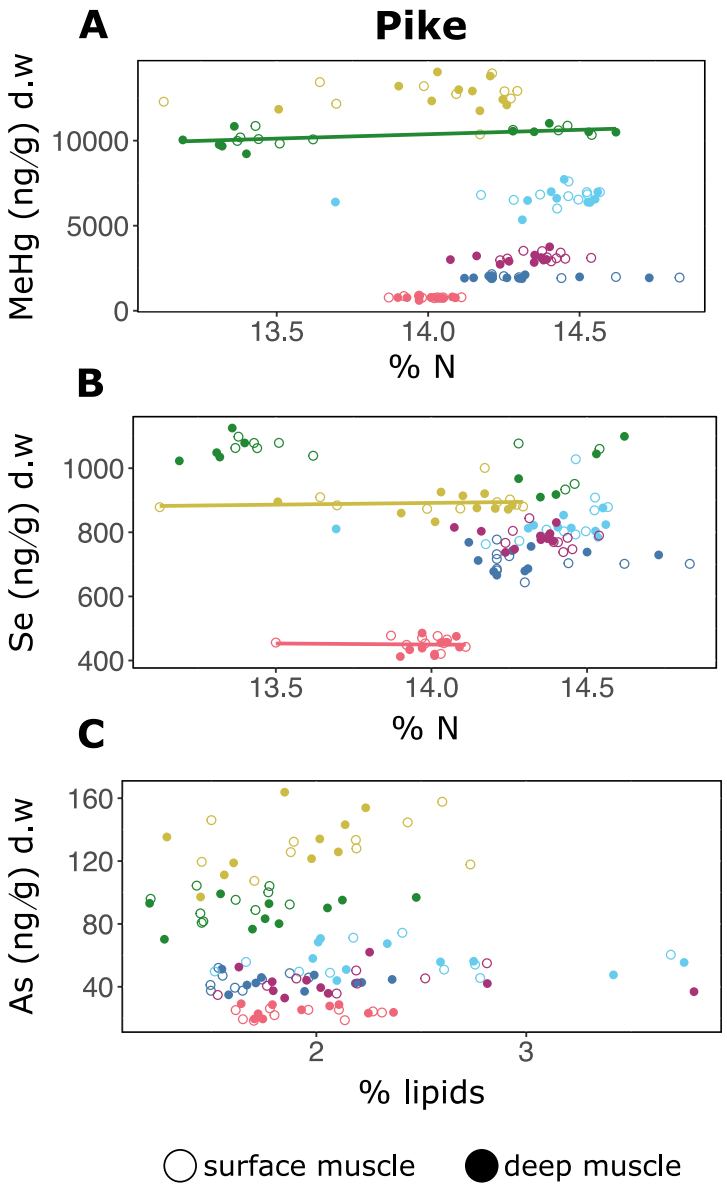


Figure S2. Relationships between metals and muscle composition in Pike. Each individual (*n* = 6) had 10 sampling areas, subdivided in surface (open symbols) and deep muscle (closed symbols), yielding 20 samples per fish. Only significant regressions are shown.

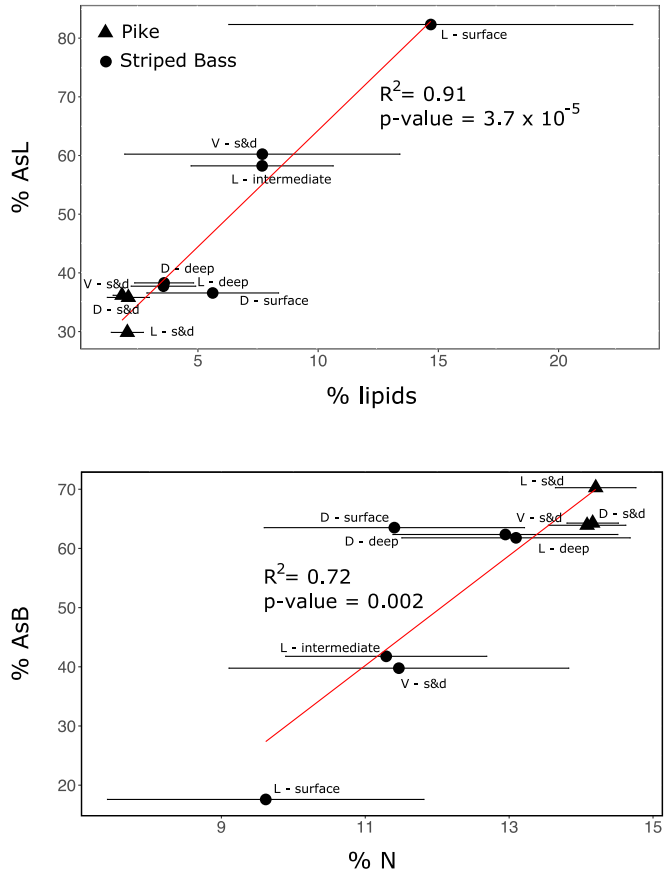


Figure S3. Linear regression between As species and muscle components according to the muscle zone and depth in Striped Bass and Pike flesh. Values of % lipids and % N are illustrated as mean ± standard error and come from Striped Bass and Pike #1 to #6. Arsenolipids and AsB data from the mean of recto/verso musculature from Striped Bass #7, whereas there is a single value per data in the case of Pike #7. D: dorsal; L: lateral; V: ventral; surface: surface muscle; deep: deep muscle; s&d: surface and deep muscle mixed.

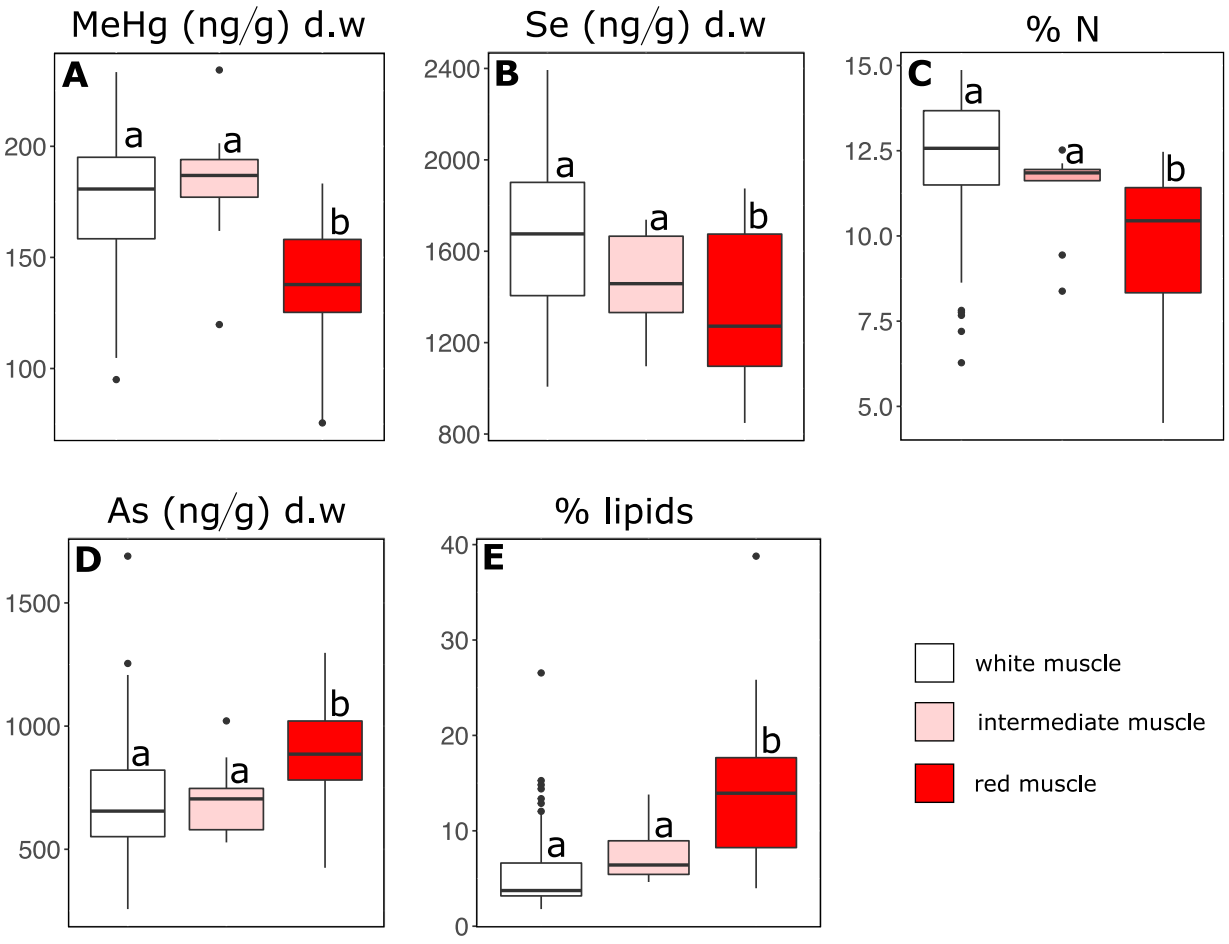


Figure S4. Distribution of metal(loid)s, % N and % lipids in Striped Bass (*n* = 3 to 6) flesh depending on muscle types, without considering muscle depth (white include white muscles from the dorsal, lateral and ventral zones (*n* = 96); intermediate (*n* = 9); red (*n* = 24). Mixed linear model was applied with « fish individual » as random effect, with Tukey test as post *hoc* procedure. Letters are used to indicate a significative difference ($p < 0.05$) between muscle types.

Chapitre 2 : Évaluation de la bioaccessibilité *in vitro* et de la biodisponibilité orale *in vivo* comme outils complémentaires pour mieux comprendre l'effet de la cuisson sur le méthylmercure, l'arsenic et le sélénium chez le thon.

Tranche de vie #2 : Mon ambiance optimisée à la rédaction

Musique : essentiellement que du piano. Artistes favoris : Elijah Bossenbroek et Michelle McLaughlin

Méthode *Pomodoro* : 50 minutes de rédaction pour 10 minutes de pause. Une moyenne de 6 blocs de rédaction quotidiens.

Application *SelfControl* : bloque l'accès à tout ce que tu lui demandes, pour un temps précis. Sites internet bloqués pendant ma rédaction : Facebook, YouTube et Amazon

Boissons : Tisane à la menthe poivrée ou café noir

Pour passer le stress et aider la concentration : gomme à mâcher à la menthe de la marque Trident de préférence.

Assessment of *in vitro* bioaccessibility and *in vivo* oral bioavailability as complementary tools to better understand the effect of cooking on methylmercury, arsenic, and selenium in tuna

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Published in Toxics, 9 (2), 27. Copyright 2021 MDPI Ltd.

DOI: 10.3390/toxics9020027

Minor edits to the published text have been made here, following suggestions by this thesis' reviewers.

Abstract

Fish consumption is the main exposure pathway of the neurotoxicant methylmercury (MeHg) in humans. The potential toxicity associated with exposure to MeHg may be modified by its interactions with selenium (Se) and arsenic (As). *In vitro* bioaccessibility studies have demonstrated that cooking the fish muscle decreases MeHg solubility markedly and, as a consequence, its potential absorption by the consumer. However, this phenomenon has yet to be validated by *in vivo* models. Our study aimed to test whether MeHg bioaccessibility can be used as a surrogate to assess the effect of cooking on MeHg *in vivo* availability. We fed pigs a single raw and cooked tuna (*Thunnus albacares*) meals and collected blood samples from catheters in the portal vein and carotid artery at -5, 30, 60, 90, 120, 180, 240, 300, 360, 420, 480 and 540 min post-meal. Metal(loid)s (MeHg, Se and As) analysis was performed in whole blood and serum. Kinetic assessment was done using the serum concentration-time profile and exploiting the following pharmacokinetic parameters: peak concentration (C_{max}) and time to peak concentration (T_{max}) were used as indirect metrics to characterize the rate of absorption. In parallel to the *in vivo* design, we conducted an *in v*itro digestion using a subsample of the raw and cooked tuna meals used in the *in vivo* experiments. Cooking decreased mean MeHg *in vitro* gastrointestinal bioaccessibility by 33%. In contrast, pig oral bioavailability of MeHg and As was not affected by cooking, although the MeHg kinetics was faster for the cooked meal than for the raw meal. Dietary treatment did not affect Se blood levels, in contrast to MeHg and As. As well, MeHg was distributed into the red blood cell fraction at 81 ± 4% and 86 ± 3% in pigs fed raw and cooked tuna meals, respectively. In distinction, Se and As were not preferentially distributed in the red blood cells of pigs fed either raw or cooked tuna. For the three metal(loid)s, these proportions were not altered by the cooking treatment. Arsenic speciation revealed that arsenobetaine were the only As species in tuna muscle and pig's blood, whereas the toxic inorganic As species (As^{3+}) was under the limit of detection. This is the first study to indicate that bioaccessibility cannot be readily used as a surrogate for *in vivo* studies and that, in contrast with the *in vitro* results, the cooking of fish flesh did not decrease the exposure of the consumer to MeHg.

Keywords: methylmercury; arsenic; selenium; oral bioavailability; bioaccessibility

Funding sources & approval for animal research

The experimental procedures followed the guidelines of the Canadian Council on Animal Care (Canadian Council on Animal Care (CCAC), 2009) and were approved by the Institutional Animal Care Committee of the Sherbrooke Research and Development Centre (Quebec, Canada). All animals were cared for according to the recommended code of practice of the National Farm Animal Care Council (National Farm Animal Care Council (NFACC), 2014).

Research was funded through the Canada Research Chair program (MA), a NSERC Discovery grant (MA, MR), a CRD NSERC/Hydro-Quebec grant (MA) and an NSERC CREATE Ecolac stipend to TC. The animal phase of this study was supported by Agriculture and Agri-Food Canada core budget.

Introduction

Fish provides a means of food security and offers an elevated nutritional value (FAO/WHO, 2010). The consumption of fish is also, however, the primary pathway of human exposure to mercury (Hg) and its organic form, methylmercury (MeHg) (National Research Council, 2000a). Even chronic low MeHg exposure, for example at concentrations observed for average fish consumption in the Western Hemisphere, could be toxic for neurological, cardiac, and immune systems (Bourdineaud et al., 2011; European Food Safety Authority, 2012). Currently in Canada, Hg is the only element on which fish consumption guidelines are based (Health Canada, 2007a).

Methylmercury is the main Hg species found in fish muscle, representing over 80% of total Hg (Magalhães et al., 2007). Along with high levels of MeHg, the muscle of fish such as tuna is also rich in selenium (Se) and arsenic (As) (Cappon & Smith, 1982; Storelli et al., 2005). Antagonistic effects of Se on MeHg toxicity have been reported (Peterson et al., 2009). In fish muscle, Se can be found in organic forms, such as selenomethionine, selenocysteine and selenoneine (Cabañero et al., 2005; Jagtap et al., 2016; Yamashita et al., 2011). Further, there is a potential interaction between Se and As which could limit the protective effect of Se against MeHg (Zeng et al., 2005). However, the main As species known to interact with Se are the inorganic forms (Zeng et al., 2005), this later representing a low proportion in fish muscle compared with that of organic species (iAs ranges from <2% to 30% of total As) (Charette et al., 2021; de Rosemond et al., 2008; Leufroy et al., 2011; Shah et al., 2010). In its organic form, As can be found as arsenobetaine (AsB) and arsenolipid (Taylor et al., 2016). Therefore, the study of MeHg behavior should include As as well since they are both interrelated by the metabolism of Se (El-Begearmi et al., 1982), and even more so since the identification of the Hg-Se-As complex by Korbas et al. (2008).

In vitro digestion has emerged as a rapid, cheap, and highly replicable tool for assessing Hg exposure from fish consumption (Moreda-Pineiro et al., 2011). This technique consists of measuring the bioaccessibility of a given component, defined as the soluble fraction that is released from the food matrix by the action of digestive processes (Van de Wiele et al., 2007). *In vitro* models are valuable but must be used with caution because they can fail to include several aspects of human digestive physiology. Pig-based studies have successfully validated the use of

in vitro digestion as a surrogate of *in vivo* studies for iAs bioavailability from soils (Juhasz et al., 2007, 2009). Because of its similar digestive system, the pig model is a relevant alternative that can be used as surrogate for humans (Rees et al., 2009). In contrast, no study has yet confirmed the reliability of *in vitro* digestion of MeHg from fish muscle to evaluate its *in vivo* bioavailability. MeHg bioaccessibility from fish muscle has been compared once with oral bioavailability using a mouse model (Li & Wang, 2019). The authors found no correlation between the two methods. Metals in fish muscle are complexed differently than in soils, which could modify the efficiency of *in vitro* models to release MeHg from fish muscle. Despite this, recent studies have started to propose changes in consumption guidelines on the basis of *in vitro* bioaccessibility results alone (Anacleto et al., 2020; Gong et al., 2018). Furthermore, numerous *in vitro* studies have reported that the cooking of fish muscle induced a significant reduction of Hg and MeHg bioaccessibility, which suggest a decreased MeHg solubility (Afonso et al., 2015; Anacleto et al., 2020; Girard et al., 2017; Ouédraogo & Amyot, 2011). If this phenomenon can be confirmed *in vivo*, current consumption guidelines could be modified.

As the assessment of MeHg bioaccessibility cannot easily be performed *in vivo* due to ethical constraints (Brodkorb et al., 2019), studies have used blood total Hg (THg) values as a biomarker of exposure to this heavy metal. Total Hg blood concentrations, a proxy for MeHg levels, are often well correlated with fish consumption (Mahaffey et al., 2004; Sanzo et al., 2001). In human blood, MeHg is mostly found trapped in red blood cells (RBCs) (European Food Safety Authority, 2012). Arsenic is also found in RBC and serum fractions mostly in the form of AsB (Ito et al., 2010; Shibata et al., 1994). Although it is known to be unbound and poorly degraded or metabolized (Taylor et al., 2016), exposure to this organic As species has not been associated with toxic effects because of its high excretion rate (Kaise et al., 1985). Furthermore, to the best of our knowledge, the effect of cooking on its oral bioavailability has yet to be studied.

Here we determined the effect of cooking on MeHg exposure after fish consumption, using both *in vivo* and *in vitro* approaches. According to literature, cooking should decrease MeHg exposure (Afonso et al., 2015; Anacleto et al., 2020; Girard et al., 2017; Ouédraogo & Amyot, 2011). In this study, pigs were fed raw or cooked tuna meals (*Thunnus albacares*), and we collected portal blood samples periodically for 540 min post-meal. Emphasis was put on the

serum fraction, since compounds must be distributed in this blood compartment prior to exit the vascular system (Carrier et al., 2001). Through this approach, we assessed the kinetics of the oral bioavailability of MeHg, As, and Se as a function of the cooking method, and we determined the partitioning of MeHg and other metal(loid)s in blood compartments. In parallel, we conducted *in vitro* digestion using the same tuna meals (raw and cooked) to assess MeHg bioaccessibility. Our research is of interest since other bioaccessibility studies have proposed using *in vitro* results to modify fish consumption guidelines.

Materials and methods

Animals, surgeries, and treatments

Five Yorkshire-Landrace × Duroc gilts females were selected at 30 kg of body weight (BW) and fed ad libitum a conventional basal diet for growing pigs (Table 1) until surgery. Average BW at surgery was 45.6 ± 3.9 kg at 80.5 ± 4.9 days of age. The surgery procedure has been described by Hooda et al. (2009) and modified by Dalto et al. (2018). Briefly, a catheter was inserted into the portal vein at approximately 2.5 cm before its entry into the liver, and another catheter was inserted through the carotid artery up to the junction between the carotid and subclavian arteries. The catheter into the portal vein allows to monitor directly what was absorbed from the digestive system, before any hepatic action, and the catheter into the carotid artery gave information on metal(loid) levels after the hepatic metabolization and extra-vascular distribution that could occur between the liver and the carotid. This is a delicate surgical procedure that was performed by experienced veterinarians following strict ethical guidelines. This imposed a limit to replication with 5 pigs included in this paper, for each of which we collected detailed data over time (see below). This is the first time that this experimental design is used to assess MeHg oral bioavailability.

Table 1. Conventional basal diet for growing pigs and treatments. Metal(oid)s values (wet weight) are presented as average ± standard error.

Basal Diet, Nutrients [1]						**Amount (%)**
Corn						55.4
Soybean meal 48%						15.1
Wheat						15.0
Barley						1.3
Distillers dried grain with solubles						9.8
Amino acids						3.5
Basal diet, contaminants [2] **(*n* = 5)**						**Levels (ng/g)**
THg						2.6 ± 1.3
TSe						58.4 ± 7.5
TAs						ND
Treatment [3] **(*n* = 6)**	**Pig number**	**MeHg (µg)**	**%MeHg** [4]	**TSe (µg)**	**TAs (µg)**	**%AsB** [5]
Raw	1R *, 2, 3	118.8 ± 26.7	94 ± 8%	319.2 ± 39.4	508.8 ± 16.3	73 ± 9%
Cooked	1C *, 4, 5	139.2 ± 9.4	93 ± 3%	409.4 ± 45.4	598.4 ± 83	96 ± 5%

[1] Basal diet contained 17.8% protein, 0.73% Ca, 0.52% P, 140 ppm Zn, 18 ppm Cu, 479 ppm Fe, 143 ppm Mn (analyzed values), and 3082 kcal (calculated value).
[2] THg for total Hg; TSe for total Se; TAs for total As.
[3] Raw tuna meal corresponded to 495 ± 2.3 g and cooked tuna meal to 375 ± 0.4 g. ND: not detected.
[4] %MeHg is the proportion of THg that is methylated. [5] %AsB si the proportion of As that is AsB.
* Pig 1 received both cooked and raw treatment a week apart.

After surgery, animals were penned individually (1 m × 1.8 m) and fed the conventional growing-phase diet described above in a single daily meal in accordance with their BW (1.0 kg/day when <50 kg BW; 1.2 kg/day from 50–60 kg BW; and 1.5 kg/day when >60 kg BW). Seven to 10 days after surgery, when the animals had fully recovered (appetite and normal growth rate), they were gradually acclimatized (3–5 days) to the metabolic cage (i.e. a specific type of cage allowing the collection and the separation of urine and feces) with free access to water. One week prior to the experimental day, animals were fed one single portion of raw (80 g) or cooked (80 g) tuna to have them adapt to the taste. Each pig received a single dietary treatment (Table 1) on the experimental day. Exceptionally, Pig 1 was fed a cooked tuna meal (1C) followed by a raw tuna meal (1R) one week later. The other animals received either a raw or cooked treatment only.

For dietary treatments, two yellowfin tuna (*Thunnus albacares*) frozen loins (1.3 to 2.3 kg each) were bought from the Odessa Poissonnier fish market (Montreal, Quebec, Canada). Once thawed at room temperature, the tuna was chopped into small pieces (1 cm × 1 cm) and

separated into ten meals of approximately 520 g wet weight. The remaining mass was used for the *in vitro* digestion procedure and was kept at −20 °C until the experimental day.

For the cooked treatment, tuna samples (427 ± 4.0 g) were placed in a previous acid-washed Pyrex™ dish and heated in an oven (VWR Scientific 1370 FM forced air oven) until an internal temperature of 70 °C was reached, corresponding to the safe internal cooking temperature according to Health Canada (Health Canada, 2019b). Raw and cooked tuna samples were kept at −20 °C until the experimental day. Each tuna sample was subsampled (± 20 g) and preserved in the freezer until metal(loid) analysis (−20 °C). The final mass of each meal was 495 ± 2.3 g for raw tuna and 375 ± 0.4 g for cooked tuna (due to moisture lost in the oven). Final metal(loid)s levels are presented at Table 1. On average, cooked tuna meals contained 14% more MeHg than the raw treatment, resulting from the heterogenous distribution of MeHg in tuna muscle. Overall, three raw (fed to pigs 1R, 2, and 3) and three cooked meals (fed to pigs 1C, 4, and 5) were administered in the experiment.

Blood collection and analysis

On the experimental day, pigs were moved to the metabolic cages, and blood samples (4 mL) were collected simultaneously from the two catheters at five minutes before the dietary treatment, every 30 min for the first two hours after the meal, and every hour for the following seven hours for a total of nine postprandial hours (total blood samples: 12 from the portal vein and 12 from the carotid artery). A total sampling time of 540 min was chosen since mean gastric emptying time and chyme transit time in the small intestine is approximately 3-4 h each, for a total of 8 h (480 min) (Davis et al., 2001; DeSesso & Jacobson, 2001; Strathe et al., 2008). Immediately after sampling, arterial and portal-drained viscera (PDV) blood was transferred through syringes into EDTA-treated tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ, USA) and trace element–free BD Hemogard™ Closure tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ, USA). Packed cell volume was measured in duplicate on fresh PDV blood by microcentrifugation. Aliquots of collected arterial and PDV blood were frozen for metal(loid) analysis as well as for determining hemoglobin according to the method of Drabkin (Manet,

1969). After at least four hours of decantation, arterial and PDV serums were then collected after centrifuging the blood samples at 1800 × *g* for 10 min at 4 °C. The serums were frozen at −20 °C until used for metal(loid) analysis.

In vitro digestion experiment

We used an *in vitro* protocol, the physiologically based extraction test (PBET), to test the metal bioaccessibility of raw vs. cooked fish. The PBET was used based on a protocol modified from Girard et al. (2017), Ouédraogo & Amyot (2011) and Ruby et al. (1996). The ratio of gastric and intestinal fluids (1:1) was set according to Smith & Morton (2010).

Digestive fluids were prepared on the same day of the experiment in acid-washed Teflon™ bottles. Chemicals were purchased from Sigma-Aldrich. Gastric fluid was composed of 1.25 g of porcine pepsin (>400 U/mg), 0.50 g of sodium citrate (>99%), 0.50 g of malic acid (>99%), 420 µL of lactic acid (>85%), and 500 µL of acetic acid (99.7%), mixed in ultrapure Milli-Q water to a final volume of 1 L. For the intestinal fluid, we mixed 0.60 g of bile salts and 0.15 g of pancreatin (4 × USP grade, lipase >24 U/mg, protease >400 U/mg) in a final volume of 250 mL 1M $NaHCO_3$. pH targets for the stomach and intestinal fluids were 2 and 7, respectively, and were adjusted using HCl (OmniTrace Ultra, EMD) before the digestion procedure.

Briefly, 1.0 ± 0.1 g of raw or previously cooked tuna was roughly homogenized and added to trace metal–free Falcon™ tubes, and each treatment was performed five times. A total of 22.5 mL of gastric fluid was added to the Falcon tubes, and they were placed in a horizontal incubator (100 rpm) at 37 °C for 1 hour. After this step, 22.5 mL of intestinal fluid was added to each tube, and pH was monitored and adjusted if needed before a second horizontal incubation (100 rpm) at 37 °C for 2 hours.

Isolation of the gastric and intestinal bioaccessible fraction was performed by a centrifugation step at 3000 × *g* for 15 minutes at 20 °C. Aliquots were then taken and kept at −20 °C until further analysis. Bioaccessibility was calculated as

$$(1)\ \frac{[MeHg]\ in\ PBET \times PBET\ volume}{[MeHg]\ in\ fish \times fish\ mass} \times 100,$$

where [MeHg] in PBET (ng/L) represents MeHg levels measured in the aliquot collected after the PBET digestive processes, PBET volume (L) is the PBET fluid volume (0.0225 L for the gastric and 0.045 L for the gastrointestinal fractions), [MeHg] in fish (ng/g) is the MeHg level in the fish samples before PBET digestion, and fish mass (g) is the mass of fish samples used for the PBET simulation. MeHg mass balance was calculated as follows

$$(2)\ \frac{mass\ of\ MeHg\ in\ supernatant + mass\ of\ MeHg\ in\ pellets}{mass\ of\ MeHg\ in\ tuna\ flesh} \times 100,$$

where mass of MeHg in supernatant is the quantity of MeHg that is bioaccessible and mass of MeHg in pellet is the quantity of non-soluble MeHg following centrifugation. We accounted for an average of 95 ± 10% (*n* = 18) of the MeHg mass added to the vessels, indicating no loss of MeHg during the experiments.

Total mercury analysis

Whole blood (WB) and tuna muscle. Total mercury was measured using a direct mercury analyzer (DMA 80, Milestone Inc., Pittsburgh, PA), in which 100 µL of WB and 0.01 g (dw) of freeze-dried tuna samples (Freezone6, Labconco) were thermally decomposed at 750 °C in an oxygen-rich furnace. Elemental mercury vapors were preconcentrated on a gold amalgamation trap for analysis by atomic absorption spectrophotometry. For WB, conversion from mass to volume was achieved using a density of 1.06 kg/L (U.S. EPA, 2007).

Serum. Prior to THg analysis in serum, 200 µL was digested in 1500 µL of 70% HNO_3 (OmniTrace Ultra™, MilliporeSigma) for 30 min on a hotplate (95 °C). The digestion was completed using OPTIMA grade H_2O_2 by completing the volume up to 2 mL and returning it to the hotplate (95 °C) for 5 h; the volume was then adjusted to 2 mL with Milli-Q water (>18.2 MΩ/cm),

diluted 1:4 with 2% HNO_3. Aliquots were analyzed using CVAFS (Tekran 2600, Tekran Instruments Corporation). Whole blood and serum were analyzed by two different protocols. Whole blood is highly viscous, and it was more convenient to use sample mass rather than volume for its analysis.

MeHg analysis

WB, serum, tuna flesh, and PBET soluble fraction. For MeHg analyses, 250 μL of WB, 500 μL of serum, 0.01 g (dw) of freeze-dried tuna samples (Freezone6, Labconco), and 100 μL of PBET soluble fraction were digested overnight at 60 °C in 5 mL of 4M HNO_3 (Fisher Scientific, ACS-pur). Samples were then analyzed with a gas chromatograph coupled to a cold-vapor fluorescence spectrometer (GC-CVAFS) (Tekran 2700, Tekran Instruments Corporation) with reference to US EPA Method 1630.

Other metal(loid)s

WB and serum. For other metal(loid)s, we performed the same digestion as for THg serum analysis. Samples were analyzed by ICP-MS/MS (8900 Triple Quadrupole, Agilent).

Tuna flesh. For other metal(loid)s, freeze-dried tuna samples were digested in 250 μL of HCl (OmniTrace Ultra, EMD) and 250 μL of ultrapure 5% HNO_3 (OmniTrace Ultra™, MilliporeSigma) for 3 h in a pressure steam sterilizer (#50X 25-quart electric sterilizer, All American). The digestion was completed with 250 μL of OPTIMA grade H_2O_2; then, ultrapure Milli-Q water (>18.2 MΩ/cm) (EMD Millipore) was added to reach a total volume of 10 mL. Samples were analyzed by ICP-MS/MS (8900 Triple Quadrupole, Agilent).

Arsenic speciation

WB and serum. Arsenic speciation was conducted on subsets of all blood samples. We selected six sampling times (t0, 90, 180, 300, 420, and 540 min) over the concentration-time

profile of serum (arterial and venous) and WB (arterial and venous) from pigs 1R and 1C (*n* = 48). Whole blood and serum were treated in the same manner. As detailed by an interlaboratory study on As speciation in whole blood (Ito et al., 2011), 200 µL was digested in a 5 mL methanol–ultrapure Milli-Q water solution (1:1). Samples were then homogenized using an ultrasonic bath (VWR Model 150 D) for 1 h and were centrifuged at 3000 × *g* for 15 min at 20 °C. Finally, the resulting supernatants were filtered through a Captiva EMR-lipid cartridge (Agilent) facilitated by a vacuum system. The water-soluble species in the aqueous phase were then injected into a HPLC-ICP-MS/MS.

Tuna flesh. Arsenic speciation was performed on ground freeze-dried tuna flesh following the protocol described in Taleshi et al. (2010). A chloroform–methanol solution (2:1) (>99.8 and 99.9%, respectively, Fisher Scientific) was used for the first fractionation. The resulting solution was filtered (Machery-Nagel, MN 85/70, 45 mm filter papers). After the evaporation of the filtrate, the oily phase was submitted to a second extraction using chloroform–methanol–ultrapure Milli-Q water solution (2:1:1). The chloroform phase was evaporated, and the oily substance containing fat-soluble As species was digested following the Quebec (Canada) environment ministry (MELCC) protocol MA. – Mét 1.2 (2000) and analyzed by ICP-MS/MS. The water-soluble species in the aqueous phase were then analyzed by HPLC-ICP-MS/MS.

Quality control for metal(oid) analyses

Intercalibration criteria followed that of the Canadian Association for Laboratory Accreditation (CALA). Various certified reference materials (CRM) were used for quality control and were submitted to the same digestion protocol as the samples. For WB and serum, we used Seronorm^{TM} Trace Elements Whole Blood L-2 and Serum L-1 certified materials (SERO, Norway) and obtained a mean recovery of 98 ± 0.8% (*n* = 28) for WB THg, 100 ± 3.4% (*n* = 7) for serum THg, 91 ± 3.5% (*n* = 13) for WB and serum MeHg combined, and 90 ± 17.4% (*n* = 34) for WB and serum for other metal(loid)s.

For tuna flesh and the PBET soluble fraction, we used TORT-2 (lobster hepatopancreas, National Research Council, Canada) as well as DORM-2 and DORM-3 (fish protein, National Research Council, Canada) reference materials. Mean recoveries for tuna flesh were 102 ± 2.8% (*n* = 23) for THg, 97 ± 6.5% for MeHg, and 92 ± 0.01% (*n* = 2) for Se and As. Finally, 101 ± 3.4% (*n* = 4) was measured for MeHg in the PBET soluble fraction. Detection limits of metal(loid) analyses are presented in Table S1.

Data handling and statistics

Venous concentrations of metal(loid)s recorded five minutes before the meal were used as basal levels (t0). The fraction (%) of intake metal(loid) in the blood compartment at time *t* was assessed as (Fournier et al., 2002; Kershaw et al., 1980)

$$(3)\ \frac{[M] \times whole\ body\ blood\ volume}{intake} \times 100,$$

where *[M]* describes the metal(loid) point concentration, *whole body blood volume* was estimated at 7% of BW (L/kg) (Ramasawmy et al., 2018; Wolfensohn & Lloyd, 2013), and metal(loid) *intake* corresponds to the quantity of the metal(loid) in tuna meal (µg) (Table 1). Mean pig BW was 56 ± 5.4 kg (CV = 9.6%) on the day of the experiment.

As described and validated by Kershaw et al. (1980), RBCs metal(loid) concentrations were calculated as

$$(4)\ Crbc = \frac{Cwb - \left(Cserum \times (1-h)\right)}{h},$$

where *Crbc*, *Cwb*, and *Cserum,* correspond to the RBCs, WB, and serum concentrations, respectively, and *h* is the hematocrit. In the case of negative *Crbc*, where *Cserum* > *Cwb*, *Crbc* was set to zero.

Kinetic assessment was done using the serum concentration-time profile and exploiting the following pharmacokinetic parameters: peak concentration (C_{max}) and time to peak concentration (T_{max}) were used as indirect metrics to characterize the rate of absorption, whereas

the systemic exposure was assessed through dose-normalized area-under-the-curve from 0 to 540 minutes post-meal ($AUC_{0\text{-}540}$), from the linear trapezoidal method (Chen et al., 2011; Cohen-Barak et al., 2017; Purves, 1992).

Metal(loid) levels as a function of time and treatment were treated with multiple linear regression models and using 'individual pig' as a random factor. The variance explained by fixed and random factors was assessed using the marginal (r^2m) and conditional (r^2c) r^2. A post hoc Tukey test was used when appropriate. Due to the non-normality of groups, the mean MeHg profiles from raw and cooked treatments were compared using nonparametric Wilcoxon tests. The comparison of the metal(loid) levels between the portal vein and the carotid artery was done using linear regression analysis when the residuals were normally distributed, and an analysis of the covariance (ANCOVA) was performed to compare the linear regression slope to a 1:1 slope. Outlier data were identified with a Cook's distance superior to 4/n (Bollen & Jackman, 1985). When the normality of residuals was not respected, the Kendall test was used. Methylmercury bioaccessibility results from raw and cooked treatments were also compared using nonparametric Wilcoxon tests because of the lack of equivariance between groups. The level of significance was set at $p \leq 0.05$.

Results

In vitro bioaccessibility of MeHg

During *in vitro* assays, gastrointestinal (GI: gastric + intestinal bioaccessibility) MeHg bioaccessibility from the raw treatment (n = 5) was 64 ± 3%, whereas we obtained a significantly lower result for the cooked treatment (n = 5) with 31 ± 2% (Fig. 1). We measured a mean gastric bioaccessibility of 41 ± 6% for the raw treatment (n = 5) and 8 ± 1% for the cooked treatment (n = 5), whereas the intestinal bioaccessibility was similar between treatments (23%). These results suggest that cooking decreased consumer exposure to MeHg by 33%.

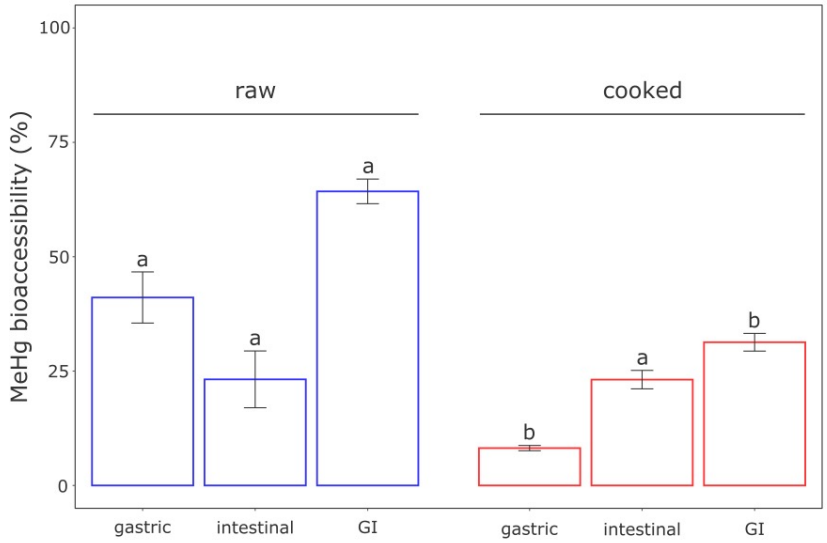

Figure 1. Methylmercury bioaccessibility (%) according to the digestive compartment assessed from raw and cooked tuna flesh (n = 5). GI: gastric plus intestinal bioaccessibility. Letters indicate a significant difference (Wilcoxon tests, $p \leq 0.05$) between treatments. Error bars present the standard deviation of the quintuplicates.

Effect of cooking on methylmercury, arsenic, and selenium kinetics in the pig digestive system

During the *in vivo* experiment, we followed venous serum MeHg concentrations in pigs as a function of time and treatment (Fig. 2). In contrast with the *in vitro* results, MeHg levels from

the serum of the raw and cooked treatments were not statistically different, with a mean (± SEM) MeHg concentration of 0.21 ± 0.12 µg/L and 0.27 ± 0.09 µg/L, for the raw and the cooked treatment respectively (multiple linear regression model; treatment effect, $p = 0.568$, $r^2m = 0.37$; $r^2c = 0.61$). Similar trends were observed for $AUC_{(0\text{-}540)}$, with no difference observed between treatments (Table 2). On the other hand, the cooking treatment influenced MeHg levels as a function of time (multiple linear regression model; time effect × treatment, $p = 0.031$, $r^2m = 0.39$; $r^2c = 0.64$), as illustrated by the mean concentrations as a function of time for both treatments (Fig. 2, All pigs panel).

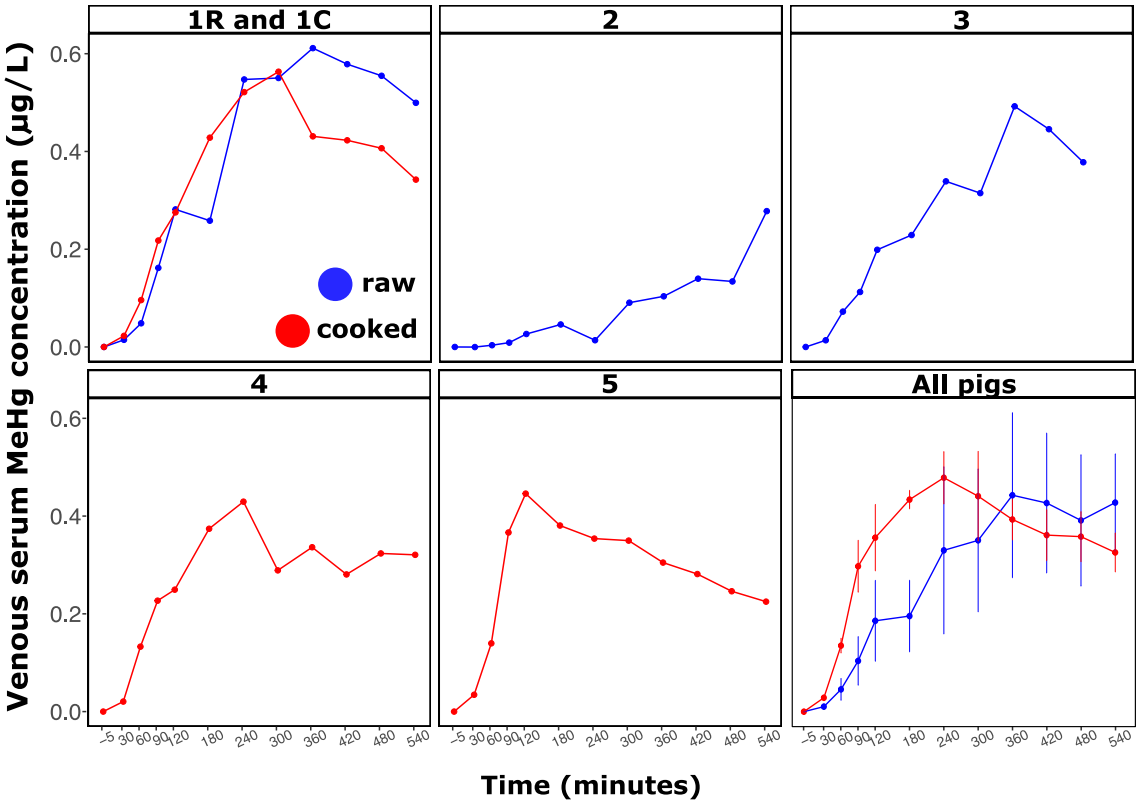

Figure 2. Venous serum MeHg concentration (µg/L) of five individuals during the 540 minutes post-consumption of a single raw (1R, 2, 3) or cooked tuna meal (1C, 4, 5). Values differed from zero (multiple linear regression model; time effect, $p < 0.05$) and were influenced by treatments (multiple linear regression model; time effect × treatment, $p < 0.05$). Panel "All pigs" present the average (± SEM) of venous serum MeHg concentration (µg/L) for raw ($n = 3$) and cooked ($n = 3$) tuna meals. Serum MeHg averages from raw and cooked treatments were similar (Wilcoxon test, $p > 0.05$). Preconsumption concentration was subtracted from each value.

The average consumption time for both treatments was 20 min. Increases in MeHg levels in serum (Fig. 2) and RBCs (Fig. S1) were observed within 30 min. All pigs, with the exception of Pig 2, reached their C_{max} within the recorded 540 min (T_{max} ranged from 120 to 360 min, Table 2). At C_{max}, the fraction of the total MeHg intake (see Equation 3) varied from 2.0 to 8.4%, 0.6 to 1.2%, and 1.6 to 7.4% in the WB, serum, and RBC compartments, respectively. These values were not influenced by the cooking treatment (multiple linear regression model; treatment effect, p = [0.083 - 0.404]; r^2m = [0.39 – 0.49]; r^2c = [0.60 – 0.67]). The blood MeHg profile suggests that the declining phase was not finished at 540 min post-meal. Pig 2 fed with raw fish did not reach C_{max} during the experiment. This pig was healthy the day of the experiment and interindividual variability is our only explanation regarding its MeHg level serum profile (Fig. 2).

Table 2. Details of the dose of MeHg ingested and MeHg changes in pig serum.

	Raw			Cooked		
Pig number	1R *	2	3	1C *	4	5
Preconsumption values (µg/L)	0.20	0.07	0.09	0.06	0.08	0.002
AUC $_{(0\text{-}540)}$ (µg x min/L/ µg)	1.7	0.3	1.8	1.5	1.2	1.1
C_{max} (t0 corrected) (µg /L)	0.6	0.3	0.5	0.6	0.4	0.5
T_{max} (min)	360	NA †	360	300	240	120
Intake (µg)	130	138	88	137	149	131
Dose (µg /kg bw)	2.5	2.4	1.7	2.6	2.3	2.3

* Pig 1 received both cooked and raw treatment a week apart.
† Pig did not reach T_{max}.

As mentioned above, treatments influenced MeHg levels in serum as a function of time, and this observation agreed with the observed difference between both treatments in terms of T_{max}. Pigs exposed to the cooked meal reached their MeHg C_{max} earlier than those fed with raw meals (Table 2). This treatment effect is supported further by the earlier C_{max} observed for the cooked treatment in Pig 1 (1C) compared with its C_{max} for the raw treatment (1R).

In addition to the treatment responses of the MeHg kinetic profiles, we also observed a generally large interindividual variability. Within the same treatment (raw or cooked), pigs showed markedly different metal(loid) serum profiles as a function of time (Figs. 2 and S2). For example, Pig 3 demonstrated MeHg and TAs oral bioavailability profiles that differed markedly

from those of Pigs 1R and 2, even though they all received similar doses of both metal(loid)s (Tables 2 and S2) (multiple linear regression model, pig effect, p <0.0001).

For TAs absorption kinetics, the response was very similar to that of MeHg, including the distinction observed for the Pig 2 profile (Fig. S2) and the early absorption recorded in the first 30 min post-meal. As for MeHg, As-serum levels were influenced by treatment as a function of time (multiple linear regression model, time effect × treatment, p = 0.049; r^2m = 0.04; r^2c = 0.48) and $AUC_{(0\text{-}540)}$ was similar between treatments (Table S2); however, T_{max} was not affected by cooking (Table S2). Before the fish meal, we did not detect any AsB in the blood of any pigs. In tuna meal and postprandial blood, AsB was the only detectable As species. In the pig's fish meal, AsB represented on average 73 ± 9% and 96 ± 5% of total As in raw and cooked treatments, respectively (Table 1). Average AsB recovery (AsB/TAs × 100) in blood for the raw treatment was 41 ± 16% (range 27–69%, n = 23 with one outlier excluded) and 63 ± 11% for the cooked treatment (range 45–95%, n = 24). Since AsB was the only As species in tuna meal, it can be used as a specific marker of the absorption of As from tuna meal. Indeed, AsB levels from WB and serum (n = 47) were highly correlated to TAs values (linear regression, r^2 = 0.74, p << 0.001). After the meal, AsB absorption profiles were similar to those of TAs, except that AsB concentrations were lower (Fig. S3).

Compared with MeHg and TAs, TSe oral bioavailability showed no clear trends (Fig. S4). According to the mixed linear model, TSe levels did not differ from zero (multiple linear regression model; time effect, p = 0.651; r^2m = 0.17; r^2c = 0.29) and were not influenced by treatments (multiple linear regression model; time effect × treatment, p = 0.171; r^2m = 0.19; r^2c = 0.30). These results suggest that tuna meal had no impact on the TSe blood levels of pigs.

Stability of methylmercury, arsenic, and selenium distribution between blood compartments

Global venous data were plotted against the arterial data (Fig. 3) to evaluate the stability of metal(loid)s within a given blood compartment. In cases where venous and arterial measures

tightly fitted the 1:1 line, it would suggest a rapid equilibrium with limited biodistribution of the metal(loid)s between blood compartments (RBCs to serum and vice versa), and from the blood toward extravascular tissues. As illustrated in Fig. 3, we obtained a strong correlation for MeHg in the RBC fraction (linear regression; r^2 = 0.98, p << 0.001) for which the linear regression slope (solid line: slope; gray: confidence interval = 0.95) did not differ from the 1:1 slope (dotted line; ANCOVA, p = 0.07). Data for MeHg in the serum fraction were not normally distributed (Shapiro test, p = 0.07); however, according to the Kendall test, venous and arterial data were well correlated (τ = 0.88, p << 0.001). Another strong correlation was observed for As in the serum fraction (linear regression; r^2 = 0.98, p << 0.001), but the regression slope differed from a 1:1 relationship (ANCOVA, p << 0.001). In contrast with serum, As in the RBC fraction displayed a significant but weak correlation (linear regression; r^2 = 0.32, p << 0.001), with many data falling beyond the 95% confidence interval. This weaker correlation is unlikely due to analytical constraints because the limit of detection for As was 0.71 ng/L (Table S1). Finally, for Se in serum (linear regression; r^2 = 0.86, p << 0.001) and RBC (linear regression; r^2 = 0.70, p << 0.001), the results were similar to those of Hg and As, showing a moderate correlation. Interestingly, extreme Se values observed in the serum all came from Pig 2 (Fig. S5). By excluding the data of Pig 2 in the statistical analysis, we obtained a weaker correlation between the venous and the arterial Se levels (data not shown: linear regression; r^2 = 0.22, p << 0.001, y = 0.48x + 75).

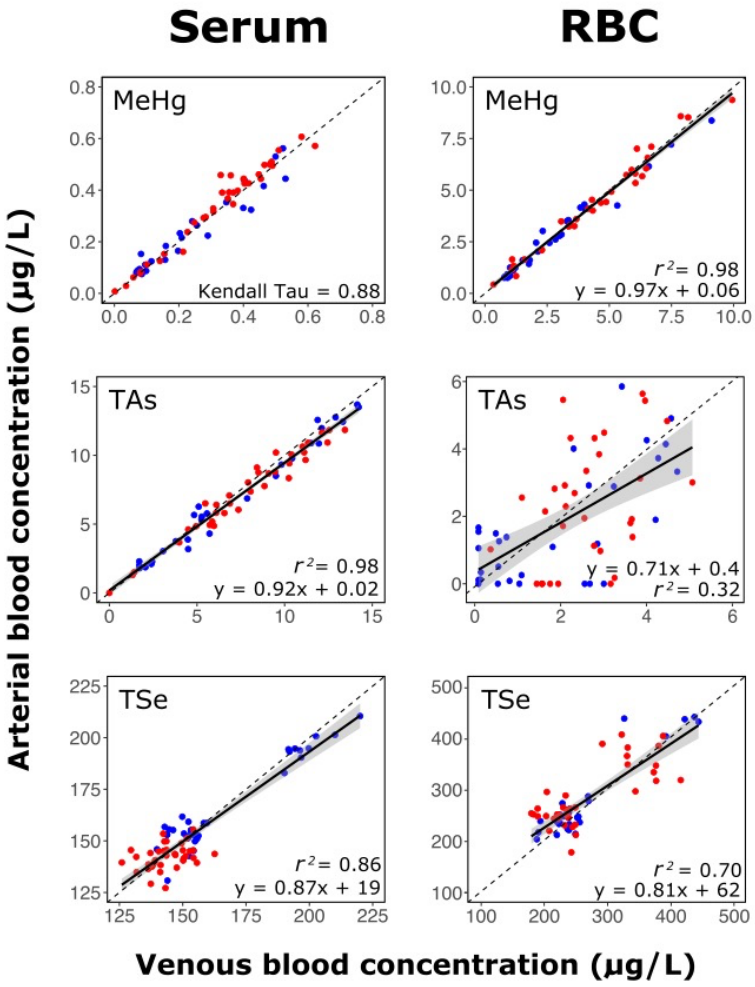

Figure 3. Relationship between the concentration of metal(loid)s in venous and arterial blood for all pigs and sampling times (*n* = 72). Blue points represent raw tuna meal, and red points represent cooked tuna meal. The dotted line shows the 1:1 slope, and the solid line signifies a significant regression slope with the gray area illustrating the confidence interval at 0.95.

Cooking effect on metal(loid) distribution between blood compartments

We investigated further whether cooking affects MeHg blood partitioning. Fig. 4 shows the proportion of metal(loid)s when the blood compartment volume was considered. RBCs constitute the main reservoir of MeHg and THg, representing, respectively, 81 ± 4% (*n* = 34) and

84 ± 5% (*n* = 35) of the total load in the blood of pigs fed raw meat and 86 ± 3% and 79 ± 13% (*n* = 36 for both), respectively, in pigs fed cooked tuna meal. Contrasting results were found for Se and As. Indeed, they were not preferentially distributed in the RBC fraction, with 38 ± 4% and 45 ± 7% for Se and 9 ± 7% and 15 ± 9% for As in pigs fed raw and cooked tuna, respectively. For THg, Se, and As, but not for MeHg (Fig. 4), the distribution between blood fractions varied as a function of time, without any clear trend, as for other elements (Fig. S6) (multiple linear regression model; time effect, p = [0.005 - 0.024]; r^2m = [0.01 – 0.12]; r^2c = [0.22 – 0.91]). For all studied metal(loid)s, these proportions did not differ statistically between treatments (multiple linear regression model; treatment effect, p = [0.206 - 0.909]; r^2m = [0.01 – 0.12]; r^2c = [0.22 – 0.91]) (Figs. 4 and S6). When comparing concentrations instead of loads, RBCs contained 11 to 15 times more MeHg, 2 times more Se, and 3 times less As than the serum (Table S3).

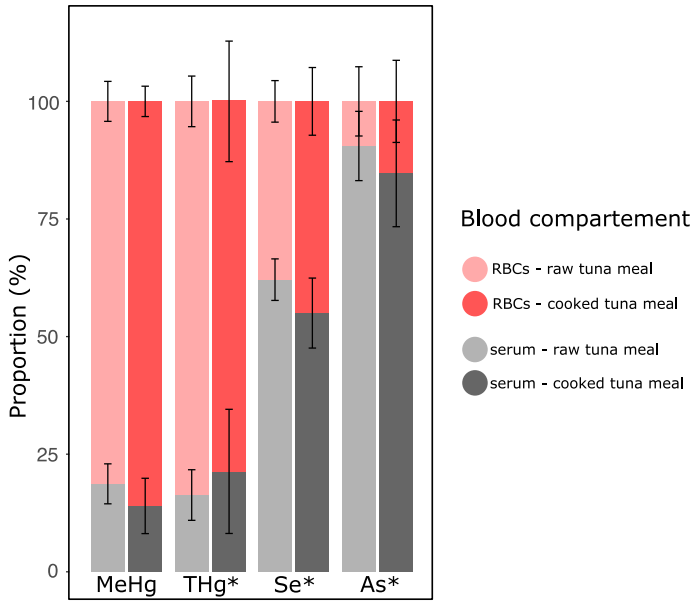

Figure 4. Mean (± SD) proportion (%) of the total quantity of different elements distributed between RBCs and the serum fraction over 540 min postprandial (*n* = 34 and 35 for the raw tuna meal, and *n* = 36 for the cooked tuna meal treatment). Total quantity of each blood compartment was adjusted to their respective volumes (RBC concentration was adjusted as a function of hematocrit ($[M_{RBC}]$ × blood volume × hematocrit) and serum concentration as a function of serum volume ($[M_{serum}]$ × blood volume × (1 − hematocrit)). * indicates that mean proportion varies as a function of time (multiple linear regression model; time effect, $p < 0.05$). No difference was measured between the raw and cooked treatments (multiple linear regression model; treatment effect, $p > 0.05$).

Discussion

Differing effects of cooking between in vitro and in vivo model results

Overall, cooking decreased *in vitro* MeHg bioaccessibility by a factor of 2, with a GI bioaccessibility of 64% and 31% for the raw and cooked tuna, respectively (Fig. 1). Other digestion models have similarly established that cooking decreases Hg and MeHg solubility within GI fluids (Afonso et al., 2018; He et al., 2010; Jadán Piedra et al., 2016; Torres-Escribano et al., 2010). A decrease of fish MeHg bioaccessibility following heat treatment is usually explained by protein oxidation produced by the heat treatment through various cooking methods, leading to the modification of the amino acids (Davies, 1987; Stadtman, 1993). Ultimately, protein polymerization and aggregation occur (Santé-Lhoutellier et al., 2008), which limit enzymatic protein degradation and digestibility (Grune et al., 2004). Because MeHg is bound to protein (Nong et al., 2020), cooking could decrease the MeHg release from fish muscle into GI fluids.

The effect of cooking on bioaccessibility likely results from processes occurring during the gastric phase, as no difference was observed for the intestinal phase. Indeed, we observed five times lower gastric MeHg bioaccessibility for the cooked treatments, which can be linked to the reduced efficacy of the gastric pepsin with the digestion of cooked fish muscle. Concordant results have been found for the *in vitro* cooking effect on myofibrillar protein digestibility (Santé-Lhoutellier et al., 2008). In that study, myofibrillar proteins were isolated from bovine meat (these proteins are also present in fish muscle; Venugopal & Shahidi, 1996). After 5 min of cooking at 100 °C in a dry bath, the proteolysis rate for gastric pepsin decreased by 42% relative to the control (Santé-Lhoutellier et al., 2008), suggesting a non-negligible effect of cooking on *in vitro* gastric protein digestibility.

In contrast to these above-mentioned studies, we found that cooking did not affect the postprandial MeHg concentrations in the serum and RBCs of pigs fed with fish. The principal observed effect in the *in vivo* experiment was a faster uptake in the cooked treatment. Similar *in vivo* report is consistent with our observations. Indeed, a study using ileostomized humans, in which the aim was to evaluate the effect of cooking temperature in a steam oven on ^{15}N-labeled bovine meat digestibility—assessed by the appearance of ^{15}N-labeled in the ileum—found no

statistical difference between barely cooked meat (55 °C for 5 min, *n* = 8) and highly cooked meat (90 °C for 30 min, *n* = 8) (Oberli et al., 2015). Comparing this human-based study to our pig-based one is relevant in the context where a pig's digestive system is comparable to the human one (Rees et al., 2009). Even from this perspective, to our knowledge, there is no other relevant literature addressing the effects of cooking on *in vivo* digestibility/absorption of metal(loid)s.

According to the *in vivo* results, cooking does not significantly modify MeHg oral bioavailability. We could explain our results by a potential intestinal membrane competition between MeHg and amino acids released from fish muscle. Indeed, in fish muscle, MeHg is believed to bind predominantly to cysteine (Harris et al., 2003), and the MeHg-Cys complexes can cross the intestinal membrane via $B^{0,+}$ amino acid transporters because of the molecular mimicry of the amino acid methionine (Ballatori, 2002; Broer, 2008). Moreover, Vázquez et al. (2014) found that MeHg transport across the intestinal barrier is a saturable mechanism. That said, a higher level of free amino acids in the intestinal lumen (in the case of a high bioaccessibility such as for the raw treatment) may reduce the absorption of MeHg by means of transport competition (Vázquez et al., 2015). As we observed a higher *in vitro* MeHg solubility and a potentially higher solubilization of amino acids in the raw treatment, this could have limited intestinal MeHg absorption, leading to a similar MeHg oral bioavailability in pig blood. Our study suggests that not all the soluble MeHg is absorbed by the intestinal barrier. We have not found any studies focused on the impact of cooking on the MeHg-Cys complex.

Together, our results suggest that the MeHg *in vitro* bioaccessibility model used in this study is not a direct surrogate for *in vivo* models. *In vitro* models remain useful complementary tools to test different gastric and intestinal processes affecting metal bioaccessibility from diet under controlled conditions. They also have the advantage of being non-invasive and highly replicable, but are not optimized to directly predict mammalian MeHg exposure. To the best of our knowledge, this is the first direct comparison of raw and cooked tuna meat using *in vitro* bioaccessibility combined to *in vivo* bioavailability approaches for MeHg.

Cooking does not influence the MeHg fraction in blood

Since Hg is slowly excreted from organisms, blood can be used as a short-term exposure biomarker (Bartell et al., 2000). Maximal MeHg blood levels (C_{max} without subtracting the concentration from t0) observed in our pig WB were considerably under the acceptable Canadian general population guidance (< 20 µg/L) (Health Canada, 2004), varying from 1.1 to 3.8 µg/L. The fraction of the total MeHg intake in the WB compartment remained low. At its highest proportions, the MeHg fraction varied from 2.0 to 8.4% (see Equation 3) and was not affected by cooking. This range or MeHg fraction is generally consistent with what has been reported for other species. For instance, at a steady state, the MeHg body burden found in human WB for persons having been exposed through fish consumption is estimated at 5.6% to 5.9% (Kershaw et al., 1980; Sherlock et al., 1984). Values of 5.5% were found in common loon (*Gavia immer*) chicks (35 days of oral or intravenous exposure) and 10.4% in Cory's shearwaters (*Calonectris diomedea*) (multiple months of oral exposure), both exposed to MeHg chloride (Fournier et al., 2002; Monteiro & Furness, 2001). The wide range of values observed in our pigs, relative to previously published values, could be due to interspecific variation in terms of MeHg blood affinity and stability (Naganuma et al., 1980), in addition to MeHg not being at a steady state.

Cooking affects methylmercury and arsenic kinetics of oral bioavailability in blood

Cooking did not change MeHg levels in blood, relative to levels in the blood from the raw tuna test; we nonetheless investigated further the potential effect of heat treatment on MeHg kinetics of oral bioavailability. Knowing that cooking can modify protein configuration, it may have an effect on its digestibility rate.

Cooking affected MeHg levels as a function of time (multiple linear regression model; time effect × treatment, $p = 0.031$, $r^2m = 0.39$; $r^2c = 0.64$). This observation was also supported by the T_{max} results (Table 2), which demonstrated an earlier C_{max} for pigs fed with cooked tuna meal. This phenomenon was also observed in Pig 1, which received both types of treatments (1R and 1C), suggesting that the observed variance would not only be related to interindividual variability but

also due to meal properties. Studies on ileostomized humans illustrate a faster ^{15}N-labeled uptake in the ileum for a highly cooked bovine meat than for a minimally cooked meat (Oberli et al., 2015). Changes in tuna meal viscosity by cooking treatment could be responsible for the observed T_{max}. Indeed, gastric emptying time is negatively correlated with meal viscosity (Ehrlein & Pröve, 1982), and cooking decreases the viscosity (Liu et al., 1982). The earlier T_{max} observed for the cooked meal could therefore be related to a lower meal viscosity and a faster gastric emptying, relative to the raw treatment.

Mean gastric emptying time and chyme transit time in the small intestine are 3-4h each, resulting in 8 hours (Davis et al., 2001; DeSesso & Jacobson, 2001; Strathe et al., 2008). Consequently, our experimental design was set at 9 hours (540 min) of blood sampling, a time period deemed sufficient to record the full MeHg bioavailability profile. However, according to Fig. 2, a longer monitoring period would have been preferable, although difficult to perform under the logistical constraints of the invasive surgery.

In our study, AsB was the only As species detected in tuna meal and blood. This As species is known to be readily absorbed (Tam et al., 1982); however, to the best of our knowledge, the effect of cooking on its absorption has yet to be studied. The kinetics of oral bioavailability for As in serum were altered by cooking. Because the AsB profile was similar to that of TAs, and AsB values correlated well with TAs, it is likely that cooking would also influence the AsB kinetics of oral bioavailability. Lehmann et al. (2001) studying blood AsB levels after a single fish meal (cooked plaice filet) in 14 women, observed a half-life distribution of 7.1 h and a half-life elimination of 63 h. This agrees well with a previous study where human volunteers (n = 15) were fed with a single meal of cooked flounder. After eight days, the subjects had 76% of the AsB doses in urine and 0.33% in feces (Tam et al., 1982). The authors suggest that the small proportion of AsB in the feces relates to AsB being readily absorbed and excreted without any metabolic change. This fast elimination rate could be related to the As distribution found mostly in the serum fraction (Fig. 4), thereby implying a higher bioavailability for a biodistribution toward the elimination pathway.

Tuna meal did not alter TSe serum levels, as the values did not differ from zero (multiple linear regression model; time effect, $p = 0.651$; $r^2m = 0.17$; $r^2c = 0.29$) (Fig. S4). Being an essential element (Kurokawa & Berry, 2013) that is normally well absorbed (Fairweather-Tait et al., 2010), our results could be related to the tight homeostasis and intense metabolic Se requirements for intestinal tissue as proposed by Dalto & Matte (2020). Furthermore, once absorbed, this metalloid may be incorporated into essential selenoproteins (Jotty et al., 2013), such as selenoprotein P (SelP) which acts as a major reservoir of Se, stored in the liver (Jotty et al., 2013). Hence, it was anticipated that such levels of Se from single tuna meals would not significantly alter Se levels in serum.

Partitioning of MeHg in blood compartments differed for As and Se

Tracking the MeHg distribution ratio between RBCs and serum is important because it could influence the distribution of MeHg in tissues and its toxicity (Carrier et al., 2001). To exit the vascular system, MeHg must be distributed in the serum fraction (Thomas & Smith, 1982), which may result in a delay. The European Food Safety Authority has proposed that the delay of the entry of MeHg into the brain could be the result of the binding of MeHg to RBCs (European Food Safety Authority, 2012).

Methylmercury was chemically stable over time in both blood compartments, without being affected by the cooking treatment and was largely distributed in the RBC fraction (Fig. 4). Since no study has assessed the effect of cooking on MeHg complexes in fish muscle, gastrointestinal juices and blood of consumers, this is a relevant result suggesting that speciation of MeHg from cooked or raw muscle is similar, resulting in a comparable metabolism when absorbed.

In blood, MeHg can form complexes mainly with sulfhydryl group–rich (-SH) biomolecules, such as hemoglobin, albumin, cysteine, and glutathione (Naganuma & Imura, 1979; Naganuma et al., 1980), as well as with selenol (-SeH) (Wang et al., 2012). The mobility of MeHg is attributed to the rapid exchange reaction between MeHg-bound ligands (Nogara et al., 2019). An *in vitro* study using human RBCs demonstrated that the exchange of MeHg between glutathione molecules is

less than 0.01 seconds (Rabenstein & Isab, 1982). However, this study used particularly high MeHg levels, which could have influenced their findings (Nogara et al., 2019). In bottlenose dolphins (*Tursiops truncatus*), RBCs had greater concentrations of the -SH group than in plasma (11,730 ± 587 µM vs. 425 ± 3 µM, respectively) (Ancora et al., 2002), which could be explained by the fact that hemoglobin found in RBCs is thiol-rich (Wang et al., 2012). Indeed, in the bottlenose dolphin study, the authors observed a direct relationship between -SH group and MeHg bioaccumulation in blood compartments, suggesting that an asymmetric -SH distribution between the plasma and RBCs is responsible for the MeHg distribution (Ancora et al., 2002).

Furthermore, the 1:1 relationship between the venous and arterial RBC values implies that MeHg levels measured immediately after intestinal absorption are very similar to those measured after the blood had passed through the hepatic, pulmonary, and cardiac systems. This suggests that the biodistribution outside of the circulatory system takes longer than 540 minutes. These observations are consistent with the observed delays between food consumption and biodistribution in humans (European Food Safety Authority, 2012).

Arsenic and Se were likely more mobile and transferred more quickly to extra-vascular tissues than MeHg in the blood system (Fig. 3). This difference could explain the observed variation of the metalloid proportions found in the RBC fraction as a function of time in Fig. 4 (multiple linear regression model; time effect, p = [0.005 - 0.024]; r^2m = [0.01 – 0.12]; r^2c = [0.22 – 0.91]). Specifically, with respect to As in the RBC venous concentrations were generally greater than the arterial levels, suggesting potential As exchange from RBCs to the serum compartment and subsequent extravascular biodistribution between the portal vein and the carotid artery. It cannot be ruled out, however, that As from dietary treatments would be absorbed continuously by the intestinal wall during the digestive processes that lasts for approximately four hours. This dietary input would possibly result in higher As levels in the portal vein compared with the carotid artery.

In our study, AsB was the only As species detected in tuna meal (Table 1) and postprandial blood, and it was preferentially found in the serum fraction (Fig. S3). AsB in human serum has been found unbound, such as its structural analog glycine betaine (Pei & Gailer, 2009) and is very

stable and not inclined to be degraded or metabolized (Taylor et al., 2016). It would therefore be unlikely that AsB would decrease the potential antagonistic effect of Se toward MeHg in blood. Metabolism of that As species in blood remains unknown (Taylor et al., 2016).

For Se, Fig. 3 suggests a biodistribution of Se toward extravascular tissue or exchange between blood compartments. Selenium partitioning between blood compartments depends highly on its speciation. In human plasma, three major species have been identified: SelP, glutathione peroxidase, and the selenoalbumin complexes (Achouba et al., 2016). Achouba et al. (2016) found that in plasma, SelP accounted for 52% of total Se and that up to 50% of Hg was associated with SelP. Another study proposed the formation of the MeHg-Se-SelP complex in rat plasma, which reduced Hg plasma bioavailability and facilitated Hg excretion (Liu et al., 2018). In RBCs, Se may be found as selenoneine, known to bind to hemoglobin and prevent Fe oxidation (Little et al., 2019). RBCs collected from Nunavik Inuit, a Canadian population exposed to high levels of selenoneine through beluga consumption, up to 92% of Se was found to be selenoneine (mean of 26%, n = 858) (Little et al., 2019). Another study found that selenoneine was the dominant Se species in pig kidneys as well as in the blood and skeletal muscle of tuna (Yamashita & Yamashita, 2010). Selenoneine is of interest in studies of MeHg because selenoneine can promote MeHg demethylation and excretion in zebra fish embryos (Yamashita et al., 2013). Therefore, MeHg in the RBC fraction of pig's blood could be bound to the selenoneine from tuna muscle and, as speculated by Achouba et al. (2019), could convert MeHg into an insoluble (nontoxic) mercury selenide (HgSe) and contribute to its excretion. Hence, Se is highly involved in multiple mechanisms, which could have enhanced its mobility between blood compartments.

Conclusion

Our study demonstrates that MeHg bioaccessibility based on *in vitro* models should be used as a complementary tool to *in vivo* studies and not as a substitute. While previous studies suggested using direct bioaccessibility results in risk assessment calculation, our results highlight

that *in vitro* digestion model may lack representativeness. The direct use of *in vitro* bioaccessibility model to inform risk assessment of MeHg exposure from fish consumption should be revisited.

Indeed, in this paper we demonstrated that cooking tuna meal decreased the *in vitro* MeHg bioaccessibility, while it did not decrease the *in vivo* oral bioavailability of MeHg. Our results suggest that not all of the soluble, bioaccessible fraction of MeHg will be further absorbed by the intestinal wall and that potential competition during membrane transport could occur with amino acids. To strengthen this hypothesis, however, more research is required that focuses on the impact of cooking on the MeHg-Cys complex.

To our knowledge, ours is the first study to assess the impact of cooking on MeHg, As, and Se nutritional metabolism and distribution in blood using the pigs as an experimental model for humans. Our results show that MeHg from cooked tuna meal was absorbed faster than raw tuna, despite the observed interindividual variability. Once in the blood compartment, MeHg was stable for at least 540 min postprandial, in contrast to As and Se for which their distribution between blood fractions varied as a function of time.

Finally, AsB was the only As species detected in tuna meal and pig's blood, whereas the toxic inorganic As species (As^{3+}) was under the limit of detection. More in-depth evaluation of the interactions between MeHg, Se and As should be conducted in blood and other tissues where these elements bioaccumulate, following a long-term exposure of raw and cooked fish meal. These studies could rely on metallomic tools such as liquid chromatography coupled to inductively coupled plasma mass spectrometry (Shi & Chance, 2008). This would increase our knowledge towards the mechanism of detoxication of MeHg coming from raw and cooked fish meal and see if cooking affects the behavior and the toxicity of MeHg.

Acknowledgements

The author thanks the animal care team under the supervision of M. Turcotte, Isabelle Audet for the technical assistance during surgeries and samplings, and Dominic Bélanger, Maria Chrifi Alaoui and Kathy St-Fort for assistance in the laboratory. Research was funded through the

Canada Research Chair program (MA), a NSERC Discovery grant (MA, MR), a CRD NSERC/Hydro-Quebec grant (MA) and an NSERC CREATE Ecolac stipend to TC; the animal phase of this study was supported by Agriculture and Agri-Food Canada core budget.

Supplementary information

Supplementary figures

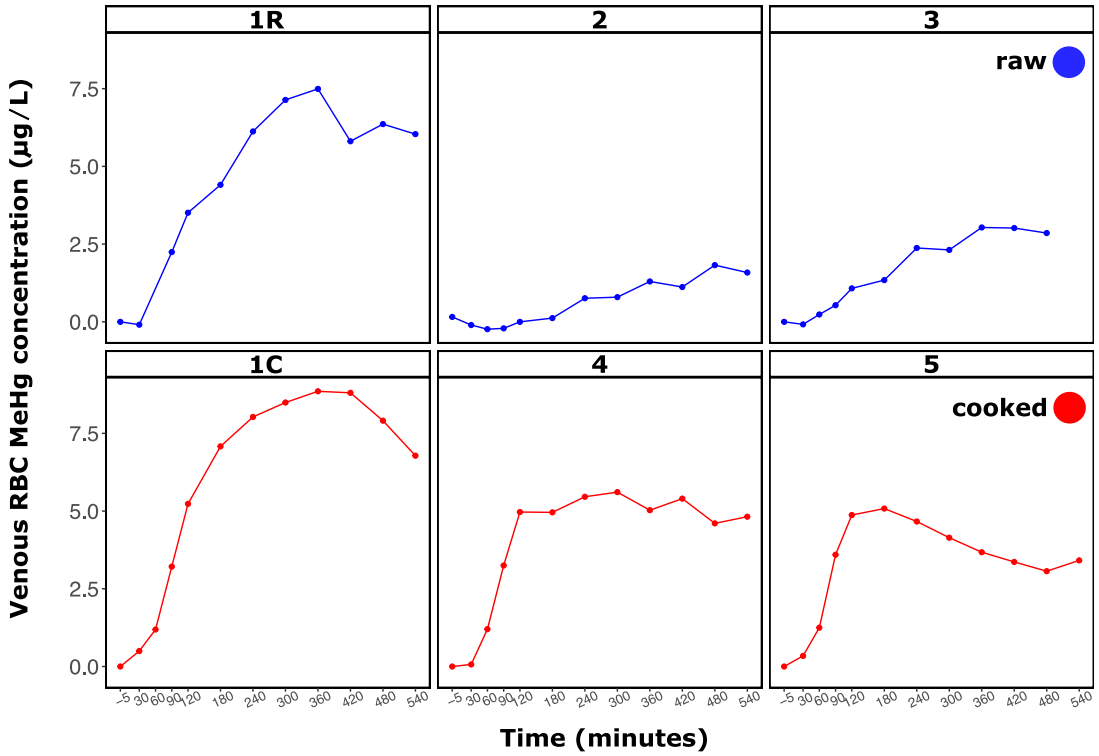

Figure S1. Venous RBC MeHg concentration (µg/L) of five individuals within 540 minutes following consumption of a single raw (1R, 2 and 3) and cooked tuna (1C, 4 and 5) meal. Preconsumption concentration was subtracted from each presented value. Values were different from zero (multiple linear regression model; time effect, $p < 0.0001$; $r^2m = 0.40$; $r^2c = 0.69$), were not influenced by treatments (multiple linear regression model; treatment effect, $p = 0.217$; $r^2m = 0.40$; $r^2c = 0.69$) and no interaction between time and treatments (multiple linear regression model; time effect × treatment, $p = 0.521$; $r^2m = 0.40$; $r^2c = 0.69$) was measured.

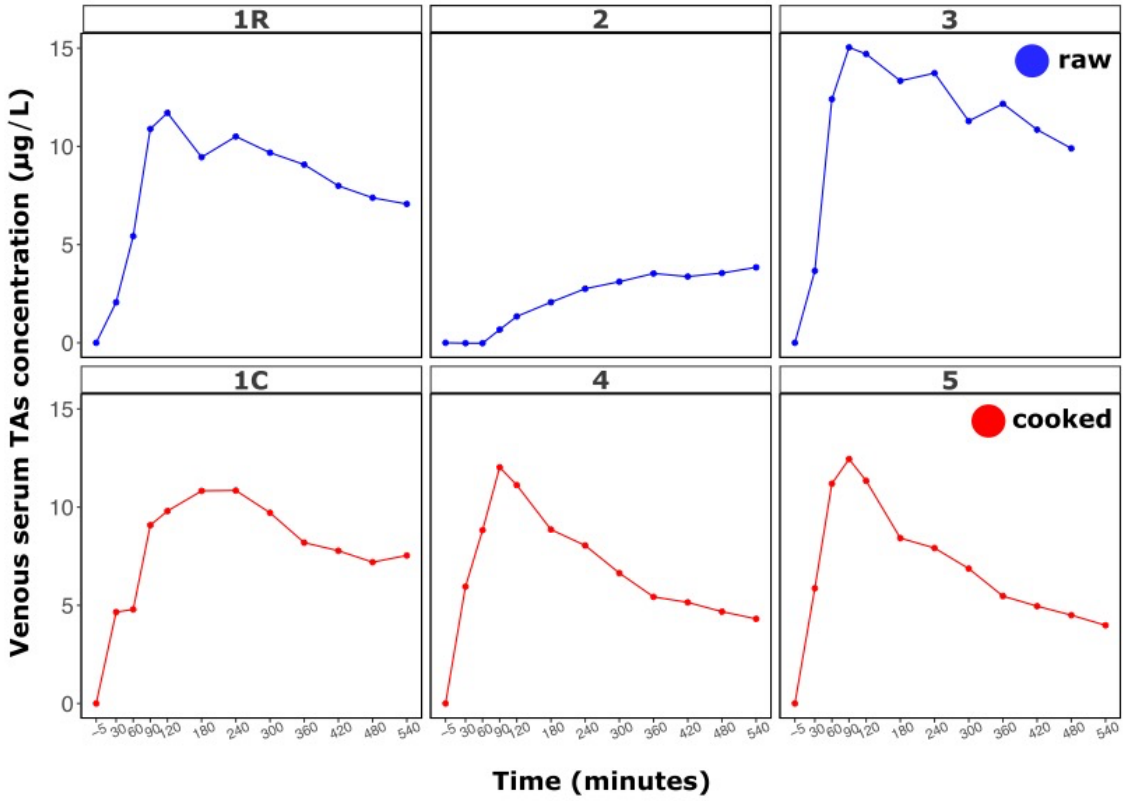

Figure S2. Venous serum TAs concentration (µg/L) of five individuals within 540 minutes following consumption of a single raw (1R, 2 and 3) and cooked (1C, 4 and 5) tuna meal. Preconsumption concentration was subtracted from each presented value. Values from raw treatment as function of time were different from zero (multiple linear regression model; time effect, $p = 0.021$; $r^2m = 0.06$; $r^2c = 0.67$), in the contrary of cooked treatment data (multiple linear regression model; time effect, $p = 0.659$; $r^2m = 0.01$; $r^2c = 0.01$). Treatment influenced the values as function of time (multiple linear regression model; time effect × treatment, $p = 0.049$; $r^2m = 0.04$; $r^2c = 0.48$).

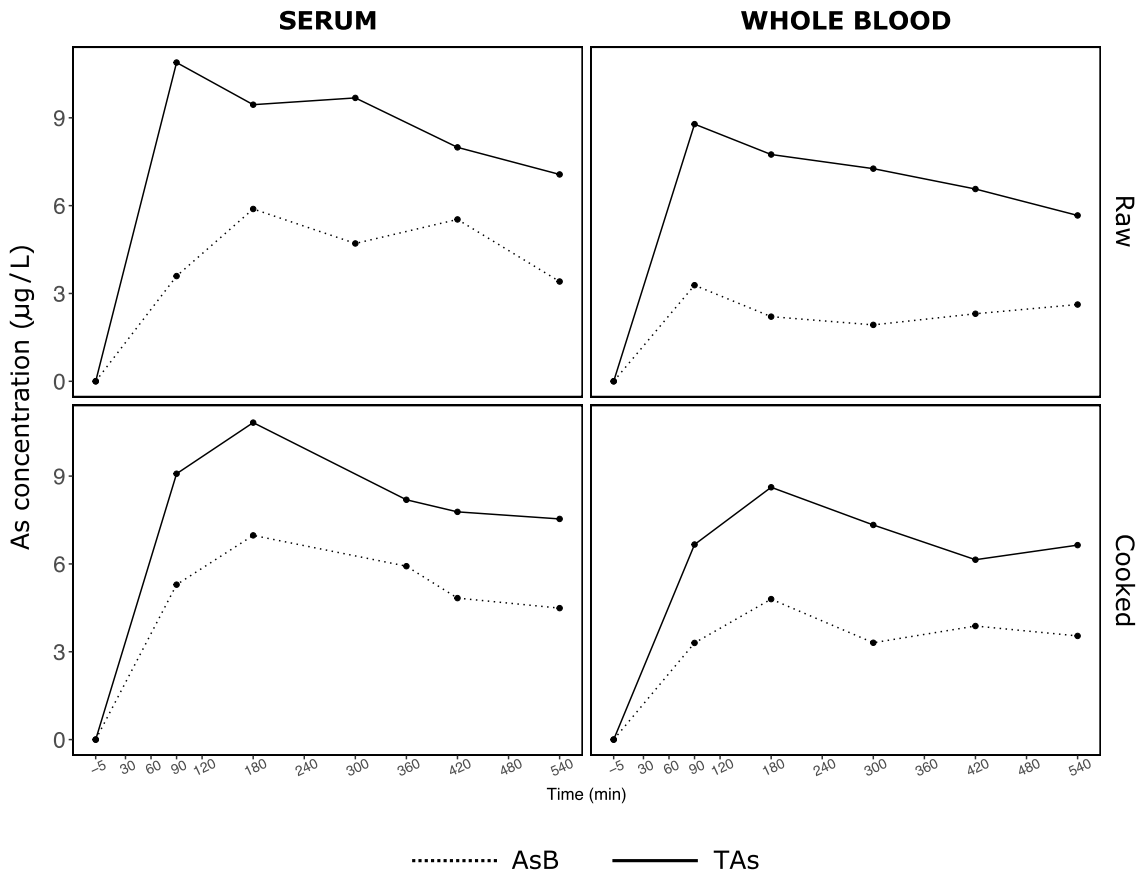

Fig. S3. Venous serum TAs and AsB concentration (µg/L) as function of time and treatment of one individual blood profile (1 single pig) within 540 minutes following consumption of a single raw (1R) and cooked (1C) tuna meal. Raw and cooked meal were spaced by one week. Preconsumption concentration was subtracted from TAs presented value. AsB preconsumption value was not detectable.

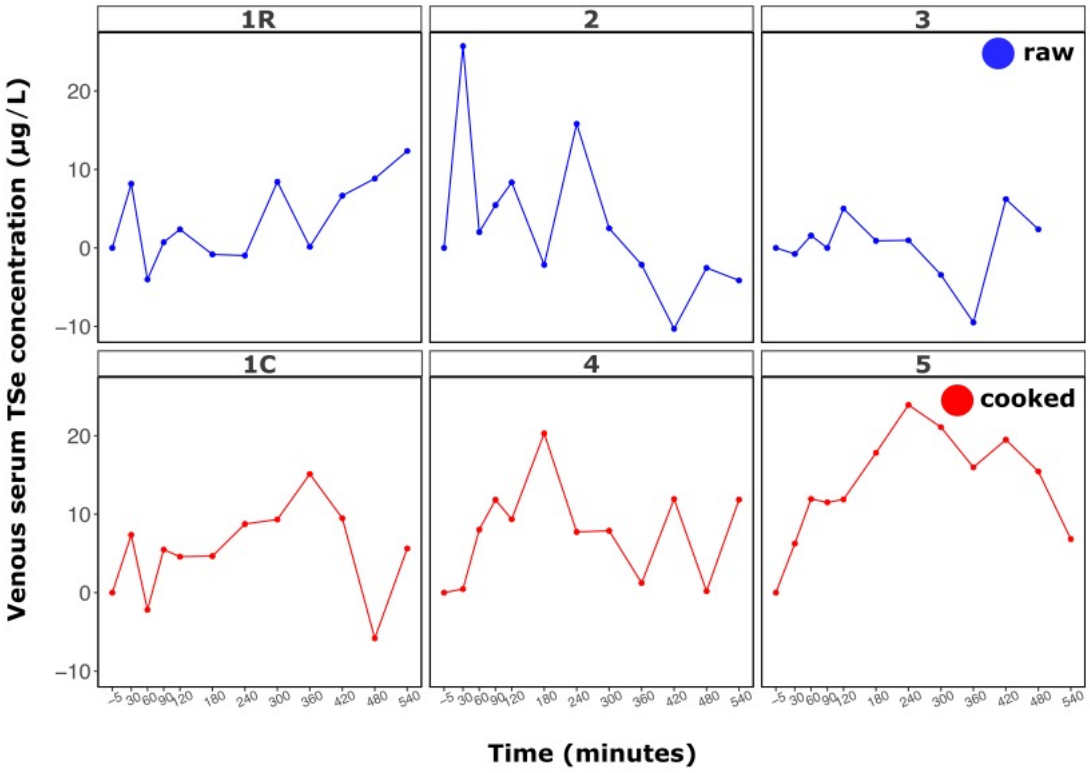

Figure S4. Venous serum TSe concentration (µg/L) of five individuals within 540 minutes following consumption of a single raw (1R, 2 and 3) and cooked (1C, 4 and 5) tuna meal. Preconsumption concentration was subtracted from each presented value. Values were not different from zero (multiple linear regression model; time effect, $p = 0.651$; $r^2m = 0.17$; $r^2c = 0.29$) and were not influenced by treatments (multiple linear regression model; time effect × treatment, $p = 0.171$; $r^2m = 0.19$; $r^2c = 0.30$).

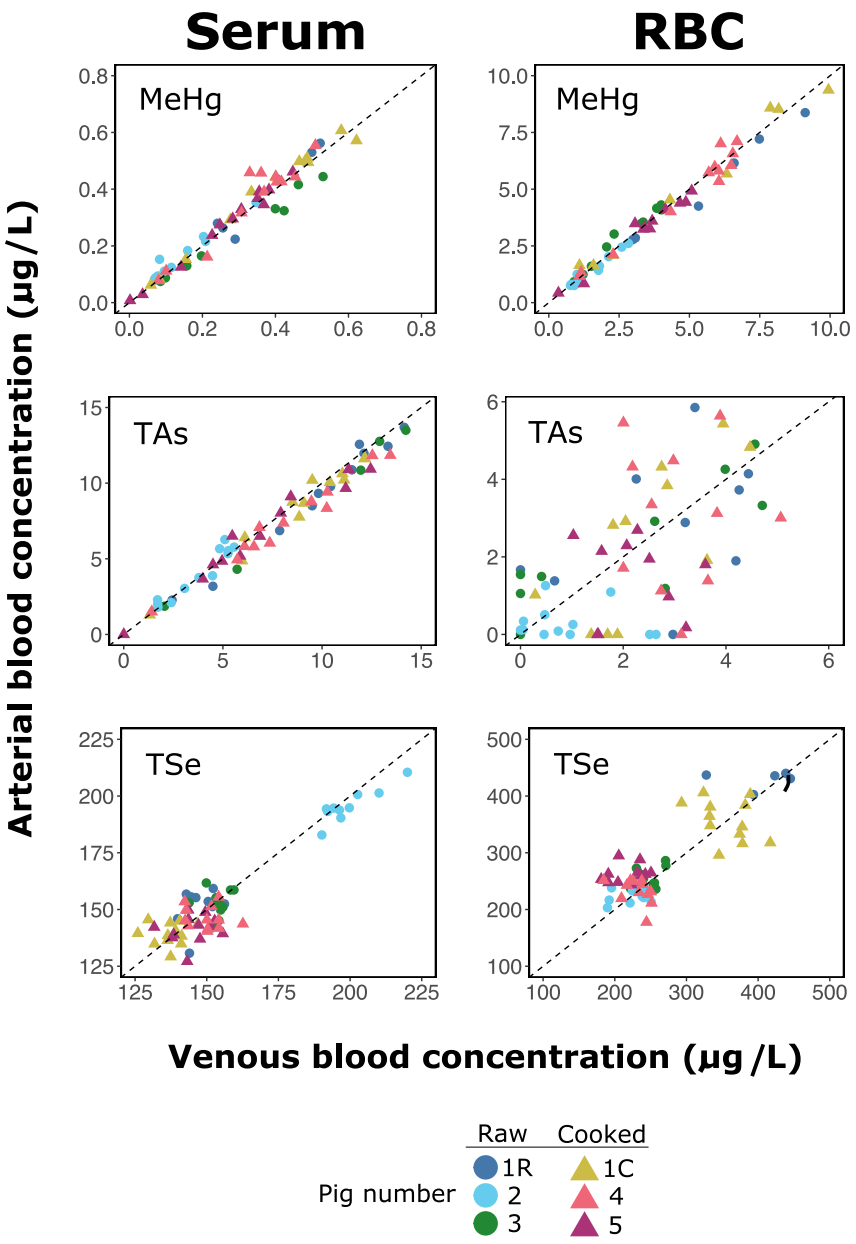

Figure S5. Relationship between the concentration of metal(loid)s in venous and arterial blood for all pigs and sampling times (*n* = 72). The dotted line shows the 1:1 slope.

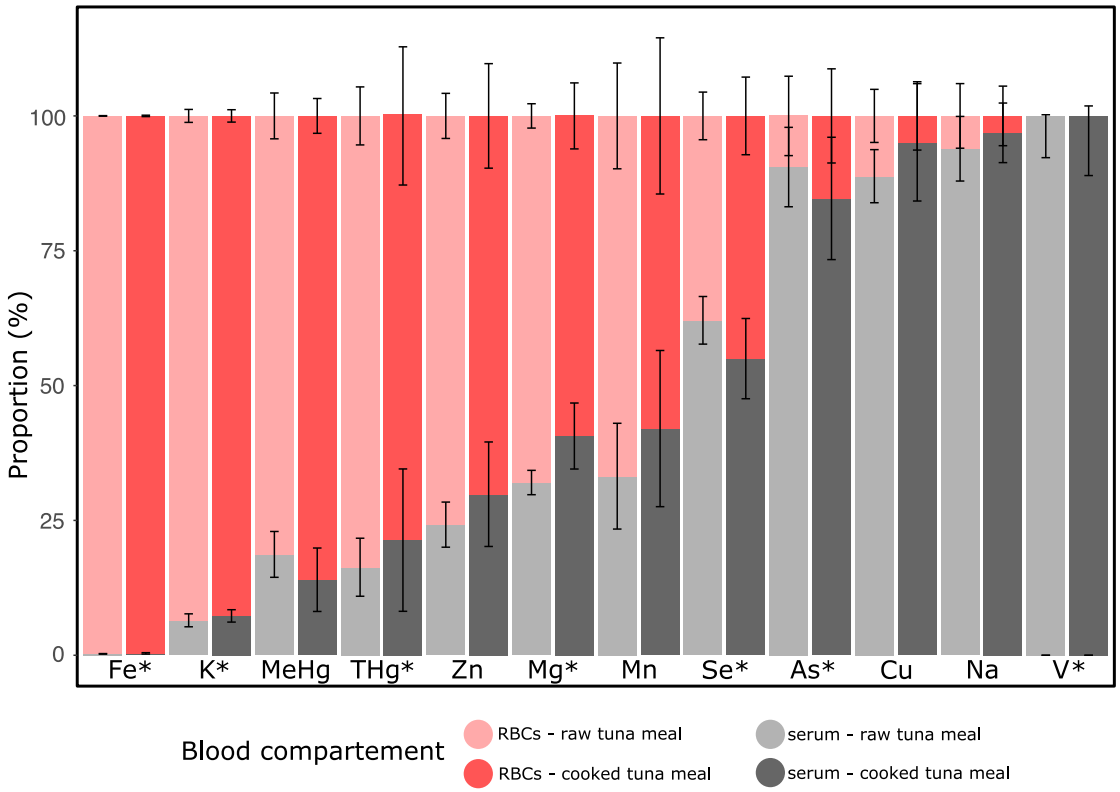

Figure S6. Mean (± SD) proportion (%) of the total quantity of different elements distributed between RBCs and the serum fraction over 540 min postprandial (n = 34 and 35 for the raw tuna meal, and n = 36 for the cooked tuna meal treatment). Total quantity of each blood compartment was adjusted to their respective volumes (RBC concentration was adjusted as a function of hematocrit ($[M_{RBC}] \times$ blood volume × hematocrit) and serum concentration as a function of serum volume ($[M_{serum}] \times$ blood volume × (1 − hematocrit)). * indicates that mean proportion varies as a function of time (multiple linear regression model; time effect, $p < 0.05$). No difference was measured between the raw and cooked treatments (multiple linear regression model; treatment effect, $p > 0.05$).

Supplementary tables

Table S1. Elements analyzed, their detection limit and method used.

Element	Detection limit	Unity	Technology/instrument
THg	0.05	ng/g	DMA 80
THg	0.04	ng/L	CVAFS, Tekran 2600
MeHg	0.01	ng/L	GC-CVAFS, Tekran 2700
Se	0.49	ng/L	ICP-MS/MS 8900 Triple Quadrupole, Agilent
Na	100		
Mg	60		
K	100		
Mn	2		
Fe	100		
Cu	2		
Zn	100		
V	2		
Sr	2.5		
Ba	0.5		
TAs	0.71		
AsB	0.01		
DMA	0.012		
MMA	0.008		
As^{5+}	0.01		
As^{3+}	0.012		

Table S2. Details of the dose of TAs ingested and TAs changes in pigs' serum.

	Raw			Cooked		
	1R*	**2**	**3**	**1C***	**4**	**5**
Preconsumption values (µg/L)	2.4	1.7	2.1	1.3	1.4	0
AUC $_{(0\text{-}540)}$ (µg x min/L/µg)	2.5	0.7	3.2	2.3	2.0	2.5
C_{max} (t0 corrected) (µg/L)	11.7	3.8	15.0	10.9	12.0	12.5
T_{max} (min)	120	NA†	90	240	90	90
Intake (µg)	499	500	528	657	635	503
Dose (µg/kg bw)	9.6	8.7	10.2	12.6	10.9	7.7

* Pig 1 received both cooked and raw treatment a week apart. † Pig did not reach T_{max}.

Table S3. Ratio of elements distribution between blood compartments in this study and from the literature. Values presented are averages and standard deviations (SD).

Elements	Medium of exposure	Model	*n*	WB : serum ratio	RBC : serum ratio or range of values	References
THg	1 fish meal (raw)	Pig	34 *	4.7 (1.8)	12.9 (5.8)	This study
	1 fish meal (cooked)	Pig	36 *	4.7 (2.6)	13.1 (8.3)	This study
	1 fish meal (cooked)	Human	7		[20;22] **	Kershaw et al. 1980
	Chronic exposure through fish consumption	Human (eldery)	1000	2.2 (1.4)		Schultze et al. (2014)
	Chronic exposure through fish consumption	Human (ado)	335	2.5 (0.92)		Bárány et al. (2002)
	Chronic exposure through fish consumption	Human	206		[2;13] **	Skerfving (1974)
	Chronic exposure through fish consumption	Human	79		estimated by the author at 4 **	Svensson et al. (1992)
	Chronic exposure through fish consumption	Women (postpartum)	9	2.1 (0.7) **		Suzuki et al. (1970)
	Chronic exposure through fish consumption	Human	27	4.8 (0.8) **	9.6 (0.8) **	Yaginuma-Sakurai et al. (2012)
MeHg	1 fish meal (raw)	Pig	34 *	4.09 (1.1)	11.01 (3.6)	This study
	1 fish meal (cooked)	Pig	36 *	5.26 (1.2)	14.7 (3.6)	This study
	1 single oral dosage of 15 mg/kg bw	Rat	4		180 **	Clausing et al. (1984)

	1 single oral dosage of 15 mg/kg bw	Quail	4		58 **	Clausing et al. (1984)
TAs	1 fish meal (raw)	Pig	34 *	0.8 (0.1)	0.25 (0.2)	This study
	1 fish meal (cooked)	Pig	36 *	0.8 (0.1)	0.39 (0.3)	This study
TSe	1 fish meal (raw)	Pig	34 *	1.3 (0.3)	1.9 (0.8)	This study
	1 fish meal (cooked)	Pig	36 *	1.3 (0.2)	1.9 (0.6)	This study
	Chronic exposure through fish consumption	Human (ado)	243	1.0 (0.02)		Bárány et al. (2002)
Cu	1 fish meal (raw)	Pig	34 *	0.8 (0.1)	0.4 (0.2)	This study
	1 fish meal (cooked)	Pig	36 *	0.7 (0.1)	0.1 (0.2)	This study
	Chronic exposure through fish consumption	Human (eldery)	1000	0.9 (0.3)		Schultze et al. (2014)
	Chronic exposure through fish consumption	Human (ado)	342	0.9 (0.3)		Bárány et al. (2002)
	Normal life exposure	Normal humans (not sick)	106		0.8 **	Herring et al. (1960)
Zn	1 fish meal (raw)	Pig	34 *	2.9 (0.5)	6.9 (1.9)	This study
	1 fish meal (cooked)	Pig	36 *	2.4 (0.4)	5.6 (1.4)	This study
	Chronic exposure through fish consumption	Human (eldery)	1000	8.6 (0.3)		Schultze et al. (2014)
	Chronic exposure through fish consumption	Human (ado)	336	6.1 (0.2)		Bárány et al. (2002)
	Normal life exposure	Normal humans (not sick)	106		3.3 **	Herring et al. (1960)
Mn	1 fish meal (raw)	Pig	34 *	2.5 (1.2)	5.6 (4.1)	This study

	1 fish meal (cooked)	Pig	36 *	1.9 (1)	3.9 (2.8)	This study
	Chronic exposure through fish consumption	Human (eldery)	1000	5.0 (1.0)		Schultze et al. (2014)
Na	1 fish meal (raw)	Pig	34 *	0.7 (0.1)	0.1 (0.1)	This study
	1 fish meal (cooked)	Pig	36 *	0.6 (0.1)	0.1 (0.1)	This study
	Normal life exposure	Normal humans (not sick)	50		0.1 (0.2) **	Valberg et al. (1965)
Mg	1 fish meal (raw)	Pig	34 *	2.1 (0.2)	4.5 (0.6)	This study
	1 fish meal (cooked)	Pig	36 *	1.7 (0.3)	3.4 (1.0)	This study
	Normal life exposure	Normal humans (not sick)	106		3.6 **	Herring et al. (1960)
	Normal life exposure	Normal humans (not sick)	50		3.7 (0.3) **	Valberg et al. (1965)
K	1 fish meal (raw)	Pig	34 *	10.8 (1.8)	32.7 (5.6)	This study
	1 fish meal (cooked)	Pig	36 *	9.7 (1.6)	28.9 (5.0)	This study
	Normal life exposure	Normal humans (not sick)	50		24.9 (0.2) **	Valberg et al. (1965)
Fe	1 fish meal (raw)	Pig	34 *	292.7 (92.2)	943.4 (281.4)	This study
	1 fish meal (cooked)	Pig	36 *	230.8 (98.3)	731.3 (275.0)	This study
V	1 fish meal (raw)	Pig	34 *	0.7 (0.1)	0.1 (0.1)	This study

	1 fish meal (cooked)	Pig	36 *	0.7 (0.1)	0.1 (0.2)	This study
Sr	1 fish meal (raw)	Pig	34 *	0.8 (0.2)	0..5 (0.5)	This study
	1 fish meal (cooked)	Pig	36 *	0.7 (0.1)	0.1 (0.3)	This study
Ba	1 fish meal (raw)	Pig	34 *	2.4 (1.9)	5.4 (6.2)	This study
	1 fish meal (cooked)	Pig	36 *	1.3 (0.7)	1.9 (2.3)	This study

* This study: from 3 pigs, for 9 hours postprandial sampling

** Plasma data were used (serum data not available)

Chapitre 3 : Effets de la spéciation, de la cuisson et des modifications de la bioaccessibilité sur l'évaluation de l'exposition au méthylmercure pour des régimes alimentaires contrastés de poissons et de mammifères marins

Tranche de vie #3 : Embuches

Décembre 2018 : Mon bébé canin Charlie soufre d'une grave hernie discale pour une raison inconnue. Ses pattes arrière deviennent complètement paralysées. Mon cœur de maman soufre comme jamais. Il se plaint de douleur du matin au soir.

Janvier 2019 : Les médicaments et le repos forcé finissent par atténuer ses douleurs. Nous débutons intensément les traitements d'acupuncture, d'ostéopathie et d'aquaphysiothérapie. Quelques semaines plus tard, un miracle se produit : mon bébé retrouve sa joie de vivre et se remet à trottiner un peu partout.

Avril 2020, soit 7 mois avant mon dépôt initial : rejet de l'article #1. Retour en laboratoire pour doubler le « n » de l'article #1.

Première et deuxième vagues de la COVID-19 : responsable de l'angoisse populationnelle, mais surtout de la suspension de *mon* gym de CrossFit. Cela peut paraître banal aux yeux de plusieurs, mais pour moi, le gym constitue depuis près de 10 ans mon exutoire, ma sortie quotidienne, mon antidépresseur, mon « vide-tête », mon social, ma famille et surtout, ma passion.

Effects of speciation, cooking and changes in bioaccessibility on methylmercury exposure assessment for contrasting diets of fish and marine mammals

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Published in International Journal of Environmental Research and Public Health, 18: 2565.

DOI: 10.3390/ijerph18052565

Minor edits to the published text have been made here, following suggestions by this thesis' reviewers.

Abstract

Uptake of the neurotoxicant monomethylmercury (MeHg) from fish and marine mammals continues to present a public health concern in Canada and elsewhere. However, fish and marine mammals are key diet items contributing to food security for some Indigenous population in Canada. Mercury (Hg) exposure is estimated assuming that 100% of Hg is methylated and that 100% will be absorbed by the consumer. However, those conservative assumptions could lead to an overestimation of MeHg exposure and development of stringent consumption advisories that may limit fish and marine mammals' consumption. The aim of this study was to assess the impact of additional variables on Hg exposure equation using a probabilistic risk analysis. The variables that are currently considered by Health Canada are (1) consumption rate (g/day), (2) Hg level in food and (3) body weight. New variables proposed and tested were (1) the proportion of methylated Hg compared to total Hg (pMeHg, %), (2) the relative absorption factor (RAF, %) expressed as bioaccessibility and (3) the mass loss factor (MLF, unitless) that represents the loss of moisture during cooking, known to increase MeHg concentration in fish and mammals. For the new variables, data from literature were used in order to set point estimate values that were further used in the probabilistic risk analysis. Modelling results for both fish and marine mammals indicates that adding these new variables significantly influenced estimates of MeHg exposure (Mood's median test, $p < 0.05$). This study highlights that the assessment of exposure to MeHg is sensitive to pMeHg, RAF and MLF, and the inclusion of these variables in risk assessment should be considered with care. Further research is needed to provide better food-dependent, population-specific estimates of RAF and MLF before formal inclusion in exposure estimates.

Keywords: methylmercury; exposure; fish; mammal; probabilistic

Introduction

Exposure to monomethylmercury (MeHg) from fish and marine mammals continues to present public health concern in Canada and elsewhere (Joint Expert Committee on Food Additives, 2004; Lemire et al., 2015). This Hg chemical species is known for its ability to cross mammal blood-brain and placental barriers (Amin-Zaki et al., 1974; Aschner & Aschner, 1990; Kerper et al., 2017). Studies have shown harmful neurological and neurodevelopmental effects due to MeHg exposure in babies and young children linked to maternal consumption of fish with elevated MeHg levels during pregnancy (Cohen et al., 2005; FAO/WHO, 2010). Populations consuming marine mammals are at greater risk, such as the Inuit population from Canada, since marine mammal meat and offal may contain high levels of MeHg and are often consumed (Lemire et al., 2015). A mean concentration of blood total Hg (THg) level of pregnant women from Nunavik has been established at 4.2 µg/L (data from 2017) (Adamou et al., 2020), compared to 0.6 µg/L for pregnant women from the general Canadian population (data from 2008 to 2011) (Arbuckle et al., 2016).

Country food can be defined as « all food within a particular culture available from local natural resources and culturally accepted » (Kuhnlein & Receveur, 1996). Its consumption may present toxicological risks but remains desirable for several reasons. Country food consumption contributes to physical, mental, spiritual, emotional and social health in Indigenous populations (Council of Canadian Academies, 2014). Country foods are rich in proteins, vitamins, minerals and poor in saturated fats and sugars (Kuhnlein et al., 1994), which are key factors in preventing chronic diseases (Council of Canadian Academies, 2014). Furthermore, access to country food contributes to food security and helps to overcome lack of money for vulnerable households (Van Oostdam et al., 1999), since commercial food in the Canadian North is much more expensive than in the South (Chan et al., 2006).

In Canada, the consumption guidelines use a tolerable daily intake (TDI) as a basis for the calculation of allowable portion size and frequency of consumption (Health Canada, 2007b). Tolerable daily intake represents the reference dose (µg × kg^{-1} body weight (bw) per day) for a non-carcinogenic compound that can be consumed daily over a lifetime without any harmful effects on human health (Rice et al., 2000). This is based on the notion that harmful effects begin beyond a certain threshold (United States Environmental

Protection Agency, 2019). Methylmercury TDIs determined by Health Canada are set at 0.2 µg × kg^{-1} bw per day for sensitive populations (women of childbearing age and children < 12 years) and 0.47 µg × kg^{-1} bw per day for the general adult population (Health Canada, 2007b). It is not identical worldwide: WHO/FAO sets a benchmark dose of 0.23 µg × kg^{-1} bw per day (Joint FAO/WHO Expert Committee, 2003), whereas U.S. EPA determined the threshold to be 0.1 µg × kg^{-1} bw per day (including sensitive subgroup) (United States Environmental Protection Agency, 1997). Three major factors could explain the difference between those thresholds : (1) the choice of the epidemiological study from which the data are sourced, (2) the choice of the biomarker of exposure and (3) the choice of the uncertainty factor applied (Mergler et al., 2007).

The equations used by Health Canada (Health Canada, 2007b) in the calculation of Hg exposure defined by the current model (*Ecm*) and hazard quotient (*HQcm*) are:

$$(1)\ Ecm\ = \frac{CR \times [THg]}{BW}$$

$$(2)\ HQcm = \frac{Ecm\ (\text{mg/kg bw per day})}{pTDI\ (\text{mg/kg bw per day})}$$

where *CR* represents the consumption rate (mg wet weight (ww) fish flesh per day), *[THg]* is the THg concentration in the fish flesh (mg × kg^{-1} ww) and *BW* is for body weight (kg). Hazard quotient is used for threshold chemical compound (non-carcinogenic), such as MeHg (Rice et al., 2000) and links the exposure and the potential risk (McAuley et al., 2018). HQ $\leq$ 1 should not cause health effects, whereas HQ > 1 could imply risks for individuals exposed. The assessment of MeHg exposure through food using Equation 1 implies that 100% of Hg is present as MeHg. However, while the proportion of MeHg compared to THg is high in fish species (> 80%) (Magalhães et al., 2007), it is lower in marine mammal meat (range from 65 to 86%) and offal (range from 1.8 to 30%) (Ewald et al., 2019; Lemire et al., 2015; Wagemann et al., 1998; Wagemann et al., 2000). Moreover, Equation 1 assumes that 100% of MeHg will be absorbed by the consumer, yet this assumption is based on a 1970s obsolete study, where humans have been exposed to a solution of MeHg nitrate (Aberg et al., 1969). Furthermore, a growing number of *in vitro* digestion studies suggest that fish flesh cooking could decrease MeHg solubilization in digestive fluids (i.e. bioaccessibility),

theoretically leading to a lower absorption rate (Alves et al., 2018; Anacleto et al., 2020; Bradley et al., 2017; Girard et al., 2017; Torres-Escribano et al., 2010).

At the moment Health Canada recommends a deterministic approach to assess MeHg exposure (see Equation 1) through consumption pathways (Health Canada, 2007b), involving the use of a point estimate value to describe each variable of Equation 1 neglecting the potential variability within population and uncertainties in the data (Health Canada, 2010a). In comparison, probabilistic analysis uses probability distributions defining one or more variables, accounting for the variability and uncertainties and resulting in a full range and frequency of hypothetical risks (Health Canada, 2010a). Often, upper-bound (worst-case scenario, 95^{th} percentile) estimate variables are used in deterministic approach to compensate for not taking the range of variability and data uncertainties into consideration. This also increases the confidence that risks have not been underestimated; however, it could lead to an important, but difficult to define, overestimation of exposure (Health Canada, 2007b; United States Environmental Protection Agency, 2001).

This paper aims to assess the impact of the inclusion of new variables in the MeHg exposure equation using a probabilistic analysis. This analysis was performed for two consumption scenarios: scenario 1 is based on the consumption patterns of the general Canadian population (consuming mostly salmon and albacore canned tuna) while scenario 2 is based on consumption practices specific to many northern Indigenous populations (seal liver, beluga meat and beluga *nikku*). For scenario 1, we assessed MeHg exposure of the general population and of the sensitive part of the population, for medium (22 g/day) and high (40 g/day) consumption rates of fish. The sensitive population is defined as women of childbearing age and children < 12 years. In scenario 2, we assessed the exposure to MeHg for the general Indigenous population and the sensitive part of this population, using consumption rates specific to each mammal species assessed.

We only considered fish and mammal meats as MeHg exposure pathways since other MeHg sources are considered as negligible (European Food Safety Authority, 2012). Furthermore, only MeHg was used in this risk assessment but it is known that fish and mammal muscles can contain other contaminants (Hinck et al., 2009; Hoekstra et al., 2005). Thus, this study does not constitute a full human health risk assessment and was intended to be more of a sensitivity analysis. The goal of this paper is to test the exposure calculation

by taking into account recent research. We consider that our study will serve as a starting point for practitioners and scientists to adjust currently used exposure equations, taking into consideration specific consumer groups and the particularity of their diet. We do not propose any health-related changes in consumption guidelines, since we simply explore the potential effect of introducing new terms in exposure assessments, in order to guide future research.

Materials and methods

Population and diet

Our study compared MeHg exposure for two different dietary intake scenarios. Scenario 1 (Table 1) reflects the general Canadian diet and considers MeHg intake from salmon and canned tuna. Salmon (all species combined) was selected because it is the fish most consumed in Canada (Health Canada, 2007b). We also chose Albacore canned tuna containing generally relatively high Hg levels, and for which Health Canada issued consumption guidelines, in contrast to canned light tuna containing less Hg (Health Canada, 2019a). For the general and sensitive population, CR values and THg levels for salmon and canned tuna were obtained from Health Canada's publication (Health Canada, 2007b), and BW from Canadian Exposure Factors Handbook (Richardson & Stantec Consulting Ltd, 2013) (Table 1). Scenario 2 (Table 1) reflects consumption by the Indigenous Canadian population for which BW, CR and mass loss factor (MLF) data were obtained from Lemire et al. (2015). Lemire et al. (2015) based their data on the 2004 Nunavik Health Survey; we reevaluated the data on MeHg exposure for key marine mammal species using a probabilistic approach. The Indigenous BW value is a geometric mean (n = 702, where women represent 48.2%) adjusted for age and gender, which could have overestimated the BW of the sensitive population in our modelling. THg concentration in marine mammals was obtained from Palaniyandi (2016). Beluga meat and *nikku* (air-dried beluga meat) and seal liver were chosen to represent the marine mammal diet because they are the most Hg-contaminated part of northern country diet (Laird et al., 2013; Lemire et al., 2015; Palaniyandi, 2016). In order to assess the effect of drying (MLF) on THg levels, beluga meat THg value was used in the modelling of risk assessment (Table 1). Note that scenario 2 is not meant to represent

any specific community but rather aims to consider a diet rich in marine mammals. No inference to risk assessments of specific communities or populations should be derived from this theoretical exercise. The variables used in each scenario are described in detail in Table S1.

Table 1. Values used in the probabilistic risk assessment

	Input variables	Point estimate		Probability distribution		Units	References
		CTE [a] (SD)	RMaE [b]	Distribution type	Parameters min;max		
CR	**Scenario 1 – Fish flesh**						
	Medium consumer	22				g/day	(Health Canada, 2007b)
	High consumer	40				g/day	(Health Canada, 2007b)
	Scenario 2 – Marine mammals						
	Beluga meat (pre-drying)	3.4				g/day	(Lemire et al., 2015)
	Beluga *nikku*	2.3				g/day	(Lemire et al., 2015)
	Seal liver	1.0				g/day	(Lemire et al., 2015)
BW	**Scenario 1 – Fish flesh**						
	General population	76.5 (15.8)	53.3	lognormal	[40;120]***	kg	(Richardson & Stantec Consulting Ltd, 2013)
	Sensitive population	69.8 (16.3)	46.5	lognormal	[35;115]***	kg	(Richardson & Stantec Consulting Ltd, 2013)
	Scenario 2 – Marine mammals						
	Indigenous population	68.4 (20)*	43.9	lognormal	[38.2–129.4]	kg	(Lemire et al., 2015)
[THg]	**Scenario 1 - Fish flesh**						
	Salmon	0.03 (0.03)*	0.07	lognormal	[0–0.12]	ug/g (ww)	(Health Canada, 2007b)
	Albacore canned tuna	0.35 (0.1) **	0.5	lognormal	[0.2–0.6]	ug/g (ww)	(Health Canada, 2007b)
	Scenario 2 - Marine mammals						
	Beluga meat (pre-drying)	2.0 (0.5)[†]	3.0	lognormal	[0.8-4.0]	ug/g (ww)	(Palaniyandi, 2016)
	Beluga *nikku*	5.0 (1.2)	7.2	lognormal	[2–10]***	ug/g (ww)	(Palaniyandi, 2016)
	Ringed seal liver	19 (11.1)	23.7	lognormal	[10–25]***	ug/g (ww)	(Palaniyandi, 2016)
pMeHg	**Scenario 1 - Fish flesh**						
	Health authorities	100				%	(Health Canada, 2007b)
	Literature mid data	90				%	(European Food Safety Authority, 2012)
	Scenario 2 - Marine mammals						
	Beluga meat	65				%	(Lemire et al., 2015)
	Ringed seal liver	11				%	(Lemire et al., 2015)
RAF	**Scenario 1 - Fish flesh**						
	Health authorities	100				%	(Health Canada, 2007b)
	Cooked - *In vitro* bioaccessibility	40				%	(Afonso et al., 2015; Anacleto et al., 2020; Girard et al., 2017; Liao et al., 2020)
	Scenario 2 - Marine mammals						
	Beluga meat	51				%	(Palaniyandi, 2016)

	Beluga *nikku*	33		%	(Palaniyandi, 2016; Yassine, 2017)
	Seal liver	27		%	(Laird et al., 2009; Palaniyandi, 2016; Yassine, 2017)
MLF	**Scenario 1 - Fish flesh**				
	Salmon - all cooking methods	1.35		unitless	(Burger et al., 2003; Costa et al., 2015; Morgan et al., 1997; Moses et al., 2009)
	Albacore canned tuna	1		unitless	
	Scenario 2 - Marine mammals				
	Beluga meat (raw)	1		unitless	(Lemire et al., 2015)
	Beluga *nikku* (Air-dried)	2.5		unitless	(Lemire et al., 2015)
	Ringed seal liver (raw)	1		unitless	(Lemire et al., 2015)

[a] Central tendency exposure expressed as arithmetic mean
[b] Reasonable maximum exposure expressed as the 95th percentile of CTE
*arbitrary SD (no data available)
** weighted average
*** arbitrarily truncated to remain realistic
† [THg] for beluga meat was estimated using the [THg] of beluga *nikku* divided by MLF
CR: consumption rate; BW: body weight; [THg]: THg concentration; pMeHg: proportion of THg that is methylated; RAF: relative absorption factor; MLF: mass loss factor

New proposed variables

We tested an alternative model to the current *Ecm* model represented by equations (1) and (2) using the following equation:

$$(3)\ Eam = \frac{CR \times [THg] \times\ pMeHg\ \times\ RAF \times\ MLF}{BW}$$

where *Eam* is the exposure estimate with the alternative model, *pMeHg* corresponds to the proportion (%) of THg that is methylated in the tissue, *RAF* relates to the relative absorption factor (RAF) (expressed as bioaccessibility, %) and MLF means mass loss factor (MLF, unitless) in order to compensate for the moisture loss during cooking. Point estimates values (central tendency exposure; CTE) were used in the modelling using Equation 3 (Table 1). For pMeHg in fish flesh, two values were used: 100% (as the value currently used by health authorities (Health Canada, 2007b)) and 90% as a value presented in the general literature (according to the European Food Safety Authority that reports 26 studies describing a mean range between 80 and 100%) (European Food Safety Authority, 2012). Values for beluga meat and seal liver were age-adjusted (16.5 and 6 years, respectively) based on study by Lemire et al. (2015). For RAF values, we have chosen 100% (value used by default by health authorities, as they consider a complete absorption of MeHg) (Health Canada, 2007b) and 40% as a mean MeHg bioaccessibility reported in studies of cooked fish using different species, such as swordfish, grouper, tuna, salmon, common smooth-hound, Atlantic wreckfish, black scabbardfish, shark, tilapia, snapper, turbot and anchovy (Afonso et al., 2015; Anacleto et al., 2020; Girard et al., 2017; Liao et al., 2020). We have allocated an RAF of 40% to canned tuna since it is cooked before being canned (Vincent, 2010). In the literature, the data on RAF for marine mammals are very scarce and no study was found describing the bioaccessibility of MeHg from this country food. However, some studies have reported bioaccessibility of THg; an RAF of 51% was reported for beluga meat (Palaniyandi, 2016), and an average of 33% for beluga *nikku* (Palaniyandi, 2016; Yassine, 2017). An average of 27% was associated with ringed seal liver (Laird et al., 2009; Palaniyandi, 2016; Yassine, 2017). In this study, bioaccessibility values were used since no *in vivo* study assessed the relative absorption of MeHg from cooked flesh. Finally, we included MLF in the Equation 3 when appropriate in order to

represent the loss of humidity during cooking, which is known to increase the MeHg concentration in fish flesh (Afonso et al., 2015; Burger et al., 2003; Costa et al., 2016; Costa et al., 2013; Maulvault et al., 2011). The value of 1.35 encompasses all cooking methods (grilling, frying, boiling, etc.) (Afonso et al., 2015; Burger et al., 2003; Costa et al., 2016; Costa et al., 2013; Maulvault et al., 2011). No MLF factor was added in the case of canned tuna that does not need further food processing before being eaten, and seal liver that is consumed raw (Lemire et al., 2015). The MLF for beluga *nikku* corresponds to the loss of moisture during air-drying of the beluga meat and was set at 2.5 (Lemire et al., 2015).

The following equation was used to calculate the CR of canned tuna that would be allowed if HQ equals to 1:

$$(4)\ CR\ limit = \frac{pTDI * BW * HQ}{[THg] * pMeHg * RAF}$$

Probabilistic risk assessment

For this study, CTE characterizes the mean whereas reasonable maximum exposure (RMaE) represents 95th percentile of CTE (Table 1). Point estimate (CTE) values were used for the new parameters of Equation 3 (RAF, pMeHg and MLF) (Table 1). On the other hand, in order to account for the potential variability in the population (Table 1, BW) and uncertainties in the data (Table 1, [THg]), we developed a probabilistic risk assessment according to Health Canada (2010a) and United States Environmental Protection Agency (2001) procedures with R software (R Core Team), using a first-order Monte Carlo one-dimensional simulation (*n* = 10,000). Typically, 10,000 iterations are appropriate to capture most of the variability of the input distributions extremities (Health Canada, 2010a). The stability of the results was tested by varying the number of iterations from 10,000 to 10,000,000 and similar results were obtained (Table S2). We generated lognormal distributions (*n* = 10,000, R: *rlnormTrunc* function from *EnvStats* package) for [THg] and BW with defined values based on literature (Table 1). According to Health Canada (2010a) and United States Environmental Protection Agency (2001) variables subject to the multiplicative effect of a large number of processes tend to yield a lognormal shape distribution. For Monte Carlo analysis,

dependencies between variables were considered as negligible. The only potential dependency that may occur is a positive correlation between the CR and the BW (Health Canada, 2007b), but since they are positioned at both sides of the exposure equation (CR at the numerator and BW at the denominator, see Equations 2 and 3) this limits the bias caused by this dependency.

Impact of RAF on risk estimates

To illustrate the impact of MeHg bioaccessibility on the level of estimated risk (expressed as HQ; Fig. 3), we extracted data from various studies (Afonso et al., 2015; Afonso et al., 2018; Afonso et al., 2015; Cano-Sancho et al., 2015; Matos et al., 2015; Siedlikowski et al., 2016a; Wang et al., 2013). We used the values of MeHg bioaccessibility and MeHg initial concentration in fish muscle in order to calculate the resulting HQ. Equation 1 (*CR-medium consumption* and *BW* of the general population, Table 1) with the addition of RAF was applied in the modelling.

Statistic and icons

The resulting HQ distributions were summarized with box-and-whisker plots. When needed, HQ distributions were compared with the Mood's median test. Significance level was set at $\alpha < 0.05$. Seal and beluga icons were downloaded from the noun project (www.thenounproject.com), from Victoruler, Valeriia Vlasovtseva artists, ProSymbols and Rfourtytwo.

Results

The impact of the proposed variables on methylmercury exposure

Table 2 summarizes the MeHg daily exposure dose as a function of diet, CR and BW for each scenario, and the impact of adding a single variable in a stepwise way to the exposure

equation. Only albacore canned tuna results are presented since salmon did not produce exposure superior to TDI. The use of the current model (Equation 1) for the general population and the medium consumption rate led to estimates of daily doses below the TDI of 0.47 µg × kg^{-1} bw per day. The addition of each single variable led to an exposure distribution significantly different from *Ecm*.

Table 2. Independent impact of adding a single variable in the equation of the exposure of MeHg. Results are expressed as median of MeHg daily exposure dose (µg × kg^{-1} bw) and the percentage of the distribution of exposure that exceeds the TDI value is in parentheses (no number signifies 0%). Alternative model shows the resulting MeHg daily exposure when all the proposed variables are integrated simultaneously into the exposure equation. See Table S1 for the details on variables used for each scenario. * are used to indicate a significative difference ($p < 0.05$) between the exposure distribution compared to the current model. pMeHg: proportion of THg that is methylated; RAF: relative absorption factor; MLF: mass loss factor.

Scenario 1 – Fish flesh Medium consumption rate	**Albacore canned tuna**				
	current model	*pMeHg*	*RAF*		*alternative model*
Canadian general population	0.100	0.090*	0.040*		0.036*
Sensitive Canadian population	0.110 (4%)	0.010 (1%)*	0.044*		0.040*
Scenario 1 – Fish flesh High consumption rate	**Albacore canned tuna**				
	current model	*pMeHg*	*RAF*		*alternative model*
Canadian general population	0.182 (0.1%)	0.164*	0.073*		0.065*
Sensitive Canadian population	0.200 (50%)	0.180 (38%)*	0.08 (0.2 %)*		0.072 (0.1 %)*
Scenario 2 – Marine mammals	**Seal liver**				
	current model	*pMeHg*	*RAF*		*alternative model*
Indigenous population	0.242 (2.7%)	0.027*	0.065*		0.007*
Scenario 2 – Marine mammals	**Beluga meat**				
	current model	*pMeHg*	*RAF*		*alternative model*
Indigenous population	0.100	0.065*	0.051*		0.033*
Scenario 2 – Marine mammals	**Beluga *nikku***				
	current model	*pMeHg*	*RAF*	*MLF*	*alternative model*
Indigenous population	0.068	0.044*	0.056*	0.169 (0.1%)*	0.036*

The TDI for sensitive population (women of childbearing age and children < 12 years) is 0.2 µg × kg^{-1} bw per day and this value has been exceeded by 4% and 50% of the population regarding the canned tuna scenario for the medium and high consumption rate respectively, when using the current model (see Equation 1). In the high consumption rate scenario, the addition of pMeHg and RAF decreased the percentage of the population exceeding the TDI by 12% and 50%, respectively. For this scenario, the use of the alternative model led to a safe MeHg exposure in 99.9% of cases. For the seal liver scenario, the addition of either pMeHg or RAF decreased the percentage of at-risk populations to zero %, in comparison to the TDI obtained with the current model (see Equation 1) which led to 2.7% of at-risk population. For the beluga *nikku*, 0.1% of the population are exceeding the TDI when MLF is considered. Overall, while pMeHg and RAF lead to a decrease in exposure, MLF increases it. Those results suggest that the proposed variables (pMeHg, RAF and MLF) should be considered in exposure modelling by health authorities.

Risk characterization

In order to assess the impact of the proposed variables on the health risk from MeHg exposure, we compared the HQ distributions obtained using *Ecm* vs. *Eam*. As observed in Figures 1 and 2, the simultaneous addition of all proposed variables (pMeHg, RAF and MLF) systematically led to a decrease of MeHg risk exposure.

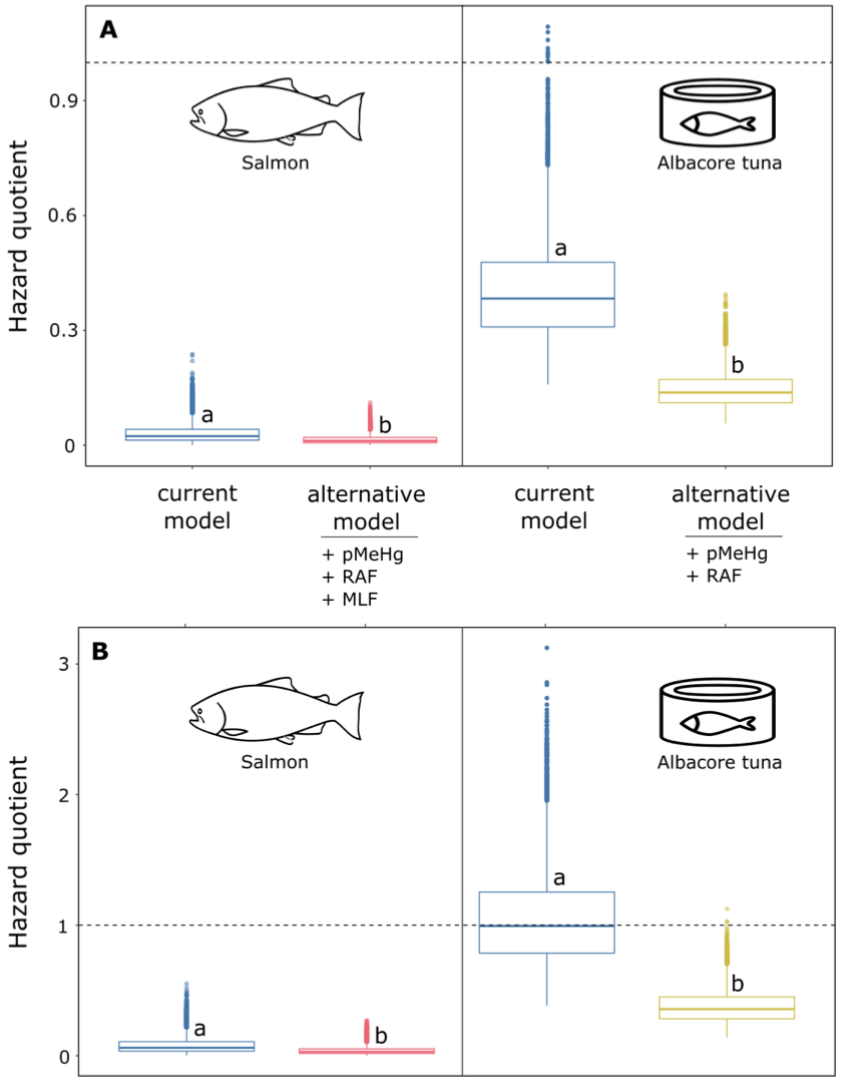

Figure 1. Hazard quotient as function of the fish species consumed and as function of the variables used in the assessment of MeHg exposure. High consumption rate scenario was used. (A) Canadian general population and (B) sensitive Canadian general population. Letters are used to indicate a significative difference ($p < 0.05$) between HQ distribution of a given fish species. See Table S1 for the details of variables used for each scenario. pMeHg: proportion of THg that is methylated; RAF: relative absorption factor; MLF: mass loss factor.

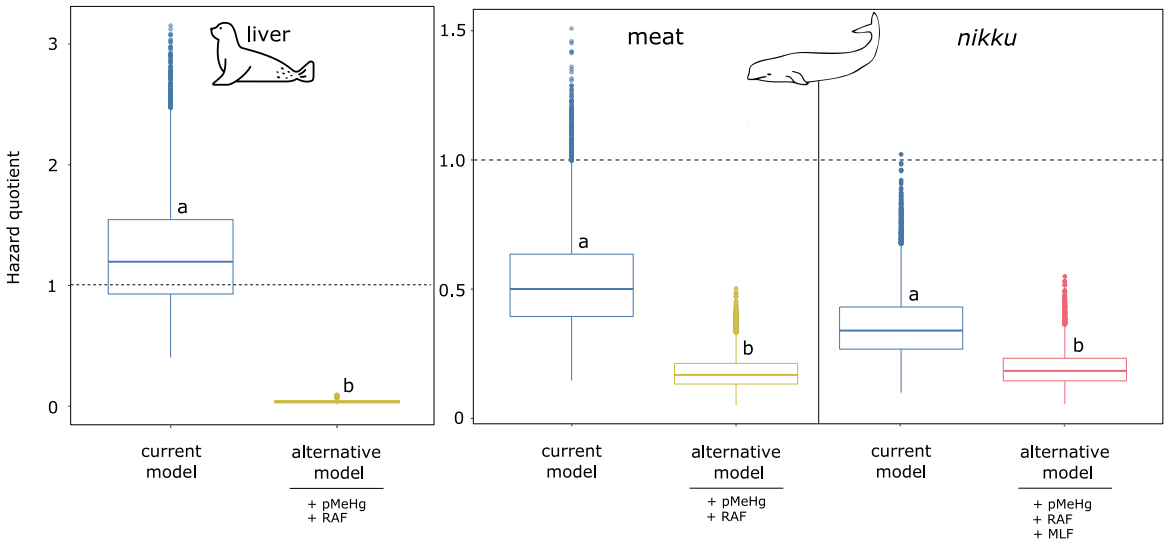

Figure 2. Hazard quotient as function of marine mammals consumed and as function of the variables used in the assessment of MeHg exposure for the sensitive Indigenous population. Letters are used to indicate a significative difference ($p < 0.05$) between a given scenario. See Table S1 for the details of variables used for each scenario. pMeHg: proportion of THg that is methylated; RAF: relative absorption factor; MLF: mass loss factor.

Salmon and canned tuna consumption scenario

Figure 1 shows the HQ distribution as a function of the fish species consumed frequently and as function of the variables used in the assessment of MeHg exposure, for the general and sensitive Canadian population. According to Fig. 1, salmon consumption should not cause any health risk; using the current model we estimated HQ (median) to be between 0.01 and 0.03 for the general and the sensitive population, respectively (Table S3). On the other hand, the consumption of canned tuna by the sensitive Canadian general population using the current model (see Equation 1) led to HQ > 1 for 50% of the population (Table S3), whereas the addition of pMeHg and RAF into the assessment produced an HQ distribution where 99.9% of the population was not exposed to any risks (HQ < 1).

Marine mammal consumption scenario

Figure 2 illustrates the health risks associated with MeHg exposure through marine mammal consumption with the example of the sensitive Indigenous population (women of childbearing age and children < 12 years). In the case of the seal liver, which is consumed raw, the current model (see Equation 1) led to HQ > 1 in 69% of cases, whereas the addition of pMeHg produced an HQ above 1 in 100% of cases (Table S3). Regarding the beluga meat and *nikku*, the use of the current model (see Equation 1) produced HQ > 1 for 1.9 and 0.01% of the population, respectively.

The implication of RAF in methylmercury risk characterization

We explored the relationship between RAF (expressed as bioaccessibility, %) and HQ, to assess the effect of cooking on HQ, since cooking has been shown to decrease the MeHg bioaccessibility in *in vitro* studies (Alves et al., 2018; Anacleto et al., 2020; Girard et al., 2017; Torres-Escribano et al., 2010). Figure 3 was created using the values (n = 45) of seven studies that have assessed the effect of cooking on MeHg bioaccessibility in the flesh of various fish (Afonso et al., 2015; Afonso et al., 2018; Afonso et al., 2015; Cano-Sancho et al., 2015; Matos et al., 2015; Siedlikowski et al., 2016a; Wang et al., 2013). Methylmercury exposure used to estimate HQ was calculated using the *CR-medium consumption* and *BW* of the general population (Table 1), the initial MeHg level in fish flesh and their respective bioaccessibility. Hence, we used the current model + RAF. As mathematically expected, HQ is linearly related to the bioaccessibility (raw: r^2 = 0.50, ρ-value < 0.05; cooked: r^2 = 0.35, p < 0.05). According to Fig. 3, the risk related to the consumption of cooked fish is higher than for raw fish, which is associated with higher MeHg levels in species chosen for the assessment of the effect of cooking on bioaccessibility. It is noteworthy that for a given fish species, bioaccessibility may largely vary.

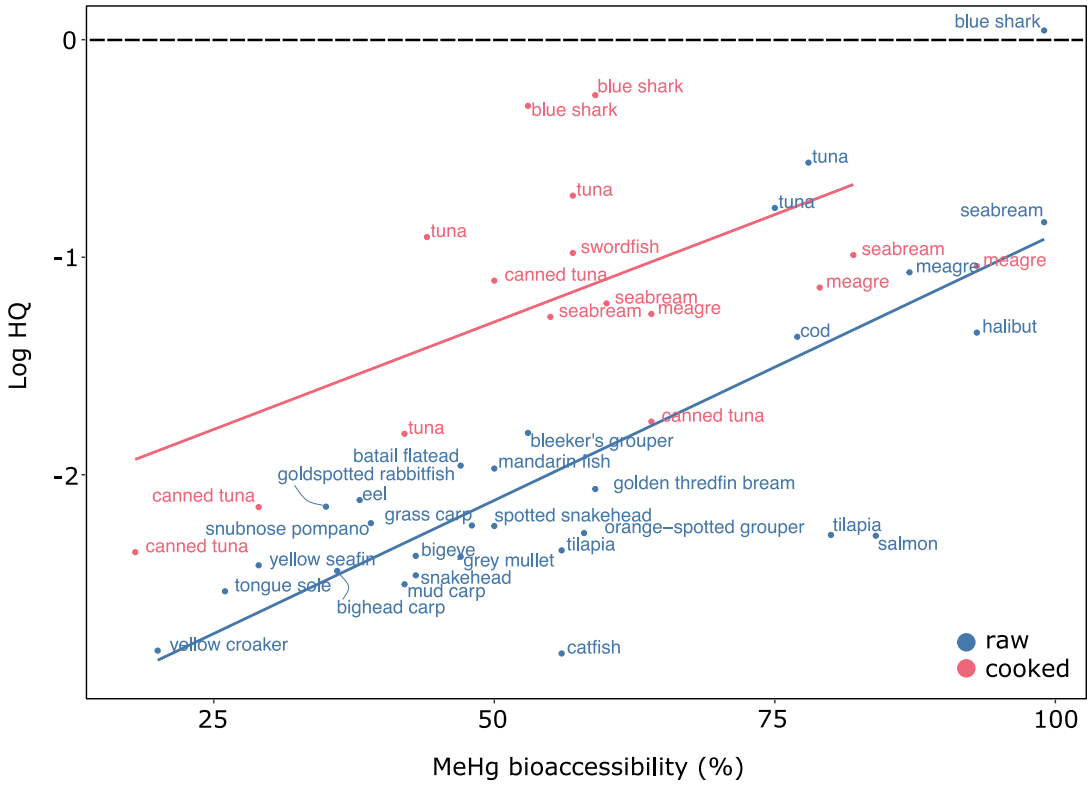

Figure 3. Impact of RAF (expressed as bioaccessibility, %) on HQ. Blue for the raw bioaccessibility value and pink for the cooked one. Dotted line represents HQ = 1. HQ was assessed using the *CR-medium consumption* and *BW* of the general population (Table 1). The initial MeHg level in fish flesh and their respective bioaccessibility were obtained from: (Afonso et al., 2015; Afonso et al., 2018; Afonso et al., 2015; Cano-Sancho et al., 2015; Matos et al., 2015; Siedlikowski et al., 2016a; Wang et al., 2013).

Discussion

The proposed model modifications impact methylmercury exposure assessment

In all cases, every suggested variable significantly impacted MeHg daily dose exposure compared to the current model (see Equation 1) (Table 2). For instance, according to Health Canada guidelines, sensitive members of the population should limit their consumption of

albacore canned tuna to 300 g per week (Health Canada, 2019a), which is seven times less than the quantity that could be allowed when the proposed variables are included in the assessment of MeHg exposure (see Equation 4, data not shown). This study therefore highlights the importance of carefully considering the proposed variables in MeHg exposure assessment and the need to better estimate these variables with care.

The value of 90% used for the pMeHg (Table 1) for fish flesh is still quite conservative since pMeHg tends to vary according to the trophic level, feeding habits and age. Lescord et al. (2018) found a pMeHg range from 40 to 100%, with an average of 84 ± 20 %, in Canadian fish flesh, and this is in agreement with the conclusion of the Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) who provided a range between 30 and 100% (FAO/WHO, 2010). The value of pMeHg for the marine mammals varies in literature as well. For instance, values for beluga muscle pMeHg range from 65 to 97% (Lemes et al., 2011; Lemire et al., 2015; Wagemann et al., 1998). Our study may underestimate MeHg exposure from beluga muscle since we chose the inferior value of this range (Table 1). In seal liver, literature shows that pMeHg can vary between 1.8 and 30 % (Ewald et al., 2019; Lemire et al., 2015; Wagemann et al., 1998; Wagemann et al., 2000). In our study, we chose a pMeHg value of 11% for the modelling, being half-way of the range of values in literature. Assessing MeHg exposure from the ringed seal hepatic tissue is complex. Indeed, Ewald et al. (2019) proposed that Hg speciation in the liver of ringed seal is function of the age and, while pMeHg in liver decreased as function of age, the formation of the chemically inert mercuric selenide (HgSe) complexes increases with it. This suggested that the hepatic demethylation ability of MeHg increases with the age of the seal. However, acquired from food, MeHg levels increases with age (Wagemann et al., 1998), complexifying the assessment of MeHg exposure. Wagemann et al. (2000) found in adult ringed seal liver that Hg speciation was HgSe at 53% and MeHg at 2%. While the Nunavut government (Canada) recommends that pregnant women avoid ringed seal liver consumption (https://nunatsiaq.com), Wagemann et al. (2000) emphasis the need to consider a recalculation of the allowable consumption limits of seal liver, due to the large proportion of HgSe and its low toxicity.

Another variable that we suggest adding in the assessment of MeHg exposure is RAF, which decreased significantly the exposure of MeHg (Table 2, Fig. 3). Bioaccessibility studies have demonstrated that MeHg solubility decreased with fish flesh cooking (Afonso et al., 2015; Anacleto et al., 2020; Girard et al., 2017; Liao et al., 2020). However, it is important to note that bioaccessibility values are notoriously variable with low inter-laboratory or even intra-laboratory reproducibility, and the lack of use of standardized protocol leads to a wide range of values (Girard et al., 2017). Also, the impact of cooking on MeHg oral bioavailability has been assessed using pigs and suggested that cooking fish muscle did not modify MeHg oral bioavailability (Charette et al., 2021). Hence, currently, *in vitro* and *in vivo* results diverge.

A standardized *in vitro* digestion protocol was developed by the COST INFOGEST network, in which skim milk powder protein digestibility has been validated using pig models (Brodkorb et al., 2019). Once optimized for metals, this model protocol could be used as a starting point to standardize *in vitro* protocols for MeHg bioaccessibility studies, since MeHg is mostly bound to proteins. It could also be used for the assessment and validation of *in vitro* digestibility of raw and cooked fish flesh.

Presently, we don't recommend using bioaccessibility (i.e., RAF) estimates in the assessment of MeHg exposure, since it could significantly underestimate the exposure of the consumer without sufficiently strong scientific evidence. Also, since pMeHg highly varies in country foods (Lemire et al., 2015), THg bioaccessibility could be a weak proxy of bioaccessibility of MeHg. Standardization and *in vivo* validation are needed before RAF is included in the alternative model.

The last variable studied was MLF. While there is no consensus regarding the impact of cooking on MeHg levels, the majority of studies points in the direction of an increase of MeHg concentration due to mass loss linked to water loss during cooking (Afonso et al., 2015; Burger et al., 2003; Costa et al., 2016; Costa et al., 2013; Maulvault et al., 2011). Including MLF in the model increases drastically the daily exposure to MeHg, but on the other hand, according to bioaccessibility literature, cooking (Afonso et al., 2015; Anacleto et al., 2020; Girard et al., 2017; Liao et al., 2020) and drying (Palaniyandi, 2016; Yassine, 2017) can decrease the solubilization of

MeHg and THg during the digestion. The interaction between those phenomena should be further investigated by researchers and health authorities.

Our findings show that there is a great range of values for the proposed variables presented in this study (pMeHg, MLF and RAF), but does not address the inter-population variability regarding BW and CR, nor the potential heterogeneity of MeHg distribution within fish muscle (Charette et al., 2021). Many of these variables (BW, CR and [THg]) vary geographically and culturally, which makes the assessment of exposure to MeHg through diet difficult. This emphasizes the importance of the probabilistic risk assessment approach, where uncertainties and inter-population variability of data are included in the assessment (Health Canada, 2010a).

The assessment of methylmercury exposure through marine mammals should be a priority

Health Canada has stated that for MeHg exposure from food the 95th percentile dose estimate obtained from probabilistic risk assessment should produce HQ < 1 (Health Canada, 2010b). Our results indicate that the inclusion of some of the proposed variables could modify the HQ for Indigenous consumers and particularly for those who consume seal liver and beluga meat (Table S3). Better assessing the values of pMeHg, MLF and RAF for foods rich in MeHg would allow for better estimates of risk and more precise consumption advisories. Presently local health services in many Canadian provinces and Territories (such as the Nunavik Regional Board of Health and Social Services (www.nrbhss.ca) and the Nunavut government (Canada) (https://nunatsiaq.com)) encourage the consumption of country food as a good source of nutrients but recommend that pregnant women and women of childbearing age limit their consumption of beluga meat and seal liver.

Assessing exposure to MeHg through country food consumption is complex as it depends on personal consumption patterns, seasonal food availability (Gaudin et al., 2014) and variability of MeHg content in different tissues of country foods (Lemire et al., 2015) and even within a same tissue (Charette et al., 2021).

We have to underline that a calculated HQ > 1 not necessarily leads to imminent health risks. Health Canada in its calculations of TDIs, included an uncertainty factor (UF) to account for inter-individual variability and to derive safe guidelines (Legrand et al., 2010). The TDI for the general population is based on Hg blood levels with no observable harmful effect from MeHg poisoning cases in Japan (1950-1960) and Iraq (1970), to which UF of 10 has been added (Legrand et al., 2010). For the sensitive population, the TDI comes from the data of three epidemiological studies conducted in the Seychelles and Faroes Island and New Zealand which assessed the effect of exposure to MeHg *in utero* on fetal neurodevelopment (Legrand et al., 2010). In this context, Health Canada used the MeHg level in maternal hair with no observable harmful effect, which was further converted into blood levels, using a ratio of 250:1 (µg MeHg/mg of hair:µg MeHg/mL of blood) (U.S. EPA, 1997). However, the ratio listed varies from 150 to 450, which could lead to an under- or overestimation of the actual exposure (Bartell et al., 2000; Liberda et al., 2014; Yaginuma-Sakurai et al., 2012). Then, an UF of 5 has been added in order to produce the TDI (Legrand et al., 2010). Thus, HQ slightly higher to 1 will very unlikely lead to health risks. But how far HQ can be exceeded without risks? At the moment, science does not allow an answer to this question.

Our study focused on MeHg exposure. However, balancing risks and benefits regarding country food consumption is essential and is not a trivial task since many benefits have to be considered. First, food scarcity, anemia and obesity are significant health issues for some communities in Canada, and country food brings many key nutrients that are needed to improve health. Lemire et al. (2015) found that most of the commonly consumed country food in Nunavik (Canada) are poor in MeHg and rich in selenium (Se), with the exception of beluga meat and seal liver.

Further, country food consumption has also social dimensions (ex. socio-cultural and spiritual) that are usually assessed qualitatively, while health risks are measured quantitatively (ex. contaminant concentration) (Watzl et al., 2012). An overall assessment of risks and benefits requires involvement of many disciplines such as nutrition, toxicology, sociology and public health practice (Van Oostdam et al., 2005). Furthermore, risks and benefits can vary as a function of multiple factors, such as animal size, species, origin (aquaculture or wild), contamination,

seasonal changes in consumption rates (g/day), consumer profile (age and gender) and more (FAO/WHO, 2010).

Conclusion

Overall, our study demonstrated the sensitivity of the MeHg exposure assessment and underlined the need to deepen our knowledge of the additional, suggested variables in order to avoid an underestimation or overestimation of the risk. Of particular importance is the need to better investigate bioaccessibility before RAF is included in a new risk assessment model.

We showed that pMeHg and RAF decreased MeHg exposure, while MLF increased it. Salmon consumption produced HQ < 1 in all cases, while the consumption of Albacore canned tuna resulted in HQ > 1 for the Canadian sensitive population, mostly when high-rate consumption is assessed. Methylmercury exposure through seal liver consumption should be investigated, since our modelling shows a great difference between the current and the alternative model. Furthermore, even though beluga *nikku* is more contaminated than beluga meat, its lesser bioaccessibility could buffer this difference and lead to similar MeHg exposure. However, validation of *in vitro* studies should be conducted before proposing changes in consumption guidelines.

Finally, benefits are to be considered before setting recommendations.

Acknowledgements

The author thanks Kevin Charette for statistical advice. Research was funded through the Canada Research Chair program (MA), a NSERC Discovery grant (MA, MR), a CRD NSERC/Hydro-Quebec grant (MA) and an NSERC CREATE Ecolac stipend to TC.

Supplementary information

Supplementary table

Table S1. Details of the variables used in the modelling of each scenario.

SCENARIO 1 – FISH FLESH **MEDIUM CONSUMPTION RATE**	**Salmon**	
Canadian general population	*current model* CR-medium consumption [THg] salmon General population BW	*alternative model* CR-medium consumption [THg] salmon General population BW +pMeHg fish flesh literature med data; +RAF cooked fish flesh; +MLF fish flesh
Canadian sensitive population	CR-medium consumption [THg] salmon Sensitive population BW	CR-medium consumption [THg] salmon Sensitive population BW +pMeHg fish flesh literature med data; +RAF cooked fish flesh; +MLF fish flesh
SCENARIO 1 – FISH FLESH **HIGH CONSUMPTION RATE**	**Salmon**	
Canadian general population	*current model* CR-high consumption [THg] salmon General population BW	*alternative model* CR-high consumption [THg] salmon General population BW +pMeHg fish flesh literature med data; +RAF cooked fish flesh; +MLF fish flesh
Canadian sensitive population	CR-high consumption [THg] salmon Sensitive population BW	CR-high consumption [THg] salmon Sensitive population BW +pMeHg fish flesh literature med data; +RAF cooked fish flesh; +MLF fish flesh
SCENARIO 1 – FISH FLESH **MEDIUM CONSUMPTION RATE**	**Albacore canned tuna**	
Canadian general population	*current model* CR-medium consumption [THg] tuna General population BW	*alternative model* CR-medium consumption [THg] tuna General population BW +pMeHg fish flesh literature med data; +RAF cooked fish flesh
Canadian sensitive population	CR-medium consumption [THg] tuna	CR-medium consumption [THg] tuna

	Sensitive population BW	Sensitive population BW
		+pMeHg fish flesh literature med data; +RAF cooked fish flesh
SCENARIO 1 – FISH FLESH		
HIGH CONSUMPTION RATE	**Albacore canned tuna**	
Canadian general population	*current model*	*alternative model*
	CR-high consumption	CR-high consumption
	[THg] tuna	[THg] tuna
	General population BW	General population BW
		+pMeHg fish flesh literature med data; +RAF cooked fish flesh
Sensittive general population	CR-high consumption	CR-high consumption
	[THg] tuna	[THg] tuna
	Sensitive population BW	Sensitive population BW
		+pMeHg fish flesh literature med data; +RAF cooked fish flesh
SCENARIO 2 – MARINE MAMMALS	**Seal liver**	
Indigenous population	*current model*	*alternative model*
	CR seal liver	CR seal liver
	[THg] seal liver	[THg] seal liver
	Indigenous population BW	Indigenous population BW
		+pMeHg seal liver; +RAF seal liver
Sensitive indigenous population	CR seal liver	CR seal liver
	[THg] seal liver	[THg] seal liver
	Indigenous population BW	Indigenous population BW
		+pMeHg seal liver; +RAF seal liver
SCENARIO 2 – MARINE MAMMALS	**Beluga meat**	
Indigenous population	*current model*	*alternative model*
	CR beluga meat	CR beluga meat
	[THg] beluga meat	[THg] beluga meat
	Indigenous population BW	Indigenous population BW
		+pMeHg beluga meat; +RAF beluga meat
Sensitive indigenous population	CR beluga meat	CR beluga meat
	[THg] beluga meat	[THg] beluga meat
	Indigenous population BW	Indigenous population BW
		+pMeHg beluga meat; +RAF beluga meat
SCENARIO 2 – MARINE MAMMALS	**Beluga nikku**	
Indigenous population	*current model*	*alternative model*
	CR beluga *nikku*	CR beluga *nikku*
	[THg] beluga meat	[THg] beluga meat
	Indigenous population BW	Indigenous population BW
		+pMeHg beluga meat; +RAF beluga *nikku*; +MLF beluga meat
Sensitive indigenous population	CR beluga *nikku*	CR beluga *nikku*
	[THg] beluga meat	[THg] beluga meat
	Indigenous population BW	Indigenous population BW
		+pMeHg beluga meat; +RAF beluga *nikku*; +MLF beluga meat

Table S2. Stability of the simulations obtained by varying the number of iterations. High consumption rate of canned tuna scenario for the general population was used.

Iteration number	Median TDI (μg × kg^{-1} bw)	% of at-risk population*
10,000	0.182	0.05
50,000	0.182	0.056
100,000	0.182	0.059
1,000,000	0.181	0.0572
10,000,000	0.181	0.05772

*when TDI obtained is superior to TDI threshold of 0.47 μg × kg^{-1} bw.

Table S3. Resulting median HQ using the current and the alternative model for the general and the sensitive population (see Figs. 1 and 2). The percentage of the distribution of HQ > 1 is in parentheses (no number signifies 0%).

SCENARIO 1 – FISH FLESH **MEDIUM CONSUMPTION RATE**	**Salmon**		**Canned tuna**	
	current model	*alternative model**	*current model*	*alternative model**
Canadian general population	0.01	0.006	0.2	0.08
Sensitive Canadian population	0.03	0.02	0.5 (4%)	0.2
SCENARIO 1 – FISH FLESH **HIGH CONSUMPTION RATE**	**Salmon**		**Canned tuna**	
	current model	*alternative model**	*current model*	*alternative model**
Canadian general population	0.02	0.01	0.4 (0.1%)	0.1
Sensitive Canadian population	0.06	0.03	1.0 (50%)	0.4 (0.1%)
SCENARIO 2 – MARINE MAMMALS	**Seal liver**			
	current model	*alternative model**		
Indigenous population	0.5 (2.7%)	0.06		
Sensitive Indigenous population	1.2 (69%)	0.1		
SCENARIO 2 – MARINE MAMMALS	**Beluga meat**		**Beluga *nikku***	
	current model	*alternative model**	*current model*	*alternative model**
Indigenous population	0.2	0.1	0.1	0.08
Sensitive Indigenous population	0.5 (1.9%)	0.2	0.3 (0.01%)	0.2

Conclusion

La consommation de poissons expose l'humain au MeHg, un contaminant bien connu pour ses effets néfastes ciblant le système nerveux, autant en situation d'exposition aiguë que chronique (Bourdineaud et al., 2011; European Food Safety Authority, 2012). En conséquence, la présente thèse était articulée en deux objectifs principaux, soit (1) d'évaluer les facteurs de modulation par rapport à l'exposition au MeHg présent dans la chair de poisson (**Chapitres 1 et 2**) et (2) d'évaluer l'impact d'ajouts de variables dans l'équation d'exposition au MeHg qu'utilise présentement Santé Canada (**Chapitre 3**). Ainsi, par le biais de cette thèse, nous avons fourni des pistes de réponses aux questions suivantes :

1. Dans la chair de poisson, la concentration de MeHg varie-t-elle en fonction de la distribution des biomolécules? Comment se bioaccumule le MeHg par rapport au Se et à l'As? (**Chapitre 1**)
2. Est-ce que la cuisson de la chair de poisson diminue la biodisponibilité orale du MeHg *in vivo*? (**Chapitre 2**)
3. L'ajout de nouvelles variables (RAF, pMeHg et MLF) dans l'équation d'exposition au MeHg modifie-t-il de façon significative l'estimation de l'exposition? (**Chapitre 3**)

La prochaine section présente les principales contributions de cette thèse aux connaissances scientifiques concernant l'exposition au MeHg, ainsi que les potentielles avenues de recherches futures.

Bioaccumulation du méthylmercure, du sélénium et de l'arsenic dans la chair de poisson

Pour l'humain, la principale voie d'exposition au MeHg se fait *via* la consommation de poissons (National Research Council, 2000b). Pour contrôler l'exposition de la population à ce

contaminant, les autorités sanitaires émettent des lignes directrices quant à la consommation de poissons. Cependant, celles-ci omettent la potentielle hétérogénéité de la distribution du MeHg, ainsi que sa probable interaction avec d'autres constituants de la chair de poisson, tels que le Se, malgré que cela pourrait avoir des impacts significatifs au niveau de l'exposition du consommateur. C'est ainsi qu'au **Chapitre 1** de cette thèse, nous avons évalué la bioaccumulation intraorgane du MeHg et sa covariation par rapport au Se, à l'As ainsi qu'aux biomolécules constituant la chair du poisson. Cette étude nous a permis d'établir que la présence de muscles blancs et rouges chez le bar rayé crée un gradient important de biomolécules à l'intérieur de la musculature induisant par ricochet un gradient substantiel par rapport à la bioaccumulation des métal(loid)es qui se distribuent en fonction de leur affinité biochimique. Par exemple, à l'intérieur du même bar rayé, la concentration en MeHg et en As peut varier jusqu'à un facteur de 3 (**Chapitre 1**, Information supplémentaire). Aussi, tandis que le MeHg et le Se covarient avec la concentration d'azote (utilisée à titre de proxy pour le contenu en protéines), l'As lui, covarie avec le contenu en lipides.

Les résultats du **Chapitre 1** entraînent plusieurs retombées qui seront abordées ici-bas. Néanmoins, il serait important de répliquer le patron expérimental tel qu'utilisé dans notre étude, afin de confirmer que ces résultats sont réplicables à plus grande échelle et extrapolables pour d'autres espèces de poissons. Au sein des prochaines expériences, il serait notamment intéressant d'explorer la distribution des métal(loide)s en utilisant des espèces contenant différentes étendues de contamination, afin de savoir si les métal(loide)s conserveront leur patron de distribution intraorgane, tel qu'observé au **Chapitre 1**.

Une des implications reliées à la distribution hétérogène du MeHg dans l'appareil musculaire serait d'un point de vue toxicologique, en ce qui a trait à la santé du poisson. Effectivement, cela signifie que pour un même poisson, certains muscles possèderont un niveau de contamination qui pourrait excéder les seuils de toxicité, alors que pour d'autres, la concentration de MeHg ne devrait pas causer d'effet nocif. Dans le cadre d'une étude utilisant le *tissue residue-based approach*, où dans certains cas, seul un échantillon musculaire est considéré pour évaluer la contamination (McCarty & Mackay, 1993), cela pourrait entraîner une mauvaise interprétation des résultats, en croyant, par exemple, qu'un individu est en santé alors qu'en

réalité il serait en détresse toxicologique. En complément, au **Chapitre 1**, nous avons observé un lien positif entre la concentration de MeHg et de Se dans la chair du bar rayé sans toutefois évaluer directement la potentielle interaction entre ces deux éléments. Sachant qu'ils peuvent former des complexes HgSe insolubles (Ralston & Raymond, 2010), diminuant ainsi le potentiel toxique du MeHg, il aurait été intéressant d'explorer à l'aide d'outils métallomiques, notamment par *hyphenated techniques* (Klampfl, 2009), leur potentielle présence (Shi & Chance, 2008). Puisque le Se est aussi connu pour son effet antagoniste envers l'AsI (Zeng et al., 2005), nous avons exploré leur covariation. Toujours dans la chair du bar rayé, nous avons détecté aucun AsI, en plus d'avoir mesuré une tendance inverse entre le Se et l'As (organique). Ces résultats nous portent à croire que l'effet antagoniste du Se envers l'As dans la chair du bar rayé était plutôt négligeable.

Une seconde retombée serait au niveau de l'interprétation des résultats de bioaccessibilité qui sont utilisés afin d'estimer l'exposition du consommateur au MeHg (Alves et al., 2018; Anacleto et al., 2020; Girard et al., 2017; Torres-Escribano et al., 2010). Les résultats produits en laboratoire varient grandement, limitant leur fiabilité et leur pertinence. Par exemple, l'équipe de Torres-Escribano et al. (2010) a mesuré la bioaccessibilité du HgT dans 27 échantillons de chair d'Espadon et a obtenu une moyenne de 64 ± 14% avec une étendue de valeurs allant de 38 à 83%. Pour expliquer cette variabilité, l'hypothèse de la variation au niveau de la teneur en biomolécules de la chair a été soulevée. Effectivement, il est bien connu que la solubilisation d'un constituant d'une matrice alimentaire soit directement liée à la composition matricielle, ainsi qu'à des effets synergiques et antagonistes existants entre les différents composés, permettant le relâchement, ou non, des constituants pour leur digestion (Fernández-García et al., 2009). Au **Chapitre 1**, nous avons effectivement observé un gradient important quant à la distribution des protéines (% N) et des lipides dans la chair du bar rayé. La prochaine étape aurait été d'évaluer la bioaccessibilité du MeHg au sein des muscles rouges et blancs de celui-ci. Dans la littérature, aucune étude répertoriée n'a trouvé de lien entre le contenu en lipides et la bioaccessibilité du MeHg (Girard et al., 2017; Wang et al., 2013). Seule l'équipe de Peng et al. (2017) a trouvé un lien entre la composition de la chair et la bioaccessibilité du MeHg : elle soupçonne que la faible bioaccessibilité observée dans la chair d'écrevisse (*Procambarus clarkii*) serait reliée à sa haute

teneur en cystéine et en arginine. Les auteurs émettent l'hypothèse que la faible bioaccessibilité serait le résultat de la grande affinité de liaison entre le MeHg et la cystéine, conférant une forte liaison entre le MeHg et les protéines, rendant la solubilisation du MeHg plus difficile. Suite à ces résultats, de plus amples recherches devraient se consacrer à établir s'il existe un lien ou non entre la bioaccessiblité du MeHg et la composition fine de la chair de poisson, c'est-à-dire, la composition en acides aminés et le type de lipide qu'on y retrouve.

Effet de la cuisson de la chair de poisson sur l'exposition du consommateur au méthylmercure, au sélénium et à l'arsenic

De nos jours, la chair de poisson est majoritairement consommée cuite (Sobral et al., 2018). Plusieurs études suggèrent que cuire la chair de poisson augmenterait sa concentration de Hg (Afonso et al., 2015; Burger et al., 2003; Costa et al., 2013, 2016; Maulvault et al., 2011) et diminuerait sa bioaccessibilité (Alves et al., 2018; Girard et al., 2017; He & Wang, 2011; Ouédraogo & Amyot, 2011; Torres-Escribano et al., 2010). Cependant, ces deux derniers énoncés ne sont pas considérés par Santé Canada lors de l'émission des recommandations de consommation de poissons. Ainsi, dans le cadre du **Chapitre 2** de cette thèse, nous avons nourri des porcs avec des repas de thons crus et cuits, afin d'évaluer l'effet de la cuisson sur l'exposition du consommateur au MeHg. En parallèle, une étude *in vitro* a été conduite en utilisant des surplus de repas de thons crus et cuits conservés dans l'optique d'évaluer la bioaccessibilité du MeHg. Selon le profil sanguin des porcs, la cuisson de la chair de thon ne semble pas diminuer l'exposition au MeHg, comparativement à la chair de thon crue. Ceci fait contraste à ce que nous avons observé avec le modèle *in vitro*, où la cuisson a engendré une diminution de bioaccessibilité du MeHg significative.

Nos résultats *in vivo* sont certainement très importants, dans l'optique où ils suggèrent que les modèles *in vitro* ne sont pas suffisamment optimisés pour être utilisés directement dans les calculs d'exposition au MeHg, tels que proposés récemment dans la littérature (Anacleto et al., 2020; Gong et al., 2018). Des études subséquentes avec une taille d'échantillonnage

substantiellement plus élevée devront être réalisées avant qu'un quelconque changement soit apporté par Santé Canada au niveau des recommandations de consommation de poissons.

Un design expérimental intéressant serait de nourrir une cohorte de porcs (où $n \geq 50$) pendant une quinzaine de jours avec des poissons ayant été précédemment exposés à des isotopes stables de MeHg, tel que le $Me^{199}Hg$ ou le $Me^{201}Hg$ (Evans et al., 2016) et de procéder à un abattage de type séquentiel, s'échelonnant sur plusieurs jours, la demi-vie du MeHg étant de 27 jours dans le sang total chez le porc (Gyrd-Hansen, 1981). Les porcs seraient nourris avec du poisson contaminé au $Me^{199}Hg$ dans les premiers jours, puis contenant du $Me^{201}Hg$ dans les jours suivants. Ce plan d'expérimentation permettrait de faire un suivi d'efficacité d'assimilation du MeHg en comparant la quantité de MeHg ingérée *versus* celle éliminée *via* les fèces (Li & Wang, 2019). L'utilisation d'isotopes stables permet de discerner le MeHg administré (repas de poissons) à celui de provenance endogène, ce qui rendrait possible le calcul du pourcentage de MeHg réellement assimilé. De plus, il serait réalisable de mesurer le temps de résidence de l'isotope à l'intérieur de chaque organe d'intérêt et de monitorer un éventuel changement de spéciation (Evans et al., 2016), tel que la potentielle déméthylation au niveau des érythrocytes médiée par la sélénonéine (Yamashita et al., 2013) ou au niveau du cerveau par exemple (Clarkson & Magos, 2006). Finalement, l'utilisation de deux isotopes stables distincts de MeHg servirait à suivre la distribution et l'élimination du premier isotope administré, pendant que le 2^{e} isotope continue d'être ingéré par le porc, permettant d'évaluer le comportement du MeHg lors d'une exposition chronique (Evans et al., 2016). L'abattage séquentiel des porcs rendrait possible l'évaluation de de la demi-vie et de la bioaccumulation du MeHg au sein de différents organes, tandis que l'utilisation d'outils métallomiques (Shi & Chance, 2008) serait utile afin d'observer la spéciation du MeHg, telle que l'attendu complexe HgSe au niveau du foie (Nigro & Leonzio, 1996) et du cerveau (Korbas et al., 2010) ou encore, le proposé complexe MeHg-Se-As au niveau des érythrocytes (Korbas et al., 2008).

Des études futures devront être réalisées afin d'explorer l'effet de la cuisson sur le complexe MeHg-Cys. Effectivement, pour le moment, nos résultats du **Chapitre 2** suggèrent que la cuisson de la chair de thon n'affecte pas la biodisponibilité orale de MeHg chez le porc, mais nous avons toutefois enregistré une cinétique d'absorption plus rapide pour le MeHg provenant

de la chair cuite, comparativement à la chair crue. Est-ce que ce phénomène est relié à un changement de spéciation du complexe MeHg-Cys lors de la cuisson et que ce dernier serait plus rapidement absorbé par les entérocytes? Ou encore, serait-ce relié à une métabolisation différente des entérocytes par rapport à ce complexe modifié, qui retiendraient moins longtemps le MeHg au niveau de la paroi intestinale? Dans le but de mieux comprendre le comportement et la toxicité du MeHg, ces questions doivent être explorées.

Finalement, il serait important de mieux établir les limites des modèles de digestion *in vitro*. Bien que nous ayons mesuré une baisse significative de la bioaccessibilité du MeHg suite à la cuisson de la chair de thon, ce phénomène ne s'est pas transposé au niveau de nos résultats *in vivo* (**Chapitre 2**). Notre étude suggère que, contrairement aux croyances, ce n'est pas la totalité du MeHg solubilisée qui sera par la suite absorbée par la paroi intestinale, ou encore, que le MeHg non solubilisé serait absorbé. Dans tous les cas, pour le moment, la seule chose dont nous sommes certains est que les modèles *in vitro* ne sont pas optimisés pour estimer directement l'exposition du consommateur de poisson au MeHg.

Également, il y a l'impact des phytoéléments sur l'absorption intestinale du MeHg qui devra être élucidé. Pour l'instant, les études *in vitro* suggèrent que la co-consommation de phytoéléments avec un repas de poissons diminuerait l'exposition au MeHg, tandis que les études *in vivo* communiquent d'autres choses (voir section *La solubilisation du méthylmercure dans le tractus digestif*). À notre connaissance, aucune expérience n'a été conduite dans le but de tester précisément l'effet des phytoéléments sur l'absorption intestinale du MeHg. Démystifier l'impact des phytoéléments sur le sort du MeHg est important dans la mesure où deux recherches distinctes ont mesuré une augmentation de la concentration du HgT et du MeHg dans la circulation sanguine suite à l'ingestion de thé chez le rat (Janle et al., 2015) et l'humain (Canuel et al., 2006). L'expérience ayant comme modèle l'humain propose que le thé ait accéléré le cycle entéro-hépatique, encourageant de façon temporaire une augmentation de la concentration du MeHg dans la circulation sanguine. Le MeHg peut effectivement être éliminé *via* la bile (Clarkson, 2002; Dutczak & Ballatori, 1992), qui se déverse au moment opportun dans le petit intestin, où le MeHg peut se faire réabsorber par les entérocytes (Fig. 1). Ainsi, par l'accélération du cycle entéro-hépatique, les phytoéléments augmentent potentiellement l'exposition du

consommateur au MeHg et devraient donc faire l'objet d'études plus importantes (Roberts et al., 2002).

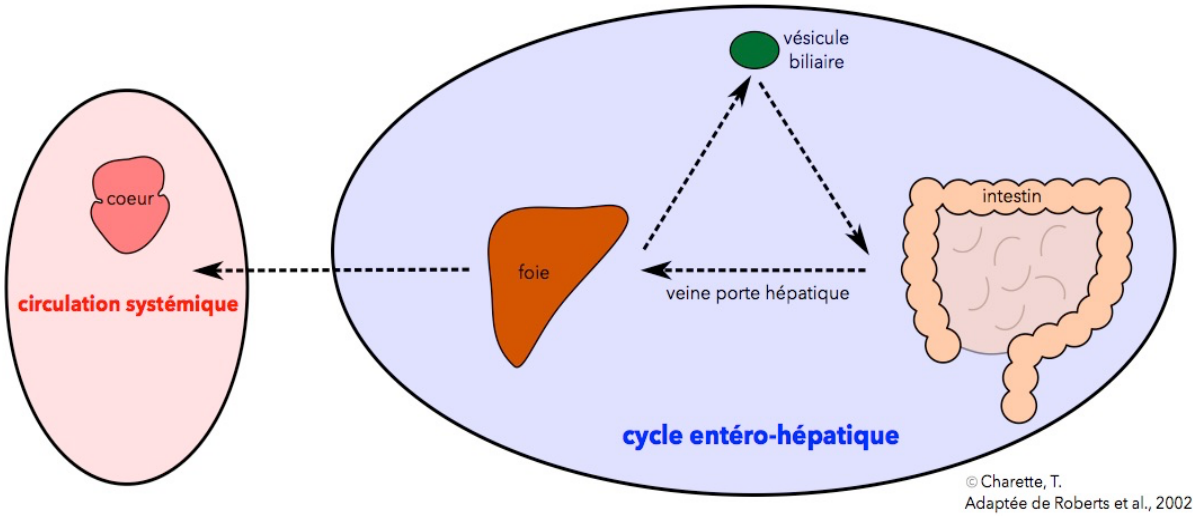

Figure 1. Schématisation du cycle entéro-hépatique.

Évaluation de l'exposition au mercure

Dans le but de protéger la population en ce qui a trait aux effets néfastes que peut causer le MeHg, Santé Canada prescrit des lignes directrices par rapport à la consommation de poissons. Cependant, dans l'objectif de vouloir protéger les populations à risques comme les enfants et les femmes enceintes, ces recommandations peuvent parfois être jugées trop limitantes dans certains cas. Au **Chapitre 3** de cette thèse, dans le but d'augmenter la représentativité de la réalité, nous avons évalué l'effet d'ajouts de nouvelles variables au niveau de l'équation actuellement utilisée par Santé Canada pour estimer l'exposition du consommateur au MeHg. Une des conclusions principales de ce chapitre est que chaque ajout individuel augmente (MLF) ou diminue (pMeHg et RAF) significativement l'exposition au MeHg résultante. Ainsi, avant d'apporter une quelconque modification à l'équation, de plus amples recherches devront être

effectuées, par souci de ne pas sous-estimer le potentiel de dangerosité que représente ce contaminant. À titre d'exemple et en lien avec le **Chapitre 2**, la proportion de MeHg qui est réellement absorbée par le consommateur est l'une des connaissances manquantes clés qui devront être approfondies.

Comme mentionné au **Chapitre 3**, l'équation d'exposition au MeHg présentement utilisée par Santé Canada arbore son lot de limites. Cependant, le calcul effectué en amont et qui mène à la DJA (0.2 μg × kg^{-1} PC × jour^{-1} pour la population sensible [femmes en âge de procréer et enfants < 12 ans] et 0.47 μg × kg^{-1} PC × jour^{-1} pour la population adulte générale) qui est utilisée dans l'estimation du QD est aussi matière à discussion.

La dose de référence n'est pas un consensus international. Par exemple, les États-Unis l'ont établie à 0.1 μg × kg^{-1} PC × jour^{-1} (U.S. EPA, 1997), tandis que de leur côté, les Européens ont déterminé une valeur de 0.19 μg × kg^{-1} PC × jour^{-1}, à la fois pour la population sensible et générale (European Food Safety Authority, 2012). Trois facteurs majeurs peuvent expliquer la différence entre les doses de références : (1) le choix de l'étude épidémiologique à partir de laquelle les données sont sourcées, (2) le choix du biomarqueur d'exposition et finalement (3) le choix du facteur d'incertitude appliqué (Mergler et al., 2007). La DJA déterminée par Santé Canada attitrée à la population générale a été calculée en utilisant les données émergeant d'enquêtes sur les cas d'intoxications au MeHg qui se sont déroulés au Japon (1950-1960) et en Irak (1970) (Legrand et al., 2010). Santé Canada a utilisé la dose sanguine sans effet nocif observable de 200 ppb pour estimer l'apport de MeHg en utilisant un modèle toxicocinétique à un seul compartiment à l'équilibre (U.S. EPA, 2019), contenant l'équation [1] suivante :

$$\text{Équation [1]} \qquad DJ = \frac{C \times E \times V}{A \times f \times PC}$$

où *DJ* correspond à la dose journalière (mg × kg^{-1} PC × jour^{-1}), *C* représente la concentration de MeHg dans le sang (ici, 0.2 mg/L), *E* est la constante d'élimination du MeHg depuis le compartiment sanguin fixée à 0.014 × jour^{-1}, *V* est le volume sanguin dans le corps (L), *A* représente la proportion de MeHg qui est absorbée (ici, 0.95 sans unité), *F* est la fraction de MeHg absorbée qui se retrouve dans le compartiment sanguin (ici, 0.05) et *PC* est le poids corporel (kg).

Après l'ajout d'un facteur d'incertitude de 10, une DJA de 0.47 μg × kg^{-1} PC × jour^{-1} en résulte (Legrand et al., 2010).

Pour sa part, la DJA déterminée par Santé Canada attitrée à la population sensible est basée sur trois études épidémiologiques conduites aux Îles Féroé, aux Seychelles et en Nouvelle-Zélande, qui visaient à évaluer l'effet de l'exposition au MeHg *in utero* sur le développement neurologique des fœtus (Legrand et al., 2010). Santé Canada a utilisé la dose sans effet nocif observable établie par Grandjean et al. (1997) de 10 ppm de MeHg mesurée dans les cheveux maternels. Cette valeur a ensuite été convertie en concentration sanguine en utilisant le ratio 250:1 (μg Hgt/mg de cheveux:μg MeHg/mL de sang) (U.S. EPA, 1997), donnant 40 ppb. Cette concentration sanguine a été incorporée à l'équation [1] et un facteur d'incertitude de 5 a été ajouté à *DJ* pour considérer la potentielle variabilité intraespèce, donnant une DJA de 0.2 μg × kg^{-1} PC × jour^{-1}.

Comme utilisé par Santé Canada, l'exposition du fœtus au MeHg peut être reconstituée en utilisant les cheveux maternels à titre de biomarqueur, puisqu'ils emmagasinent ce contaminant chronologiquement, au fur et à mesure qu'ils poussent (Boischio & Cernichiari, 1998; European Food Safety Authority, 2009). Cependant, le taux de croissance du cheveu, estimé habituellement à 1 cm × mois $^{-1}$, tend à varier intra- et interindividuellement (National Research Council, 2000b) et le taux d'excrétion du MeHg *via* les cheveux n'est pas toujours représentatif de la fréquence de consommation de poissons (Canuel et al., 2006). Ensuite, le facteur de conversion 250:1 qui est appliqué pour transformer la concentration de HgT dans les cheveux vers une concentration sanguine est sujet à débat. Effectivement, les ratios répertoriés varient de 150 à 450, ce qui pourrait mener à une sous- ou surestimation de l'exposition réelle (Bartell et al., 2000; Liberda et al., 2014; Yaginuma-Sakurai et al., 2012).

La suite logique du **Chapitre 3** est intimement reliée au **Chapitre 2**. Effectivement, il serait primordial d'effectuer une étude *in vivo* d'envergure menant à la quantification de la proportion de MeHg qui est absorbée par le consommateur (équation [1], constante *A*). En second lieu, il sera essentiel d'évaluer le taux d'élimination du MeHg (équation [1], constante *E*), ainsi que ses facteurs de modulation, sachant qu'il varie à l'intérieur de la population (Rand & Caito, 2019). Le

taux d'élimination est couramment exprimé par le paramètre toxicocinétique de la demi-vie biologique qui est importante à considérer dans la mesure où elle influence directement la courbe dose-réponse (Fig. 2). Actuellement, la demi-vie du MeHg chez l'humain est estimée à 50 jours, résultant en un taux d'élimination quotidien de 1.4%. Cette longue demi-vie confère au MeHg la tendance naturelle à se bioaccumuler dans le corps, de plus en plus, en fonction des expositions subséquentes (Rand & Caito, 2019).

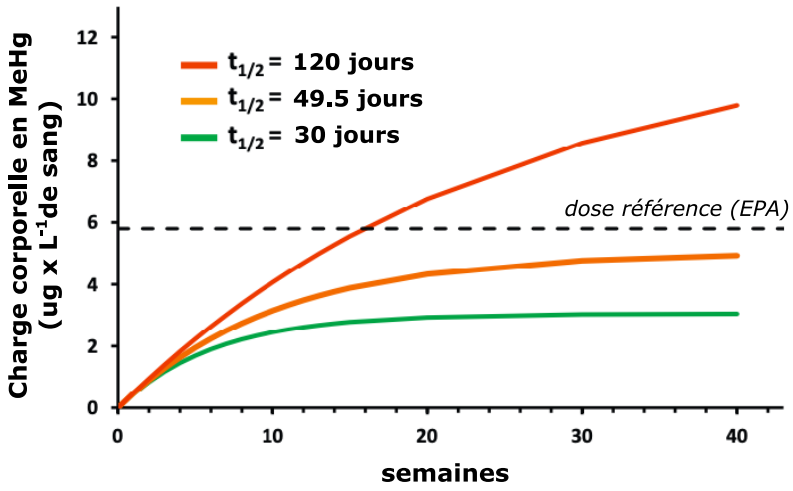

Figure 2. Influence de la demi-vie du MeHg sur la charge corporelle, lors d'une consommation hebdomadaire de thon. Adaptée de Rand & Caito (2019).

La figure 2 représente bien l'importance de la demi-vie au niveau de la charge corporelle en MeHg. Dans cet exemple tiré de Rand & Caito (2019), ils ont simulé la concentration sanguine en MeHg en utilisant les paramètres de l'équation [1], en supposant une consommation d'un repas de thons hebdomadaire et en faisant varier la demi-vie du MeHg en fonction des valeurs humaines trouvées dans la littérature. Sur la figure 2, la dose référence correspondant à une concentration sanguine de 5.8 $\mu g \times L^{-1}$ (représentée par une ligne pointillée) a été calculée rétrospectivement en utilisant l'équation [1] pour trouver *C*, dans le cas où la *DJ* est l'équivalente de la DJA états-unienne (0.1 $\mu g \times kg^{-1}$ PC × jour $^{-1}$). On peut observer qu'une demi-vie de 120 jours mène à une charge corporelle qui dépasse la dose référence de 5.8 $\mu g \times L^{-1}$. Ainsi, dans la mesure où les lignes directrices établies par les instances de la santé peuvent parfois mener à un risque

pour certains individus, les facteurs de modulation reliés au taux d'élimination du MeHg devraient être davantage élucidés.

Perspectives

Bien que Santé Canada travaille avec beaucoup d'ardeur dans le but d'émettre des lignes directrices adéquates par rapport à la consommation de poissons, reste que celles-ci contiennent des failles, principalement en raison de la grande variabilité interpopulationnelle observée quant à la métabolisation du MeHg, menant à un manque important de données probantes. Malgré que cette thèse représente l'équivalent d'une goutte d'eau dans l'océan par rapport à tout ce qui reste à élucider, elle aura contribué à l'avancement des connaissances quant à la bioaccumulation du MeHg, du Se et l'As dans la chair de poisson en fonction de sa composition en biomolécules (**Chapitre 1**), à l'utilisation du modèle porcin conjugué au modèle *in vitro* pour évaluer l'effet de la cuisson de la chair de thon sur le sort du MeHg (**Chapitre 2**) et finalement, aux limites que comportent l'équation déterministe actuellement utilisée par Santé Canada pour évaluer l'exposition au MeHg (**Chapitre 3**). Les résultats de cette thèse, particulièrement les **Chapitres 2 et 3**, agiront à titre de base pour la conception de projets futurs, notamment en ce qui a trait à l'évaluation du taux d'absorption et d'élimination du MeHg et de leurs facteurs de modulation associés.

Un enjeu qui n'a pas été abordé dans cette thèse, mais qui est de haute importance, consiste à considérer la balance entre le risque et les bénéfices reliés à la consommation de poissons. Pour le moment, Santé Canada recommande de choisir judicieusement les espèces de poissons consommées, en donnant la priorité aux moins contaminées en MeHg et aux plus riches en acides gras polyinsaturés n-3 à longues chaînes, comme le saumon et l'anchois (Santé Canada, 2007, 2019b). Les acides gras polyinsaturés n-3 à longues chaînes sont connus pour leurs bienfaits sur la santé cardiovasculaire (Santé Canada, 2019b). Pour cette raison, des analyses du risque et des bénéfices se conduisent en comparant le contenu en acides gras à sa dose quotidienne

recommandée, tout en considérant le contenu en MeHg, en utilisant l'équation suivante (Ricketts et al., 2019; Strandberg et al., 2016):

$$QD = \frac{dose\ recommandée\ acides\ gras\ \times\ [MeHg]}{[acide\ gras]\ \times\ DJAMeHg\ \times\ PC}$$

où *QD* représente le quotient de dangerosité, *dose recommandée acides gras* est la dose d'acides gras polyinsaturés n-3 à longues chaînes recommandée quotidiennement, *[MeHg]* est la concentration en MeHg dans le poisson, *[acide gras]* est la concentration en acides gras polyinsaturés n-3 à longues chaînes dans le poisson, *DJAMeHg* représente la dose journalière admissible de MeHg *et PC* est le poids corporel. Dans le cas échéant où QD > 1, l'équipe de Strandberg et al. (2016) suggère que la toxicité du MeHg excède le potentiel effet bénéfique des acides gras.

Une seconde méthode répertoriée dans la littérature permettant de quantifier le risque et les bénéfices est de comparer le ratio molaire du HgT au Se. Ceci provient du fait que plusieurs expériences ont confirmé que la toxicité du MeHg provenait de son habileté à séquestrer le Se (Huggins et al., 2009; Korbas et al., 2010), menant à l'inhibition des sélénoenzymes qui sont essentielles au bon fonctionnement du cerveau (Ralston & Raymond, 2010). En prenant pour acquis qu'une mole de Hg séquestre une mole de Se, on peut utiliser l'équation du *selenium Health-Benefit-Value (HBV$_{Se}$)* (Ralston et al., 2016) :

$$HBV\ Se = \left(\frac{Se - Hg}{Se}\right) \times (Se + Hg)$$

L'utilisation du *HBV$_{Se}$* permet d'estimer si la nourriture ingérée augmentera ou diminuera le statut du Se physiologiquement disponible : un *HBV$_{Se}$* positif signifie que le Se est en excès par rapport au Hg, tandis qu'un résultat négatif désigne l'inverse (Ralston et al., 2016). Une façon intéressante de bonifier de cette équation serait de considérer la spéciation chimique du Se et du Hg.

Bien que les deux méthodes ci-haut présentées soient intéressantes, elles ne considèrent pas la présence de contaminants additionnels avec lesquels le MeHg pourrait avoir une potentielle interaction. Par exemple, une étude *in vitro* a suggéré une interaction synergique

probable entre MeHg et les polychlorobiphényles, qui pourrait créer un effet nocif sur le système neurologique du consommateur (Bemis & Seegal, 1999). D'ailleurs une étude récente portant sur les poissons des quatre Grands Lacs (Canada) stipule qu'en considérant la présence du MeHg et des polychlorobiphényles dans la chair de ceux-ci, près de 50% des recommandations de consommation ne seraient pas suffisamment protectrices (Gandhi et al., 2017). Ceci souligne bien le besoin de développer un modèle d'évaluation du risque et des bénéfices allant au-delà de la simple observation des contaminants considérés individuellement.

Impossible de clore cette thèse sans aborder la problématique de la sécurité alimentaire actuellement vécue dans le nord du Canada, principalement dans les communautés autochtones (Conseil des académies canadiennes, 2014). La question du risque et des bénéfices est particulièrement importante dans ce contexte, où la consommation de la nourriture traditionnelle est parfois essentielle à l'atteinte d'une sécurité alimentaire adéquate (Van Oostdam et al., 1999). La nourriture traditionnelle peut être décrite comme étant [traduction] « tous les aliments d'une culture particulière disponibles à partir de ressources naturelles locales et culturellement acceptés » (Kuhnlein & Receveur, 1996). Bien que la nourriture traditionnelle possède une valeur nutritive hautement supérieure à la nourriture commerciale, elle présente aussi dans certains cas son lot de contaminants (AMAP, 2009). Particulièrement, certaines espèces de mammifères marins sont connues pour contenir de fortes teneurs en MeHg (Lemire et al., 2015), alors qu'un grand éventail d'animaux terrestres et marins sont contaminés en acides per- et polyfluoroalkyles (PFASs) à longues chaînes, un enjeu grandissant dans l'Arctique canadien (AMAP, 2017). Cependant, les bénéfices qu'apporte la nourriture traditionnelle aux communautés sont de nature qualitative. Notamment, elle contribue à la santé physique, mentale, spirituelle, émotionnelle et sociale (Conseil des académies canadiennes, 2014). De son côté, le risque est plutôt quantifiable (ex. concentration d'un contaminant), ce qui rend très complexe l'estimation de l'impact net de la consommation de la nourriture traditionnelle sur la santé du consommateur (Watzl et al., 2012). Le développement d'un outil qui permettrait de balancer le risque et les bénéfices quantitatifs et qualitatifs urge.

Finalement, comme mentionnée ci-haut, la consommation de nourriture traditionnelle comporte son lot de risques et bénéfices, rendant la communication du risque complexe qui doit

être faite avec prudence (Kuntz et al., 2010). Effectivement, une communication inadéquate de ces recommandations envers la population peut mener à une mauvaise interprétation de celles-ci et engendrer de la confusion, de la peur et un changement drastique des choix alimentaires orientés davantage vers la nourriture commerciale de moindre qualité nutritionnelle (Furgal et al., 2005). Little et al. (2020) stipule que l'élaboration de stratégies d'évaluations du risque et de communications adaptées à la culture qui seraient fondées sur les connaissances traditionnelles locales, ainsi que sur les connaissances scientifiques, est cruciale. Beaucoup de travail reste à faire quant à la gestion du risque sanitaire d'origine environnementale dans le nord du Canada. En attendant, les scientifiques encouragent les communautés à continuer de consommer la nourriture traditionnelle contenant une faible teneur en MeHg, dans le but de maintenir l'apport important en nutriments qu'elle apporte (Laird et al., 2013; Lemire et al., 2015).

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