Retrospective mercury exposure and current symptoms of nervous system dysfunction in adults of a First Nation community (Canada)

Aline Philibert

Universite du Quebec a Montreal

Myriam Fillion

TÉLUQ University

Judy Da Silva

Grassy Narrows First Nation

Tanya Suvendrini Lena

Women's College Hospital

Donna Mergler (✉ mergler.donna@uqam.ca)

Universite du Quebec a Montreal <https://orcid.org/0000-0002-5205-5180>

Research

Keywords: mercury, past exposure, First Nation, nervous system dysfunction, symptom clusters, clustering, structural equation modelling, mixed effects model

Posted Date: October 18th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-952530/v1>

License: (cc) (i) This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

**Retrospective mercury exposure and current symptoms of nervous system dysfunction in
adults of a First Nation community (Canada)**

Aline Philibert⁽¹⁾, Myriam Fillion^(2,1), Judy Da Silva ⁽³⁾, Tanya Suvendrini Lena ⁽⁴⁾, Donna
Mergler⁽¹⁾

⁽¹⁾ Université du Québec à Montréal, Centre de recherche interdisciplinaire sur le bien-être, la
santé, la société et l'environnement (Cinbiose), CP 8888, Succ. Centreville, Montréal, Québec,
Canada, H3C 3P8

⁽²⁾ Département Science et Technologie, Université TÉLUQ, 5800 Saint Denis St, Montréal,
Québec, Canada H2S 3L4

⁽³⁾ Grassy Narrows First Nation, General Delivery, Grassy Narrows, Ontario, Canada
P0X 1B0

⁽⁴⁾ Women's College Hospital 76 Grenville St, Toronto, Ontario, Canada M5S 1B2

corresponding author:

Donna Mergler

mergler.donna@uqam.ca

CINBIOSE

Université du Québec à Montréal

CP 8888, succ Centreville

Montréal, Québec

Canada H3C 3P8

Abstract

Background: The watershed in Asubpeeschoseewagong Netum Anishinabek (Grassy Narrows First Nation) territory has been contaminated by mercury (Hg) since 1962, resulting in very high Hg concentrations in fish, central to the community's culture, traditions, economy and diet. Biomarkers of Hg (umbilical cord blood and hair/blood samples), monitored between 1970 and 1997, decreased over time. A recent Grassy Narrows Community Health Assessment (GN-CHA) survey included current symptoms of nervous system dysfunction. The present study aimed to cluster self-reported symptoms and examine their relation to past Hg exposure.

Methods: The GN-CHA included 391 Registered adult Band members. Symptom clustering used a two-step segmentation approach. Umbilical cord Hg and yearly measurements of hair Hg were available for 242 participants. Longitudinal Mixed Effects Models (LMEM) served to examine past hair Hg with respect to clusters. Structural Equation Models (SEM) displayed direct and indirect pathways between Hg exposure and clusters, with Hg exposure modelled as a latent variable or in separate time periods (prenatal, childhood and having had hair Hg $\geq 5\mu\text{g/g}$ at least once).

Results: A total of 37 symptoms bonded into 6 clusters, representing Extrapyrarnidal impairment, Sensory impairment, Cranial nerve disturbances, Gross motor impairment, Neuro-cognitive deficits and Affect/Mood disorders. Median Hg concentrations were $5\mu\text{g/L}$ (1-78.5) and $1.1\mu\text{g/g}$ (0.2-16) for umbilical cord and childhood hair, respectively. More than one-third (36.6%) had hair Hg $\geq 5\mu\text{g/g}$ at least once. LMEM shows higher long-term hair Hg among those with higher scores

for most clusters. In SEM, latent Hg is directly and indirectly associated with all clusters. The contribution of prenatal exposure is mediated by childhood exposure, except for Affect/Mood disorders, where the association is direct. Hair Hg $\geq 5 \mu\text{g/g}$ is directly related to Extrapyramidal and Sensory impairment.

Conclusion: Our findings provide evidence that in this First Nation community, past Hg exposure from fish consumption was associated with later-life clusters of coexisting symptoms of nervous system dysfunction. Given the complexity of the interrelations between the various determinants of chronic health problems in this and other First Nation communities, LMEM and SEM methods provide the opportunity to trace direct and/or indirect effect routes of potential “causal impact”.

Keywords: mercury, past exposure, First Nation, nervous system dysfunction, symptom clusters, clustering, structural equation modelling, mixed effects model

Background

In the 1960's, a chloralkali plant discharged approximately 10,000 kg of mercury (Hg) into the Wabigoon-English River system in Northwestern Ontario, contaminating fish resources as far as 250 km downstream (1). Asubpeeschoseewagong Netum Anishinabek (Grassy Narrows First Nation) and Wabaseemoong Independent Nation (previously known as Whitedog and Islington Bands), for whom walleye (*Sander vitreus*) is central to their traditions, culture, economy and health, were seriously affected by this disaster (2). Between 1970 and 1997, governmental agencies carried out biomonitoring programs in these communities to assess Hg concentrations in blood and hair (3). Another program (1970-1992) assessed Hg concentrations in umbilical cord bloods collected at the local hospital (3, 4). Biomarker Hg concentrations from Grassy Narrows First Nation followed a similar pattern to Hg levels in local fish from the contaminated river system, with extremely high concentrations in the early 1970's, a sharp decline until 1977 and a less pronounced decline until 1987, after which, mean concentrations remained relatively stable (5, 6).

In 1975, neurological examinations of 89 residents from the two communities, carried out by Dr. Masazumi Harada and colleagues (7), showed a high prevalence of signs and symptoms similar to those reported by patients with Minamata Disease. The team revisited the communities in 2002 and 2004 and examined 175 individuals. Sixty (60) persons were diagnosed with Minamata Disease, 54 with Minamata Disease with complications due to other diseases, and 25 with "suspicion of Minamata Disease" (7). Self-reported symptoms included numbness, pain in limbs, joints and back, decreased vision, impaired hearing, cramps in limbs, dizziness, tendency to fall,

forgetfulness, impaired finger movement, tremor and speech impairment. For 27 persons who had been examined in 1975, symptoms had worsened (7). Takaoka and co-authors (8) compared self-reported symptoms of volunteers from the Grassy Narrows First Nation community from examinations performed in 2010, with residents from the Minamata area and a reference group from other areas of Japan. The authors observed that the prevalence of specific and non-specific complaints of older persons from Grassy Narrows was similar to that of those from the Minamata area, while the prevalence among younger persons from Grassy Narrows was lower, but higher than the Japanese reference group (8). While signs and symptoms reported in this study are consistent with methylmercury poisoning (9), the findings are limited by the recruitment strategy and the absence of measurements of mercury exposure.

In 2015, Grassy Narrows First Nation undertook a Community Health Assessment survey (GN-CHA), which included a list of 59 symptoms, as well as questions on current and past fish consumption. Although understanding one single symptom is worthwhile *per se*, people rarely present with one single symptom, but rather with an array of multiple coexisting symptoms (10). Symptom clusters represent a group of two or more related concurrently occurring symptoms (10), whose pattern reflects the nature of the underlying dysfunction (11). New multidimensional approaches and statistical techniques have been proposed to facilitate the integrated analysis of multiple symptoms (10, 12-15). Over the same period, a growing body of research has used advanced statistical techniques to examine the contributions of Hg exposure (16-26). The objective of the present study was to bond GN-CHA reported symptoms of nervous system dysfunction into clusters and to examine their association to long-term and past Hg exposure.

Methods

The present study, carried out in collaboration with the Grassy Narrows First Nation community in Northern Ontario, Canada, uses clustering, longitudinal mixed effect models (LMEM) and structural equation models (SEM) to examine the associations between current reported symptoms from GN-CHA survey and retrospective Hg exposure, using historic biomarker data from a governmental monitoring program.

GN-CHA Survey

The GN-CHA survey for adults (18 years old and over), initiated in 2015, included 266 questions that covered many aspects of their life and health: demographics, education, generational attendance of residential school, work and income, food consumption, health status, diabetes care, wellness and mental health, injuries, disability, health care access, physical activity, smoking, drinking and drug use, community wellness and traditional culture. Most questions were taken from the First Nation Regional Health Survey 2008/2010 (FNRHS 2008/2010) (27), which provided a basis for comparison with other First Nation communities in Canada. Specific questions were added about fish consumption at different periods of one's life, as well as illnesses and symptoms consistent with Hg poisoning. The questionnaire was pre-tested with 10 community members in July 2016. A house-to-house survey design was used, with the questionnaire on a web-based platform.

Survey administration was supervised by two field coordinators from Grassy Narrows; nine local interviewers went from house-to-house between December 2016 and March 2017. A total of 213

houses were identified on the reserve; 83.6% of houses were surveyed. Persons living off-reserve were recruited using convenience sampling. The results of the survey were presented to and discussed with community members during several small group and community meetings. The final report was approved by Chief and Council and a summary was made public on May 24, 2018.

A total of 425 adults participated in the survey, of whom 391 (92%) are Registered Grassy Narrows First Nation members (Band members). At the time of the survey, 303 were living on-reserve and 88 off-reserve.

Biomarkers of mercury exposure

At the request of Chief and Council of Grassy Narrows First Nation, the authors obtained from the First Nations and Inuit Health Branch (FNIHB) of the Ministry of Indigenous Services Canada and the Ontario Ministry of Health and Long-term Care, archived Band members' historic biomarker data (hair and/or blood Hg concentrations), gathered between 1970 and 1997 and Hg umbilical cord Hg data, collected between 1970 and 1992 at the hospital where Grassy Narrows women gave birth.

The blood and hair samples were taken as part of a monitoring program of the Medical Research Branch of Health Canada. The objective of the program was to identify persons whose Hg biomarker concentrations surpassed the guidelines of the time. There was no sampling strategy. The program primarily targeted fishing guides and their families, but anyone could volunteer and provide a sample (4). Some years the sampling period was longer than others and there was no consistency in the month that sampling was carried out.

Samples were analysed for total mercury at Health Canada laboratories (4, 28, 29). According to Farant et al. 1981 (28), in the initial years, samples were analyzed according to the Magos method (29) and later, a more efficient and less time-consuming method used an improved cold-vapor atomic absorption technique. The two methods were highly correlated for blood ($r = 0.98$) and hair ($r = 0.97$) (28).

We assembled a retrospective database using the highest measure of equivalent hair Hg concentration for each year sampled (6). Since fish consumption varied throughout the year, the corresponding month was also recorded. For the present study, year-based hair Hg measurements ($n = 1018$) were available for 208 (53.2%) of the 391 Grassy Narrows Band member participants in the GN-CHA. Of the 211 cord blood samples collected for newborns from Grassy Narrows, 99 participated as adults in the GN-CHA, making up 48.5% of participants born in Kenora between 1970 and 1992. A total of 242 GN-CHA participants had at least one hair and/or umbilical cord blood Hg measurement.

Figure 1 shows the breakdown of biomarker sampling among GN-CHA participants.

[Place Figure 1 here](#)

Hg exposure over time

Direct measurements of hair Hg over time are presented in Figure 2a. The number of times each person was sampled varied between 1 and 23; 75 persons (36.1%) had 5 or more measurements, while 32 (15.4%) had 10 or more.

Prenatal Hg exposure

Direct measurements (n = 99) of umbilical cord blood were used where available. Estimated Hg values were predicted from linear regression models using the 211 umbilical cord data (Figure 2b) on year of birth for the three periods of Hg exposure (1970-1976, 1977-1987 and 1988-1992). These estimates were then adjusted on where the person's mother had spent her pregnancy and/or gave birth. For those who were born or whose mother spent her pregnancy in the Wabigoon-English River region between 1970 and 1992, the mean measured umbilical cord blood Hg for their year of birth was used. For those born prior to the Hg discharge in 1962, we attributed 1 µg/L for cord blood Hg, while for those born between 1962 and 1970, mean cord blood Hg in 1970 was used (65.7µg/L), and for those born after 1992, mean cord blood Hg from 1992 was applied (4.48 µg/L). Finally, for individuals, whose mother spent her pregnancy and delivered elsewhere, cord blood Hg was set at 1.0 µg/L.

[Place Figure 2 here](#)

Childhood Hg exposure

Direct childhood hair Hg measurements were available for 137 GN-CHA participants, for whom we used the mean Hg concentration between 5 and 15 years of age. Figure 3 shows children's

measured hair Hg concentrations for the year in which they were 10 years old, with respect to fish consumption (several times a month vs less). Multiple regression analyses with direct measurements of hair Hg show significant associations with GN-CHA data on fish consumption at around 10 years of age and residential school attendance (i.e. being outside the community).

[Place Figure 3 here](#)

Childhood estimated Hg was derived from the overall mean hair Hg in children on the year in which they turned 10 years of age, and then adjusted on reported child fish consumption and having attended a residential school. For participants, who were 10 years old before 1962, hair Hg was set at 0.1 µg/g. For those who were 10 years of age between 1962 and 1970, the mean measured hair Hg in children in 1970 was used. Since there were few data after 1990 and there appears to be a plateau over this last period (Figure 3), we attributed 0.81 µg/g for those who were 10 years of age after 1990. For the 39 participants, who were missing hair Hg data between 5 and 15 years old and who did not answer the question about childhood fish consumption in the GN-CHA survey, we were unable to estimate childhood Hair Hg exposure.

Validity

To assess the validity of the estimated measures, we examined the correlations between direct and estimated (indirect) values for both umbilical cord and childhood hair Hg, using non-parametric tests (Spearman).

220 *GN-CHA questionnaire data*

221 For the present study, we used the following variables from the GN-CHA: Demographics: age,
222 sex, living on/off reserve, schooling, generational attendance of residential school in the family.
223 Work: currently working (yes/no), currently looking for work (yes/no), reasons for not working
224 (disability or illness/other). Food security: struggle to pay for food once a month or more in the
225 last 6 months. Fish consumption: childhood fish consumption at 10 years of age (5 categories
226 grouped into once a month or less/at least several times a month); walleye consumption over the
227 past year (not at all/a few times/often). Lifestyle: current smoking (yes/no) and current alcohol
228 consumption (heavy drinking (yes/no), defined in the FNRHS 2008/2010 (27) as 5 drinks in one
229 drinking occasion at least once/month in the past 12 months. Health status: obesity (yes/no) was
230 categorized using Body Mass Index ≥ 30 kg/m² and at least one reported diagnosed chronic health
231 conditions (yes/no), as listed in the FNRHS 2008/2010 (allergies, arthritis, asthma, cancer, chronic
232 back pain, chronic bronchitis, diabetes, emphysema, heart disease, hepatitis, high blood pressure,
233 liver disease, osteoporosis, rheumatism, stomach or intestinal problems, thyroid problems, and
234 tuberculosis) and at least one diagnosed nervous system disorder (blindness, epilepsy, Bell's palsy,
235 cerebral palsy, muscular dystrophy, Kennedy's disease, Parkinson's diseases, Alzheimer, senile
236 dementia, psychological/nervous disorders, cognitive/mental disorders, Attention deficit
237 hyperactivity orders (ADHD), learning disability).

238

239 The GN-CHA included 59 self-reported symptoms, rated on a 5-point Likert rating scale ("*Never*",
240 "*Rarely*", "*From time to time*", "*Very often*", "*All the time*"). Higher scores on the Likert rating
241 scale indicate greater frequency of symptoms.

242

Symptoms' clustering

Self-reported symptoms from the GN-CHA survey (n =391) were clustered based on their Likert scale, using a simultaneous two-step multivariate segmentation approach (30-32). One is agglomerative and aims at maximising a homogeneity criterion by successively aggregating the variables into clusters using a hierarchical ascendant clustering algorithm. The other is representative and creates a composite variable from a weighted linear combination of symptoms (synthetic variable that can be read as a gradient) for each cluster using a mixed factorial approach (PCAMIX method) (30, 32, 33). For each composite variable (cluster), all participants receive a score based on his/her symptom frequency.

A bootstrap approach was used for maximizing the homogeneity criterion within clusters and determining the suitable number of clusters. The desired number of clusters (K) was determined from the analysis of aggregation levels, stability of the partitions via bootstrapped mean-adjusted Rand Index and boxplots. The mean-adjusted Rand index was based on the generation of 60 bootstrap samples among all 391 participants. Each cluster was carefully inspected based on its proportion of variation explained (a minimum of 50% was mandatory) and on degree of “closeness” amongst the symptoms being clustered (a minimum of 0.6 squared correlation of the variable with its composite variable was required). Some symptoms add noise while providing little or no information in identifying the underlying pattern inherent to the cluster (34). Finally, we tested for the “driving” symptoms that best reflect the cluster. We validated the cluster results, using the VARCLUS function of the SAS computer application (JMP Professional 15.0 software).

A confirmatory factor analysis (CFA) was conducted to evaluate the construct validity of symptom clustering using a series of fit parameters (35-39). To achieve this, we used the following: Chi-square divided by the degrees of freedom (χ^2/df), Standardized Root Mean Square Residual (SRMR), Root Mean Square Error of Approximation (RMSEA), Comparative Fit Index (CFI) and Tucker Lewis Index (TLI). Internal consistency (scale reliability) for each cluster was tested using the test of Cronbach alpha (35, 40-42). Construct validity for each cluster was also confirmed by the neurologist (T. S. Lena).

The composite variable serves to validate the clusters based on a factorial approach and provides a measure (gradient). The composite variable, as a measure, is used in the LMEM, but a latent variable, which bonds the symptoms in the cluster into a construct is used in the SEM.

Longitudinal mixed effects models

LMEMs were performed using direct measurements of longitudinal hair Hg with respect to the composite variable for each cluster. We chose mixed effect modeling (MEM) as it is robust to missing data and irregularly spaced sampling; it also handles both time-invariant and time-varying covariates (43). MEM enables multilevel modeling and partitioning of the covariance structure (random effects). We tested the following fixed and/or random effects for covariates (age category, sex, year of sampling, socioeconomical status, lifestyle and medical history, age at sampling and sampling season). The covariates were kept in the MEM if they showed a p-value ≤ 0.10 or if they substantively altered the cluster ($\geq 20\%$ change). The normality of residuals was tested using

a q-q plot. The most appropriate model was selected using the Akaike Information Criterion (AIC), the Bayesian Information Criteria (BIC) and the likelihood ratio (LR) test at $p \leq 0.05$.

For the longitudinal approach, LMEM requires repeated measures. Because of the absence of a biomarker sampling strategy, over half (56%) of the 208 persons with hair Hg measurements had 3 repeated measurements or less. Despite the flexibility of MEM with missing data, we preferred not to impute Hg levels for people whose diet and environmental context varied over time, particularly when living off-reserve. For the LMEM, we only included participants with at least hair Hg measurements (17 men and 15 women; total of 447 hair Hg measurements, covering a period from 10 to 23 years (median 21 years)).

To ensure that there were sufficient observations for the analyses, we estimated the minimal required sample size, based on formulas from Hedeker and co-authors (44) and direct calculations using the G*power software (45-47). Since one centimeter of a hair sample represents an accumulation of Hg during approximately one month (48), we used a low correlation of repeated measures between yearly-based samples (ρ 0.1 and 0.2). Because the effect size was unknown, 0.25 was chosen. Power analyses were set at 80%, with a two-tailed 5% hypothesis test, and 10 time points; the minimal number of participants required was between 29 and 33.

Structural equation models

SEM is a comprehensive methodology for representing, estimating, and simultaneously testing a network of complex relationships. This multivariate technique can incorporate observed (measured) and unobserved variables (latent), while traditional techniques only allows for

measured variables. The latent variable is an unobservable multidimensional construct that cannot be directly measured (49, 50).

In the SEM for the symptom clusters, we use the latent variable rather than the composite variable because it better reflects the structural concept of co-occurring symptoms. A latent variable is likewise used for Hg exposure at different times (prenatal, childhood and having had $\geq 5\mu\text{g/m}$ hair Hg at least once over the monitoring period).

SEM allows for data from multiple exposures and multiple outcomes to be considered simultaneously (51). It is based on an interrelated system of linear regression equations that extend the possibility of relations between observed and latent variables, as well as direct and indirect (mediation) relations and allows for flexibility in modeling covariance structures (19, 39, 52, 53). Finally, SEM provides path diagrams that are useful to understand and interpret the contribution of the various variables. The standardized factor loadings allow for pathway comparison.

For the SEMs, we tested the associations between each cluster and Hg exposure, represented either by a latent variable (Latent Hg) or by separate prenatal, childhood and having had $5\mu\text{g/g}$ hair Hg at least once in their lifetime (Hair Hg $\geq 5\mu\text{g/g}$) in the same model. Covariates (age category, sex, year of sampling, residence, socioeconomic status, lifestyle, obesity and medical history) were kept in the model at a p-value ≤ 0.10 or if they substantively altered the model ($\geq 20\%$ change).

For each cluster, we verified possible mediation between covariates and Hg exposure (individual variables or Latent Hg) and the dependent latent cluster symptoms variable. Given the expected

association of prenatal Hg exposure with childhood Hg exposure in the context of a high fish-consuming population, we tested whether childhood Hg exposure mediated the relation between prenatal Hg exposure and the dependent latent cluster symptoms variable. Mediation through hair Hg $\geq 5 \mu\text{g/g}$ was also tested. Finally, we tested whether sex modified the relation between past Hg exposures, covariates, and the latent cluster symptoms variable (moderation). Moderated mediation was also tested in SEM with age and sex variables.

Variables with a skewed distribution were log (base 10) transformed. Because latent variables, Hg exposures and covariates, are all on different scales, we used standardized path coefficients to test the magnitude of relations. Standardized coefficients indicate the expected amount of change in standard deviation (SD) in the dependent latent symptoms cluster for an increase of one SD in the predictor, while other predictors (covariates) are kept constant. The adequacy of model fit to the data was determined using multiple tests, χ^2/df , CFI, TLI, RMSEA, SRMR and Akaike's Information Criterion (AIC) and Schwarz's Bayesian information criterion (BIC) (35-39). Diagonally weighted least squares (DWLS) adjustments in SEM were used to take into account the ordinal nature of symptom frequency outcomes. To improve the reliability of the latent construct's scale or the models, we examined residual variances between indicators. Modification indices (MI) were used to assess the addition or deletion of variables and/or associations to improve the goodness of fit of the SEM. The sequence of initial fit, modification (MI) and refitting were repeated until the best fit SEM was attained.

To ensure that the database of 242 participants was sufficient to run SEM, two types of power analyses were performed: i) to detect model misspecification and ii) to detect target effect

(influence of Hg exposure on the symptom cluster) (54). *A-priori*, post-hoc, and compromise power-analyses were run simultaneously to detect model misspecification based on Chi-square likelihood-ratio and RMSEA tests of close and not-close fit, (55-58). The power for detecting specific target effect was determined with simulated data in SEM (54).

For the SEM, validated prenatal and childhood Hg exposure estimates, described above, were used when there was no measurement, while for GN-CHA variables, missing data were handled and imputed using Multivariate Imputation by Chained Equations (MICE).

To support the SEM, we ran a series of Directed Acyclic Graphs (DAG) for sensitivity analyses. DAG is a visual representation of causal assumptions that makes underlying relations explicit. It is used to detect overt bias, notably through backdoor paths that identify the presence of confounding (59, 60). Modifications to DAG-implied adjustment sets include conditioning confounding and variables with ambiguous causing roles (61, 62).

Threshold of statistical significance in all analyses was set at $p \leq 0.05$.

Database management and descriptive statistical analyses were performed using JMP Professional 15.0 (Statistical Analysis Hardware (SAS Institute). LMEM were conducted with Stata 16 software (StataCorp. 2019. Stata Statistical Software: Release 16.0. College Station, TX: Stata Corporation). All other analyses were computed using the R statistical software version 3.6.1. (R Core Team, 2016). We used the R package ClustOfVar (31) for clustering analyses and the MICE (Multiple Imputation by Chained Equations) package for multiple imputation of missing data in

CFA and SEM. PairedData and dplyr packages were used for paired-t tests. CFA and SEM models were computed with the R package Lavaan (39). The dagitty and ggdag R packages were used for analysing SEM and DAG. *A-priori*, post-hoc, and compromise power-analyses were computed using the semPower package. To assess power for detecting a target effect in SEM we used an online tool: yilinandrewang.shinyapps.io/pwrSEM/ (63).

Results

Relevant characteristics of the 391 adults who participated in the GN-CHA study and whose data was used for symptom clustering, are presented by age category in Tables 1 and 2. In this community, age also reflects changes in Hg exposure over time. Almost half of the participants are between 30 and 49 years of age, with no difference in age distribution between women and men. There are important age differences in schooling. Proportionally fewer older persons had attended high school, however, over half of those 30 years and older had furthered their education through post-secondary training programs (data not shown). In Canada, First Nation children were placed, often forcibly, in residential schools, with harmful effects on themselves and their families (64, 65). In the present study, no one born after 1978 had attended residential school; for those born prior to this date, 58 persons (50.0%) had been in a residential school, while for their mothers and/or fathers, it was 95.4%. At the time of the survey, 51.1% of women and 43.1% of men are working, excluding persons studying, retired or at home with children ($n = 32$). Among participants who report not working, 78% of those between 18 and 49 years of age are looking for work, while

31 persons (68.9%) of those over 30 years of age, who are not looking for work, report that it is due to illness or disability.

There is no age difference for difficulty paying for food, which is reported by almost 25% of participants. The prevalence of current smokers and heavy drinkers significantly decreases with age. Obesity is most prevalent among those 30-49 years old, of whom two-thirds are women. Sixty percent of participants report that a health professional had told them that they have at least one chronic health condition; the prevalence increases significantly with age.

Table 1. The relative frequency of the characteristics of the population within each age category.

	N ^a	18-29 y n (%)	30-49 y n (%)	50+ y n (%)	Total n (%)	Chi-square (LR) ^b p-value
Sex	391					
Women		66 (53.3%)	97 (51.6%)	48 (60.8%)	211 (54%)	1.94 0.380
Men		58 (46.7%)	72 (48.4%)	31 (39.2%)	180 (46%)	
Living on reserve	390	95 (76.6%)	144 (77.0%)	62 (78.5%)	301 (77.2%)	0.10 0.949
Schooling						
Attended school	382	122 (100%)	180 (98.4%)	75 (97.4%)	377 (98.7%)	4.13 0.257
Attended high school	366	115 (98.3%)	166 (94.9%)	48 (64.9%)	329 (89.9%)	52.6 <0.0001
Other education	386	33 (27.1%)	108 (58.4%)	43 (54.4%)	184 (47.7%)	31.69 <0.0001
Residential school						
Attended residential school	376	0	6 (3.4%)	52 (66.7%)	58 (15.4%)	171 <0.0001
Parents in residential school	326	36 (40.5%)	148 (89.7%)	68 (94.4%)	252 (77.3%)	89 <0.0001
Work activities						
Currently working ^c	349	39 (37.5%)	94 (52.2%)	32 (48.5%)	165 (49.2%)	5.90 0.052
Currently looking for work ^c	165	45 (81.8%)	58 (75.3%)	7 (21.2%)	110 (66.7%)	38.2 <0.0001
Struggle to pay for food monthly or more in the last year	332	28 (26.9%)	38 (24.2%)	17 (23.9%)	83 (25.0%)	3.65 0.392
Current smoker	376	54 (46.2%)	90 (49.7%)	24 (30.8%)	168 (44.7%)	0.28 0.016

Heavy drinker	360	79 (71.2%)	100 (56.8 %)	20 (27.4%)	199 (55.3%)	35.3 <0.0001
Obese	375	47 (39.8%)	93 (51.7%)	36 (46.8%)	176 (46.9%)	4.03 0.133
At least one diagnosed chronic health condition	385	46 (38.3%)	117 (62.9%)	67 (84.8%)	230 (59.7%)	46.6 <0.0001
At least one diagnosed nervous system disorder	381	20 (17.1%)	37 (19.9%)	35 (44.9%)	92 (24.2%)	32.6 <0.0001

^aNumber (N) of valid responses (does not include don't know or don't remember and refused).

^bLikelihood Ratio Chi-square compares the relative frequency between age categories.

^cExcludes students, stay at home parents and retirees.

There are no differences between men and women for age distribution, schooling, having attended or having parents who attended residential school, difficulty paying for food, and current smoking. Proportionally more men report heavy drinking compared to women (62.7% vs 48.7%; Chi-square LR 7.17; $p = 0.007$), while women have a significantly higher prevalence of obesity (54.0% vs 38.9%; Chi-square LR: 14.01, $p = 0.003$) and at least one chronic health condition diagnosed by a health professional (68.5% vs 49.7%; Chi-square LR: 14.01, $p = 0.0001$).

In this community, fish, especially walleye, is the source of Hg exposure (4, 5). Table 2 contains the responses to the GN-CHA questions on past and current fish consumption frequency. In response to the question about fish consumption at around 10 years old, 46 persons (12.1%) respond that they do not remember. Among the others, over half of those 50 years and older (born before 1968) report that, as a child, they ate fish daily. Childhood fish consumption frequency significantly decreased over time. Fish consumption questions were species specific. Walleye is still the fish the most consumed; 30.6% of GN-CHA participants report eating walleye often compared to 4.7% for whitefish and 3.2% for northern pike. No differences are observed between

women and men for the frequency of childhood or current fish consumption.

Table 2. The relative frequency of fish consumption by age category

	N	18-29 y n (%)	30-49 y n (%)	50+ y n (%)	Total n (%)	Chi-square (LR) p-value
Fish consumption at 10 y of age	333					
None		9 (9.4%)	10 (6.1%)	4 (5.5%)	23 (6.9%)	101 <0.0001
Hardly ever/occasionally		32 (33.3%)	26 (15.9%)	3 (4.1%)	61 (18.3%)	
Once a month		24 (25.0%)	38 (23.2%)	2 (2.7%)	64 (19.2%)	
Several times a month		28 (29.2%)	96 (42.1%)	24 (32.9%)	121 (36.4%)	
Every day		3 (3.1%)	21 (12.8%)	40 (54.8%)	64 (19.2%)	
Walleye consumption over past year	382					
Not at all		23 (19.5%)	29 (15.7%)	9 (11.4%)	61 (16.0%)	3.44 0.486
A few times		60 (50.9%)	96 (51.9%)	48 (60.8%)	204 (53.4%)	
Often		35 (29.7%)	60 (32.4%)	22 (27.9%)	117 (30.6%)	

Likelihood Ratio Chi-square compares the relative frequency between age categories

Biomarkers of Hg exposure decrease over the time period (Figure 2a, 2b, 3, and Table 3), paralleling the decrease in fish consumption. There is a positive association between childhood fish consumption and measured Hair Hg, adjusted for sex and year of sampling (MEM: Wald Chi-square = 96.85; p = 0.003). Paired t-tests, comparing mean hair Hg at 10 years of age, for each year, between those who report eating fish during childhood at least several times a month and those who report eating less, support the MEM result (Wilcoxon Signed-Rank prob>|S|, S: 37.5; p = 0.02).

443

444

Table 3. Measured mercury concentrations by age category

	N	18-29 y Median (min-max)	30-49 y Median (min-max)	50+ y Median (min-max)	Total Median (min-max)	Wilcoxon test p-value
Umbilical cord (ppb)	99	3.5 (1.5-10)	5.6 (1-78.5)	-	5 (1-78.5)	0.023
Childhood Hair Hg (µg/g)	137	0.9 (0.5-2)	0.7 (0.2-16)	2.9 (0.6-15)	1.1 (0.2-16)	< 0.0001
Hair ≥ Hg 5 µg/g at least once	208	0	27 (22.9%)	49 (74.2%)	76 (36.6%)	< 0.0001

445

446

447 Figure 4 presents the aggregation levels and construct stability of the clusters. Aggregation
448 distances support a cutting threshold between 5 and 8 clusters (Figure 4a), while the bootstrapped
449 mean-adjusted R and criterion suggest 6, 7, 11, or 12 (Figure 4b). The boxplots (Figure 4c) show
450 that the highest stability of the partition solution varies between K5 and K9, with K7 showing a
451 slightly higher median value, but a lower dispersion around the mean. After verification of all of
452 the above criteria, we kept a total of six clusters. Goodness of fit for CFA was acceptable for the
453 six clusters (CFI = 0.85, TLI = 0.84, RMSEA = 0.08, SRMR = 0.05); with a narrow 90% CI,
454 representing a high degree of precision. The Cronbach alpha (> 0.8) for each cluster confirmed
455 internal consistency (Table 4).

456

457 [Place Figure 4 here](#)

458

459 The six clusters include a total of 37 symptoms. Table 4 presents the list of symptoms retained in
460 each cluster with its correlation. Cluster 1 (Extrapyramidal impairment), which aggregates the
461 highest number of symptoms (n = 9), includes tremors, balance impairment, functional limb

weakness and pain in arms and legs, reflecting an extension of extrapyramidal neurological symptoms. Cluster 2 (Sensory impairment) groups peripheral neuropathic deficits, notably upper and lower limb sensory impairments including numbness, dullness and tingling. Cluster 3 (Cranial nerve disturbances) includes anosmia, ageusia, dysphagia and tingling around the mouth. Cluster 4 (Gross motor impairment) aggregates symptoms that affect carrying, lifting and walking. Cluster 5 (Neuro-cognitive deficits) groups cognitive dysfunction associated with memory loss, hearing impairment and speech disorders. Finally, Cluster 6 (Affect/Mood disorders) includes anxiety, irritability, depression and sleeping issues. For each cluster, there is no particular driving symptom. Most symptoms are highly related to their own cluster (Table 4).

[Place Table 4 \(large table\)](#)

For the large majority of symptoms, no significant difference is observed in the proportion of men and women who report its frequency as very often/ all the time (Table 4). Symptom frequency increases significantly with age (data not shown), with the exception of difficulty pronouncing words, forgetting to do things, doing nothing, irritability, anxiety, difficulty falling asleep, waking up at night, depressed, tiredness and difficulty concentrating (Fisher's Exact Test; all $p \geq 0.08$).

Table 5 presents the descriptive characteristics of the composite variable for each cluster for women and men. A higher score in the composite variable reflects a higher frequency of coexisting symptoms. Scores are significantly higher in women than in men, with the exception of Neuro-cognitive deficits. Scores increase significantly with age (Rho: 0.138-0.409; $p < 0.01$), with the exception of Affect/Mood disorders (Rho: 0.108; $p = 0.095$).

Table 5. Descriptive characteristics of the composite variable in each cluster

Clusters	Component Score Mean, Median (min-max)			Wilcoxon p-value
	All	Women	Men	
Cluster 1 (Extrapyramidal impairment)	2.7, 2.2 (0-11.2)	3.0, 2.6 (0-1.2)	2.3, 1.7 (0-9.1)	0.002
Cluster 2 (Sensory impairment)	2.6, 2.4 (0-8.3)	2.8, 2.8 (0-8.3)	2.3, 2.1 (0-8.3)	0.012
Cluster 3 (Cranial nerve disturbances)	1.4, 0.9 (0-7.9)	1.6, 1.0 (0-7.9)	1.1, 0.4 (0-6.9)	0.017
Cluster 4 (Gross motor impairment)	1.4, 0.7 (0-6.6)	1.8, 1.1 (0-6.6)	1.0, 0.3 (0-6.2)	0.000
Cluster 5 (Neuro-cognitive deficits)	2.3, 2.1 (0-8.0)	2.4, 2.3 (0-8.0)	2.1, 2.0 (0-7.2)	0.199
Cluster 6 (Affect/Mood disorders)	4.3, 4.3 (0-10.4)	4.7, 4.9 (0-10.4)	3.8, 4.0 (0-10.1)	0.000

Table 6 presents the results of the longitudinal MEMs, which include the 32 participants with at least 10 direct hair Hg measurements. The associations between longitudinal hair Hg and the median of the composite variable for each cluster are shown; age, sex and year of sampling are included as fixed factors, and age at time of sampling as random factor. In these analyses, 5 of the 6 clusters are significantly associated ($p \leq 0.05$) or show a tendency (< 0.10) with longitudinal hair Hg. No association is observed between longitudinal hair Hg and Affect/Mood disorders.

Table 6. Longitudinal Mixed Effect Model results for hair Hg concentrations (dependent variable) from 1970 to 1997 for persons whose composite symptom cluster variable scores are above the median compared to those below above the median.

Composite symptom cluster variable^{a, b}	Hair Hg Estimate^c	% Confidence Interval	p - value
Cluster1 (Extrapyramidal impairment)	1.76	0.0 - 3.6	0.061
Cluster2 (Sensory impairment)	1.57	-2.7 – 3.4	0.096
Cluster3 (Cranial nerve disturbances)	2.60	1.0 – 4.2	0.001
Cluster4 (Gross motor impairment)	2.70	0.4 – 5.1	0.023
Cluster5 (Neuro-cognitive deficits)	2.41	0.6 – 4.2	0.009
Cluster6 (Affect/Mood disorders)	-0.33	-0.3 - 1.0	0.758

500

501 ^a Only participants with 10 measurements and more of hair Hg are included in the analyses (32 persons; 447 Hair Hg
502 measurements).

503 ^b Significant covariates include age, sex, year of sampling (random effect: age of sampling nested in year of
504 sampling).

505 ^c Hair Hg estimates represent the increase in Hair Hg for each unit of the cluster score over the sampling period.

506

507 The 242 GN-CHA participants selected for the SEMs present similar characteristics to the 391
508 participants presented in Tables 1 and 2, although they are slightly older (median: 39 years old;
509 25th – 75th percentile: 32-50), with proportionally fewer persons in the 18-29 category (11.5% vs
510 28.8%). In these analyses, both measured and estimated Hg values are used. Validation of
511 estimated Hg values show significant associations with direct measurements for both prenatal and
512 childhood Hg exposure (n = 99; Rho = 0.22; p = 0.023 and n = 137; Rho = 0.73; p < 0.0001,
513 respectively). Most participants (n = 197 (81.4%)) were born after 1962 (when exposure began)

and had both pre- and post-natal exposures. Only 8 of the 242 participants (3.3%) were over 15 years of age in 1962.

For each symptom cluster, SEMs were performed (i) with latent Hg and (ii) with prenatal, childhood and hair $\text{Hg} \geq 5\mu\text{g/g}$ separately. Path diagrams, showing the configuration of the different pathways and the one-to-one relation between variables, are illustrated in Figures 5 and 6 and in Supplementary Figures 1-10 in Additional File 1. SEM standardized path coefficients, representing the total quantified contribution of significant direct and indirect associations, are presented in Figures 7 and 8.

[Place Figures 5 and 6 here](#)

[Place Figures 7 and 8 here](#)

For all latent Hg-based SEMs, childhood Hg exposure and hair $\text{Hg} \geq 5\mu\text{g/g}$ are the main drivers in the construct of latent Hg (Figures 5 and 7). Latent Hg shows a direct contribution on most symptom clusters, but not Neuro-cognitive deficits and Gross motor impairment. For all clusters, there is also an indirect pathway for Latent Hg, mediated through having at least one diagnosed nervous system disorder.

In every SEM with separate Hg exposure variables for prenatal, childhood and hair $\text{Hg} \geq 5\mu\text{g/g}$, there is a significant relation between prenatal and childhood exposure (Figures 6 and 8 and Supplementary Figures 6 –10, Additional File 1). For Extrapyrimalid Impairment (Figure 6) and

for Cranial Nerve Disturbances (Supplementary Figure 7, Additional File 1), standardized path coefficients show that the contribution of prenatal exposure to the cluster is mediated by childhood exposure.

For Sensory Impairment (Supplementary Figure 6, Additional File 1), Gross Motor Impairment (Supplementary Figure 8) and Neuro-cognitive Deficits (Supplementary Figure 9, Additional File 1) there is a chain of mediation from prenatal exposure to childhood exposure and from childhood to being diagnosed with at least one nervous disorder. When the same chain of mediation is modelled for Extrapyramidal and for Cranial Nerve Disturbances, the models' fit parameters are weaker. In these cases, the direct association of childhood Hg with the symptom cluster is higher than its indirect association through diagnosed nervous disorder.

Prenatal Hg exposure is directly associated with the cluster of Affect/Mood disorders (Supplementary Figure 10, Additional File 1). Having had $\text{Hg} \geq 5 \mu\text{g/g}$ directly influences Extrapyramidal Impairment and Sensory Impairment (Figure 6 and Supplementary Figure 6, respectively).

Analyses of separate SEM for each type of Hg exposure show direct and significant relationships for prenatal Hg exposure with Extrapyramidal impairment, Cranial nerve disturbances and Affect/Mood disorders (data not shown). Childhood Hg exposure shows significant associations for Extrapyramidal impairment, Cranial nerve disturbances and Neuro-cognitive deficits. Having had $\geq 5\mu\text{g/g}$ hair Hg at least once shows significant associations for Extrapyramidal and Sensory Impairment and a tendency for Cranial nerve disturbances (data not shown).

The best fit models are those that include childhood exposure as mediator for prenatal exposure. Childhood is not a confounding variable in the relation between prenatal exposure, diagnosed nervous system disorder or any symptom cluster. Moreover, there is no moderated mediation with age category or Hg exposure in the SEM.

Although of different magnitudes, age category, sex and obesity, at least one diagnosed chronic health condition and at least one diagnosed nervous system disorder, directly and/or indirectly influence symptom frequency in all SEMs. Positive associations are observed with age category with respect to diagnosed nervous system and health conditions, with the exception of Affect/mood disorders, for which age is both negatively (direct) and positively (indirect) associated with symptom frequency reporting. Older persons tend to report fewer symptoms of Affect/mood disorders (direct), unless they suffer from chronic health conditions and/or nervous system disorders (indirect). Past Hg exposure mediated the contribution of age for all clusters, except for Gross motor disorders (Supplementary Figure 8, Additional File 1) and Neuro-cognitive deficits (Supplementary Figure 9, Additional File 1).

Discussion

To our knowledge, this is the first study to link past Hg exposure from freshwater fish consumption with clusters of current multiple coexisting symptoms. Clustering analysis enabled us to characterize the various symptom profiles, which constitute the physical and/or mental translation of structural phenomena. The non-random distribution of symptoms and their strong correlation in

each cluster suggests a common mechanism or etiology (10, 12, 66). Here, the six clusters, derived from statistical empirical validation, reflect the involvement of different aspects of nervous system dysfunction, which we classified as representing primarily Extrapyramidal impairment, Sensory impairment, Cranial nerve disturbances, Gross motor impairment, Neuro-cognitive deficits, and Affect/Mood disorders. The symptoms that make up these clusters are similar to those reported by persons from Grassy Narrows in 2010 (8, 67) and consistent with the many descriptions of methyl Hg poisoning through fish consumption (68-75).

Hg biomarker data, collected in Grassy Narrows First Nation between 1970 and 1997 provided a unique opportunity to examine these clusters with respect to past exposure over a long-term period and at various time points. The findings of longitudinal exposure, based on participants with at least 10 year-based hair Hg measurements over the sampling period, show that long-term Hg exposure is associated with higher symptom frequency for all the clusters of nervous system dysfunction, with the exception of Affect/Mood disorders. This is consistent with SEM findings where Affect/Mood disorders show a significant relation only with prenatal exposure.

Studies on Minamata Disease have shown differences in fetal, non-fetal infantile and adult Hg damage to the brain (76). In the present study, the large majority of participants experienced both prenatal and postnatal exposure. The mediated contribution of prenatal exposure on symptom clusters through childhood exposure, observed in most SEMs, suggests that their independent contributions are difficult to assess.

SEM provided the opportunity to simultaneously examine covariance, mediation and moderation between variables through a domino effect of successive pathways from past exposure to current symptoms. The Hg exposure latent variable, which groups different periods of exposure (prenatal Hg, childhood Hg and hair Hg $\geq 5\mu\text{g/g}$ at least once), enabled us to examine their combined contributions. Latent Hg exposure variables have been used in other studies. Choi et al. (23) showed that the grouped Hg exposure variable, including current Hg nail and blood concentrations and hair Hg concentration 7 years previously, was associated with adverse cardiovascular outcomes in Faroese whaling men. In a study by Grandjean and co-authors (77), the combined prenatal and childhood Hg exposures were negatively related to children's neurobehavioral outcomes. In the present study, the combined past Hg exposures are directly and indirectly, associated with all clusters.

Independent Hg exposures may provide insight into the relative importance of exposure at different time periods. Prenatal exposure is directly associated with Affect/mood disorders, while for other clusters, its contribution is mediated by childhood exposure. Fetal Minamata patients present with psychiatric disorders (Harada, 1964 cited in (78)). A re-analysis of 1971 data from Minamata patients showed that psychiatric symptoms peaked at 20 years of age (78). This may be similar to the present study where young adults (≤ 29 years of age) report a higher frequency of altered mood symptoms. The importance of prenatal and childhood Hg exposure has been raised by many authors (25, 79). Children are more vulnerable to toxic exposure than adults because their brains are in a state of rapid growth, with relatively higher absorption rates to body weight, and their body systems are not prepared to properly metabolize, detoxify, and excrete toxic substances (80-82).

Although the GN-CHA did not include the age of onset of symptoms, the association between past exposure and later-life symptoms raises the issue of delayed and/or progressive neurotoxicity. Delayed neurotoxicity was reported in 13-year-old monkeys, treated with methyl Hg from birth to seven years of age (83). A silent latency period for methyl Hg toxicity has been proposed using examples from the Minamata and Iraq disasters (84). Newland and co-authors (85) discuss mechanisms by which neuronal development may have long-lasting behavioral consequences that appear in adulthood and, in some cases, may not appear until aging.

The FNRHS 2008/2010 included few nervous system disorders in their list of diagnosed health conditions (27). The GN-CHA added a series of neurologic disorders which share some of the signs and symptoms of Hg poisoning. These disorders mediate the relation between latent Hg exposure and all symptom clusters and between childhood exposure and Sensory impairment, Gross Motor impairment and Neuro-cognitive deficits.

In Canada, chronic health conditions are disproportionately higher among Indigenous communities compared to the non-Indigenous population (86). The most commonly reported chronic health conditions in the GN-CHA are similar to other First Nation communities across Canada: high blood pressure, allergies, arthritis, diabetes, and chronic back pain (27). Results of the SEM show the expected associations of having at least one chronic health condition with age and obesity, with women presenting a higher prevalence compared to men. It is noteworthy that the highest direct contribution to all symptom clusters is having at least one chronic health condition and/or having at least one nervous system disorder.

SEM remains one of the best approaches to assess simultaneously direct and indirect associations by investigating all relevant regression pathways. Although it cannot prove causation between Hg exposure and clusters, the positive relations between past Hg exposure and current symptoms frequency reveal links that are not explainable by reverse causation. The concordant results in longitudinal MEM support these findings. Our findings are consistent with follow-up studies of persons that had been affected by Hg pollution from the Chisso Company in Minamata, decades after the company had halted the dumping of methyl Hg into the bay (74, 87-89). The Japanese studies compared persons who had lived in polluted and non-polluted regions since they did not have actual measures of Hg exposure for the individuals who were evaluated.

Some limitations need to be considered in interpreting the present results. The study reposes on self-reported questionnaire data from the GN-CHA, raising the question of recall bias, and under- or over-reporting. To offset these limitations, the Cronbach alpha and CFA confirmed the construct reliability of self-reported symptom frequency. Reported diagnosed health conditions and nervous system disorders reflect whether a person has consulted a health professional who has made the diagnosis of this condition, as well as the accuracy of the diagnosis. Further studies would benefit from clinical assessments in relation to historic Hg exposure.

A further limitation is with respect to Hg biomarkers obtained from a government monitoring program (3). Sampling was not carried out regularly and samples were not collected yearly from everyone, resulting in many missing values. Moreover, there are seasonal and yearly differences in fish consumption frequency (3, 6). Hg exposure imprecision is also associated with blood and hair measurements over the sampling years, notably laboratory measurement imprecision and

biological variation (90). Ideally, one would like continuous Hg biomarker measurements over all participants' lifetime, but this is not possible. To address the challenges posed by the biomarker database, for the longitudinal MEMs, we limited the analyses to persons with a minimum of 10 hair Hg measurements, representing exposure for 10 years or more. For the SEMs, the estimated umbilical cord and childhood Hg values were validated, and we used latent variables of Hg exposure to manage residual error terms between variables.

Finally, the Grassy Narrows Hg disaster began in 1962 and after 1970, fish Hg concentration decreased over time and fish consumption, paralleled by a decrease in biomarker Hg concentrations. The association of Hg exposure in the models may be partially masked by age. The inverse relation between year of birth and Hg exposure makes age an ambiguous variable. It is a risk factor for the symptom clusters, covaries with Hg exposure, but is not a consequence of Hg exposure.

Conclusions

Since the disaster, Grassy Narrows First Nation has expressed concern about the high rate of symptoms of nervous system dysfunction in their community. This is the first study to demonstrate links between retrospective Hg exposure and current coexisting symptoms. Given the complexity of the interrelations between the various determinants of chronic health problems in this and other First Nation communities, LMEM and SEM methods enabled us to trace direct and/or indirect routes of potential "causal impact" manifest after many years of Hg exposure.

Declarations

Ethics approval and consent to participate

Ethics approval for the GN-CHA was obtained from the Manitoulin Anishinaabek Research Review Committee (MARRC), who issued an Ethics Certificate on September 1, 2016. Informed consent forms were signed by all participants. Consent included survey participation and the linking of the information from the GN-CHA to data obtained from previous surveillance programs and/or studies of Hg exposure. Ethics approval for the present study was obtained from the Université du Québec à Montréal (2016_e_1350) and Health Canada Research Ethics Board (REB 2017-0006).

Consent for publication

All participants provided consent for publication. The present study was presented to Grassy Narrows' Chief and Council of, who approved the manuscript for publication on May 12, 2021.

Availability of data and materials

Archived mercury biomarker data was obtained from the First Nations and Inuit Health Branch (FNIHB) of the Ministry of Indigenous Services Canada, at the request of Grassy Narrows First Nation. The datasets generated and analysed in the present study are the property of Grassy Narrows First Nation. Permission for use of the data lies with Grassy Narrows Chief and Council.

720 *Competing interests*

721 The authors declare that they have no competing interests

722

723 *Funding*

724 The GN-CHA received financial support from Health Canada and the Ontario Ministry of Health
725 and Long-Term Care and technical support from these ministries and the Ontario Agency for
726 Health Protection and Promotion, the Ontario Ministry of Indigenous Relations and Reconciliation
727 and the Northwestern Health Unit of Ontario. The present study was funded by the Canadian
728 Institutes for Health Research (#152882).

729

730 *Authors contributions*

731 AP created the database, determined the statistical approaches and performed the analyses. She
732 co-wrote the manuscript with DM. JDS is the initiator and organizer of the GN-CHA survey. She
733 provided input for data interpretation. MF participated in the analyses and dissemination of the
734 GN-CHA. She participated in the writing and editing of the manuscript. TSL provided the
735 neurological interpretation of the symptom clusters and verified the manuscript. DM was invited
736 by Grassy Narrows First Nation as scientific advisor for the GN-CHA. She was responsible for
737 over-seeing the fieldwork, analyzing the data and preparing the reports. She is principal
738 investigator on the present research. All authors read and approved the final manuscript

739

740 *Acknowledgements*

741 We thank all of the people of Grassy Narrows who organized and carried out the Grassy Narrows
742 Community Health Assessment (CHA): the community advisory committee, the fieldwork

coordinators and the surveyors. A special thank you to the people of Grassy Narrows who participated in the CHA and agreed to share their biomarker data with us.

Table 4. Characteristics of symptom clusters

	Chronbach alpha (IC 95%)	Correlation within cluster	Reported Very often or All the time			Difference Chi-square/ Fisher's Exact test p-value
			Overall	Women	Men	
Cluster 1 : Extrapyrarnidal Impairment						
	0.91 (0.90-0.92)					
Pain in arms		0.80	44 (11.5%)	28 (13.7%)	16 (9.0%)	0.19
Pain in legs		0.80	75 (19.6%)	52 (25.2%)	23 (13.1%)	0.003
Drop things		0.75	53 (13.9%)	37 (17.9%)	16 (9.2%)	0.017
Feet tremors		0.77	25 (6.6%)	13 (6.4%)	12 (6.8%)	0.873
Stiff shoulders		0.75	51 (13.4%)	33 (16.1%)	18 (10.2%)	0.099
Burning feet		0.75	48 (12.7%)	34 (16.8%)	14 (8.0%)	0.01
Stumble		0.78	39 (10.1%)	21 (10.2%)	18 (10.1%)	0.992
Stop or freeze		0.74	14 (3.7%)	10 (4.9%)	4 (2.3%)	0.275
Fall		0.75	19	14	5	0.099

			(5.0%)	(6.8%)	(2.8%)
<hr/>					
Cluster 2: Sensory Impairment					
	0.94 (0.93-0.95)				
Numbness in hands	0.88	53	37	16	0.01
		(14.0%)	(18.1%)	(9.1%)	
Numbness in feet	0.89	56	34	22	0.268
		(14.7%)	(16.5%)	(12.5%)	
Dull feeling in hands	0.88	44	27	17	0.285
		(11.7%)	(13.3%)	(9.8%)	
Dull feeling in feet	0.87	46	30	16	0.102
		(12.1%)	(14.6%)	(9.1%)	
Tingling in hands	0.87	54	33	21	0.243
		(14.2%)	(16.1%)	(12.0%)	
Tingling in feet	0.87	53	34	19	0.1
		(13.8%)	(16.5%)	(10.7%)	
<hr/>					
Cluster 3 : Cranial nerve disturbances					
	0.85 (0.83-0.88)				
Loss of taste	0.85	23	17	6	0.053
		(6.0%)	(8.3%)	(3.4%)	
Loss of smell	0.82	26	16	10	0.423
		(6.8%)	(7.8%)	(5.7%)	
Difficulty swallowing	0.81	33	23	10	0.068
		(8.6%)	(11.2%)	(5.7%)	
<hr/>					

Tingling around mouth	0.73	24	17	7 (	0.094
		(6.3%)	(8.3%)	4.0%)	
Choking	0.81	15	12	3	0.061
		(3.9%)	(5.8%)	(1.7%)	

Cluster 4: Gross motor impairment

	0.89 (0.88-0.91)				
Difficulty walking 5 minutes	0.91	38	29	9	0.004
without rest		(9.9%)	(14.0%)	(5.1%)	
Difficulty climbing stairs	0.92	47	37	10	0
		(12.3%)	(17.8%)	(5.68%)	
Trouble lifting	0.89	77	50	27	0.025
		(20.3%)	(24.5%)	(15.3%)	
Trouble lifting 10 lbs	0.82	36	32	4	0
		(9.5%)	(15.5%)	(2.3%)	

Cluster 5: Neuro-cognitive deficits

	0.82 (0.79-0.85)				
Trouble hearing	0.74	48	28	20	0.485
		(12.5%)	(13.6%)	(11.2%)	
Difficulty having his/her speech	0.77	27	17	10	0.424
understood		(7.1%)	(8.3%)	(5.7%)	
Difficulty pronouncing words	0.77	39	21	18	0.992
		(10.1%)	(10.1%)	(10.1%)	
Forget where to put things	0.79	76	50	26	0.018
		(19.8%)	(24.3%)	(14.7%)	
Forget doing things	0.8	35	23	12	0.158
		(9.1%)	(11.1%)	(6.8%)	

Cluster 6: Affect/Mood disorders

	0.9 (0.89-0.91)				
Anxious	0.81	57	39	18	0.016
		(14.9%)	(18.9%)	(10.2%)	
Tired	0.81	111	74	37	0.001
		(28.9%)	(35.9%)	(20.9%)	
Irritable	0.81	50	36	14	0.006
		(13.1%)	(17.5%)	(7.9%)	
Depressed	0.77	58	36	22	0.151
		(15.4%)	(17.9%)	(12.6%)	
Difficulty concentrating	0.78	60	39	21	0.054
		(15.9%)	(19.2%)	(12.0%)	
Wake up at night	0.77	141	86	55	0.037
		(36.8%)	(41.6%)	(31.2%)	
Trouble falling asleep	0.75	138	74	64	0.901
		(36.0%)	(35.8%)	(36.4%)	
Doing nothing	0.72	65	45	20	0.005
		(17.0%)	(21.9%)	(11.2%)	

748

749

750

Figure captions

Figure 1. Breakdown of GN-CHA participants with Hg biomarker measurements

Figure 2. Distribution of measured Hg biomarkers over time

2a. Hair Hg ($\mu\text{g/g}$) 1970 - 1997 ($n = 208$)

2b. Umbilical cord blood Hg ($\mu\text{g/L}$) 1970 – 1992 ($n = 211$)

Figure 3. Distribution of mean hair Hg ($\mu\text{g/g}$) at 10 years of age with respect to childhood fish consumption: $\geq$ several times a month ($n = 76$) and less ($n = 42$).

Note: The fitted line represents second order polynomial (quadratic), with a shaded confidence band.

Figure 4. Graphic outputs for cluster decision

4a. Plot of the aggregation levels of symptom clusters

4b. Plot of stability of the partitions of clusters

4c. Plot of dispersion of the adjusted Rand index

Figure 5. SEM path diagram linking retrospective latent Hg exposure and covariates to the latent symptoms cluster variable for Extrapiramidal impairment.

Abbreviations: χ^2/df : Chi-square divided by the degrees of freedom (χ^2/df), CFI: Comparative Fit Index, TLI: Tucker Lewis Index, RMSEA: Root Mean Square Error of Approximation, SRMR: Standardized Root Mean Square Residual.

Figure 6. SEM path diagram linking retrospective Hg exposure parameters (prenatal and childhood Hg exposure, and having had $\geq 5\mu\text{g/g}$ hair Hg at least once between 1970 and 1997) and covariates to the latent symptoms cluster variable for Extrapyrmidal impairment.

Abbreviations: χ^2/df : Chi-square divided by the degrees of freedom (χ^2/df), CFI: Comparative Fit Index, TLI: Tucker Lewis Index, RMSEA: Root Mean Square Error of Approximation, SRMR: Standardized Root Mean Square Residual.

Figure 7. SEM standardized path coefficients for direct and indirect contributions of latent Hg and covariates on the symptom latent variable for each cluster

Figure 8. SEM standardized path coefficients for direct and indirect contributions of prenatal, childhood and $\geq 5\mu\text{g/g}$ hair Hg (1970 – 1997) and covariates on the symptom latent variable for each cluster

References

1. Rudd JWM, Turner MA, Furutani A. The English-Wabigoon River system: I. A synthesis of recent research with a view towards mercury amelioration. *Canadian Journal of Fisheries and Aquatic Sciences*. 1983;40(12):2206-17.
2. Etkin D. *Disaster Theory: An Interdisciplinary Approach to Concepts and Causes*. Disaster Theory: An Interdisciplinary Approach to Concepts and Causes: Elsevier, Butterworth-Heinemann; 2016. p. 138-42.
3. Wheatley B, Paradis S. Exposure of Canadian aboriginal peoples to methylmercury. *Water, Air, and Soil Pollution*. 1995;80(1):3-11.
4. Wheatley B, Paradis S, Lassonde M, Giguere MF, Tanguay S. Exposure Patterns and Long Term Sequelae on Adults and Children in Two Canadian Indigenous Communities Exposed to Methylmercury. *Water, Air and Soil Pollution*. 1997;97(1-2):63-73.
5. Neff MR, Bhavsar SP, Arhonditsis GB, Fletcher R, Jackson DA. Long-term changes in fish mercury levels in the historically impacted English-Wabigoon River system (Canada). *J Environ Monit*. 2012;14(9):2327-37.
6. Philibert A, Fillion M, Mergler D. Mercury exposure and premature mortality in the Grassy Narrows First Nation community: a retrospective longitudinal study. *The Lancet Planetary Health*. 2020;4(4):e141-e8.
7. Harada M, Hanada M, Tajiri M, Inoue Y, Hotta N, Fujino T, et al. Mercury Pollution in First Nations Groups in Ontario, Canada: 35 years of Canadian Minamata Disease (English translation). *Journal of Minamata Studies*. 2011;3:3-30.

814 8.Takaoka S, Fujino T, Hotta N, Ueda K, Hanada M, Tajiri M, et al. Signs and symptoms of
815 methylmercury contamination in a First Nations community in Northwestern Ontario, Canada. *Sci*
816 *Total Environ.* 2014;468-469:950-7.

817 9.Eto K, Marumoto M, Takeya M. The pathology of methylmercury poisoning (Minamata
818 disease): The 50th Anniversary of Japanese Society of Neuropathology. *Neuropathology.*
819 2010;30(5):471-9.

820 10.Miaskowski C, Dodd M, Lee K. Symptom clusters: the new frontier in symptom management
821 research. *J Natl Cancer Inst Monogr.* 2004;32:17-21.

822 11.Skerman HM, Yates PM, Battistutta D. Multivariate methods to identify cancer-related
823 symptom clusters. *Res Nurs Health.* 2009;32(3):345-60.

824 12.Barsevick AM, Whitmer K, Nail LM, Beck SL, Dudley WN. Symptom cluster research:
825 conceptual, design, measurement, and analysis issues. *J Pain Symptom Manage.* 2006;31(1):85-
826 95.

827 13.Aktas A, Walsh D, Rybicki L. Symptom clusters: myth or reality? *Palliat Med.* 2010;24(4):373-
828 85.

829 14.Miaskowski C. Future Directions in Symptom Cluster Research. *Semin Oncol Nurs.*
830 2016;32(4):405-15.

831 15.Miaskowski C, Barsevick A, Berger A, Casagrande R, Grady PA, Jacobsen P, et al. Advancing
832 Symptom Science Through Symptom Cluster Research: Expert Panel Proceedings and
833 Recommendations. *J Natl Cancer Inst.* 2017;109(4).

834 16.Weihe P, Grandjean P, Debes F, White R. Health implications for Faroe islanders of heavy
835 metals and PCBs from pilot whales. *Sci Total Environ.* 1996;186(1-2):141-8.

836 17.Budtz-Jørgensen E, Keiding N, Grandjean P, Weihe P. Estimation of health effects of prenatal
837 methylmercury exposure using structural equation models. *Environ Health*. 2002;1(1):1-2.

838 18.Budtz-Jørgensen E, Grandjean P, Jørgensen PJ, Weihe P, Keiding N. Association between
839 mercury concentrations in blood and hair in methylmercury-exposed subjects at different ages.
840 *Environ Res*. 2004;95(3):385-93.

841 19.Sánchez BN, Budtz-Jørgensen E, Ryan LM, Hu H. Structural Equation Models. *Journal of the*
842 *American Statistical Association*. 2005;100(472):1443-55.

843 20.Grandjean P, Budtz-Jørgensen E, Jørgensen PJ, Weihe P. Umbilical cord mercury
844 concentration as biomarker of prenatal exposure to methylmercury. *Environ Health Perspect*.
845 2005;113(7):905-8.

846 21.Debes F, Budtz-Jørgensen E, Weihe P, White RF, Grandjean P. Impact of prenatal
847 methylmercury exposure on neurobehavioral function at age 14 years. *Neurotoxicol Teratol*.
848 2006;28(5):536-47.

849 22.Choi AL, Budtz-Jørgensen E, Jørgensen PJ, Steuerwald U, Debes F, Weihe P, et al. Selenium
850 as a potential protective factor against mercury developmental neurotoxicity. *Environ Res*.
851 2008;107(1):45-52.

852 23.Choi AL, Weihe P, Budtz-Jørgensen E, Jørgensen PJ, Salonen JT, Tuomainen TP, et al.
853 Methylmercury exposure and adverse cardiovascular effects in Faroese whaling men. *Environ*
854 *Health Perspect*. 2009;117(3):367-72.

855 24.Grandjean P, Weihe P, Nielsen F, Heinzow B, Debes F, Budtz-Jørgensen E. Neurobehavioral
856 deficits at age 7 years associated with prenatal exposure to toxicants from maternal seafood diet.
857 *Neurotoxicol Teratol*. 2012;34(4):466-72.

858 25. Grandjean P, Landrigan PJ. Neurobehavioural effects of developmental toxicity. *Lancet*
859 *Neurol.* 2014;13(3):330-8.

860 26. Debes F, Weihe P, Grandjean P. Cognitive deficits at age 22 years associated with prenatal
861 exposure to methylmercury. *Cortex.* 2016;74:358-69.

862 27. FNIGC. First Nations Regional Health Survey (RHS) 2008/10: National report on adults, youth
863 and children living in First Nations communities. Ottawa, Canada: The First Nations Information
864 Governance Centre/ Le Centre de la Gouvernance de L'information des Premières Nations; 2012.

865 28. Farant JP, Brissette D, Moncion L, Bigras L, Chartrand A. Improved cold-vapor atomic
866 absorption technique for the microdetermination of total and inorganic mercury in biological
867 samples. *Journal of Analytical Toxicology.* 1981;5(1):47-51.

868 29. Magos L. Selective atomic-absorption determination of inorganic mercury and methylmercury
869 in undigested biological samples. *Analyst.* 1971;96(149):847-53.

870 30. Chavent M, Kuentz V, Lique B, Saracco L. ClustOfVar: An R Package for the Clustering of
871 Variables 2011 December 01, 2011: [arXiv:1112.0295 p.]. Available from:
872 <https://ui.adsabs.harvard.edu/abs/2011arXiv1112.0295C>.

873 31. Chavent M, Genuer R, Kuentz-Simonet V, Lique B, Saracco J. ClustOfVar : an R package for
874 dimension reduction via clustering of variables. Application in supervised classification and
875 variable selection in gene expressions data. *Statistical Methods for (post)-Genomics Data*
876 (SMPGD 2013); 2013-01-24; Netherlands 2013.

877 32. Kuentz Simonet V, Lyser S, Candau J, Deuffic P. ClustOfVar-based approach for unsupervised
878 learning: Reading of synthetic variables with sociological data. *Electronic Journal of Applied*
879 *Statistical Analysis.* 2015;8(2):170-97.

880 33.Kuentz-Simonet V, Labenne A, Rambonilaza T. Using ClustOfVar to Construct Quality of Life
881 Indicators for Vulnerability Assessment Municipality Trajectories in Southwest France from 1999
882 to 2009. *Social Indicators Research*. 2017;131(3):973-97.

883 34.Xie B, Pan W, Shen X. Penalized model-based clustering with cluster-specific diagonal
884 covariance matrices and grouped variables. *Electronic journal of statistics*. 2008;2:168-212.

885 35.Hu Lt, Bentler PM. Cutoff criteria for fit indexes in covariance structure analysis: Conventional
886 criteria versus new alternatives. *Structural Equation Modeling: A Multidisciplinary Journal*.
887 1999;6(1):1-55.

888 36.Enders CK, Bandalos DL. The Relative Performance of Full Information Maximum Likelihood
889 Estimation for Missing Data in Structural Equation Models. *Structural Equation Modeling: A*
890 *Multidisciplinary Journal*. 2001;8(3):430-57.

891 37.Schreiber JB, Nora A, Stage FK, Barlow EA, King J. Reporting Structural Equation Modeling
892 and Confirmatory Factor Analysis Results: A Review. *The Journal of Educational Research*.
893 2006;99(6):323-38.

894 38.Hooper D, Coughlan J, Mullen MR, editors. *Structural equation modelling: guidelines for*
895 *determining model fit*2008.

896 39.Rosseel Y. lavaan: An R Package for Structural Equation Modeling. 2012. 2012;48(2):36.

897 40.Cronbach LJ. Coefficient alpha and the internal structure of tests. *Psychometrika*.
898 1951;16(3):297-334.

899 41.Tavakol M, Dennick R. Making sense of Cronbach's alpha. *International journal of medical*
900 *education*. 2011;2:53-5.

901 42.Taber KS. The Use of Cronbach's Alpha When Developing and Reporting Research
902 Instruments in Science Education. *Research in Science Education*. 2018;48(6):1273-96.

903 43.Gibbons RD, Hedeker D, DuToit S. Advances in analysis of longitudinal data. Annual review
904 of clinical psychology. 2010;6:79-107.

905 44.Hedeker D, Gibbons RD, Waternaux C. Sample size estimation for longitudinal designs with
906 attrition: comparing time-related contrasts between two groups. Journal of Educational and
907 Behavioral Statistics. 1999;24(1):70-93.

908 45.Faul F, Erdfelder E, Buchner A, Lang A-G. Statistical power analyses using G*Power 3.1: Tests
909 for correlation and regression analyses. Behavior Research Methods. 2009;41(4):1149-60.

910 46.Faul F, Erdfelder E, Buchner A, Lang A. G* Power Version 3.1. 7 [computer software].
911 Universität Kiel, Germany. 2013.

912 47.Kyonka EGE. Tutorial: Small-N Power Analysis. Perspectives on behavior science.
913 2018;42(1):133-52.

914 48.National Research Council Committee on the Toxicological Effects of M. Toxicological
915 Effects of Methylmercury. Washington (DC): National Academies Press (US)
916 Copyright 2000 by the National Academy of Sciences. All rights reserved.; 2000.

917 49.Bollen KA. Latent Variables in Psychology and the Social Sciences. Annual Review of
918 Psychology. 2002;53(1):605-34.

919 50.MacCallum RC, Austin JT. Applications of Structural Equation Modeling in Psychological
920 Research. Annual Review of Psychology. 2000;51(1):201-26.

921 51.Mogensen UB, Grandjean P, Heilmann C, Nielsen F, Weihe P, Budtz-Jørgensen E. Structural
922 equation modeling of immunotoxicity associated with exposure to perfluorinated alkylates.
923 Environ Health. 2015;14:47.

924 52.Schumacker RE, Lomax RG. A beginner's guide to structural equation modeling: psychology
925 press; 2004.

926 53.Shook-Sa BE, Chen DG, Zhou H. Using Structural Equation Modeling to Assess the Links
927 between Tobacco Smoke Exposure, Volatile Organic Compounds, and Respiratory Function for
928 Adolescents Aged 6 to 18 in the United States. *Int J Environ Res Public Health*. 2017;14(10).

929 54.Wang IE, Rhemtulla M. Power Analysis for Parameter Estimation in Structural Equation
930 Modeling: A Discussion and Tutorial. 2021.

931 55.Satorra A, Saris W. Power of the likelihood ratio test in covariance structure analysis.
932 *Psychometrika*. 1985;50(1):83-90.

933 56.MacCallum RC, Browne MW, Cai L. Testing differences between nested covariance structure
934 models: Power analysis and null hypotheses. *Psychol Methods*. 11. United States: (2006 APA,
935 all rights reserved). 2006. p. 19-35.

936 57.MacCallum RC, Browne MW, Sugawara HM. Power analysis and determination of sample size
937 for covariance structure modeling. *Psychological Methods*. 1996;1(2):130-49.

938 58.Moshagen M, Erdfelder E. A New Strategy for Testing Structural Equation Models. *Structural*
939 *Equation Modeling: A Multidisciplinary Journal*. 2016;23(1):54-60.

940 59.Suttorp MM, Siegerink B, Jager KJ, Zoccali C, Dekker FW. Graphical presentation of
941 confounding in directed acyclic graphs. *Nephrol Dial Transplant*. 2015;30(9):1418-23.

942 60.Ferguson KD, McCann M, Katikireddi SV, Thomson H, Green MJ, Smith DJ, et al. Evidence
943 synthesis for constructing directed acyclic graphs (ESC-DAGs): a novel and systematic method
944 for building directed acyclic graphs. *International Journal of Epidemiology*. 2019;49(1):322-9.

945 61.Textor J, van der Zander B, Gilthorpe MS, Liskiewicz M, Ellison GT. Robust causal inference
946 using directed acyclic graphs: the R package 'dagitty'. *Int J Epidemiol*. 2016;45(6):1887-94.

947 62.Tennant PWG, Murray EJ, Arnold KF, Berrie L, Fox MP, Gadd SC, et al. Use of directed
948 acyclic graphs (DAGs) to identify confounders in applied health research: review and
949 recommendations. *Int J Epidemiol*. 2020.

950 63.Wang YA, Rhemtulla M. Power Analysis for Parameter Estimation in Structural Equation
951 Modeling: A Discussion and Tutorial. *Advances in Methods and Practices in Psychological
952 Science* 2021;4(1):1-17.

953 64.Chief Moon-Riley K, Copeland JL, Metz GAS, Currie CL. The biological impacts of
954 Indigenous residential school attendance on the next generation. *SSM Popul Health*.
955 2019;7:100343.

956 65.Nguyen VK. An Epidemic of Suspicion - Ebola and Violence in the DRC. *N Engl J Med*.
957 2019;380(14):1298-9.

958 66.Barsevick AM. The elusive concept of the symptom cluster. *Oncol Nurs Forum*.
959 2007;34(5):971-80.

960 67.Harada M, Hanada M, Tajiri M, Inoue Y, Hotta N, Takehiko F, et al. Mercury Poisoning in
961 First Nations Groups in Ontario , Canada 35 years of Minamata Disease in Canada. *Journal of
962 Minamata Studies*. 2011;3:3-30.

963 68.McAlpine D, Araki S. Minamata disease: an unusual neurological disorder caused by
964 contaminated fish. *Lancet*. 1958;2(7047):629-31.

965 69.Harada M. Minamata disease: methylmercury poisoning in Japan caused by environmental
966 pollution. *Crit Rev Toxicol*. 1995;25:1-24.

967 70.Ninomiya T, Ohmori H, Hashimoto K, Tsuruta K, Ekino S. Expansion of methylmercury
968 poisoning outside of Minamata: an epidemiological study on chronic methylmercury poisoning
969 outside of Minamata. *Environ Res*. 1995;70(1):47-50.

970 71.Clarkson TW, Magos L. The toxicology of mercury and its chemical compounds. Crit Rev
971 Toxicol. 2006;36(8):609-62.

972 72.Ekino S, Susa M, Ninomiya T, Imamura K, Kitamura T. Minamata disease revisited: an update
973 on the acute and chronic manifestations of methyl mercury poisoning. J Neurol Sci. 2007;262(1-
974 2):131-44.

975 73.Taber KH, Hurley RA. Mercury exposure: effects across the lifespan. J Neuropsychiatry Clin
976 Neurosci. 2008;20(4):iv-389.

977 74.Yorifuji T, Tsuda T, Takao S, Harada M. Long-term exposure to methylmercury and neurologic
978 signs in Minamata and neighboring communities. Epidemiology. 2008;19(1):3-9.

979 75.Jackson AC. Chronic Neurological Disease Due to Methylmercury Poisoning. Can J Neurol
980 Sci. 2018;45(6):620-3.

981 76.Korogi Y, Takahashi M, Okajima T, Eto K. MR findings of Minamata disease--organic
982 mercury poisoning. J Magn Reson Imaging. 1998;8(2):308-16.

983 77.Grandjean P, Weihe P, Debes F, Choi AL, Budtz-Jørgensen E. Neurotoxicity from prenatal and
984 postnatal exposure to methylmercury. Neurotoxicol Teratol. 2014;43:39-44.

985 78.Yorifuji T, Tsuda T, Inoue S, Takao S, Harada M. Long-term exposure to methylmercury and
986 psychiatric symptoms in residents of Minamata, Japan. Environ Int. 2011;37(5):907-13.

987 79.Karagas MR, Choi AL, Oken E, Horvat M, Schoeny R, Kamai E, et al. Evidence on the human
988 health effects of low-level methylmercury exposure. Environmental Health Perspectives.
989 2012;120(6):799-806.

990 80.Landrigan PJ, Kimmel CA, Correa A, Eskenazi B. Children's health and the environment:
991 public health issues and challenges for risk assessment. Environ Health Perspect. 2004;112(2):257-
992 65.

993 81.Rauh VA, Horton MK, Miller RL, Whyatt RM, Perera F. Neonatology and the Environment:
 994 Impact of Early Exposure to Airborne Environmental Toxicants on Infant and Child
 995 Neurodevelopment. *Neoreviews*. 2010;11:363-9.
 996 82.Heyer DB, Meredith RM. Environmental toxicology: Sensitive periods of development and
 997 neurodevelopmental disorders. *Neurotoxicology*. 2017;58:23-41.
 998 83.Rice DC. Evidence for delayed neurotoxicity produced by methylmercury. *Neurotoxicology*.
 999 1996;17(3-4):583-96.
 1000 84.Weiss B, Clarkson TW, Simon W. Silent latency periods in methylmercury poisoning and in
 1001 neurodegenerative disease. *Environ Health Perspect*. 2002;110 Suppl 5(Suppl 5):851-4.
 1002 85.Newland MC, Reed MN, Rasmussen E. A hypothesis about how early developmental
 1003 methylmercury exposure disrupts behavior in adulthood. *Behavioural processes*. 2015;114:41-51.
 1004 86.Adelson N. The embodiment of inequity: health disparities in aboriginal Canada. *Can J Public*
 1005 *Health*. 2005;96 Suppl 2(Suppl 2):S45-61.
 1006 87.Ninomiya T, Imamura K, Kuwahata M, Kindaichi M, Susa M, Ekino S. Reappraisal of
 1007 somatosensory disorders in methylmercury poisoning. *Neurotoxicol Teratol*. 2005;27(4):643-53.
 1008 88.Takaoka S, Fujino T, Kawakami Y, Shigeoka SI, Yorifuji T. Survey of the Extent of the
 1009 Persisting Effects of Methylmercury Pollution on the Inhabitants around the Shiranui Sea, Japan.
 1010 *Toxics*. 2018;6(3).
 1011 89.Yorifuji T, Takaoka S, Grandjean P. Accelerated functional losses in ageing congenital
 1012 Minamata disease patients. *Neurotoxicology and Teratology*. 2018;69:49-53.
 1013 90.Budtz-Jørgensen E, Keiding N, Grandjean P. Effects of exposure imprecision on estimation of
 1014 the benchmark dose. *Risk Anal*. 2004;24(6):1689-96.
 1015

Figures

Figure 1

Breakdown of GN-CHA participants with Hg biomarker measurements

Figure 2

Distribution of measured Hg biomarkers over time 2a. Hair Hg ($\mu\text{g/g}$) 1970 - 1997 ($n = 208$) 2b. Umbilical cord blood Hg ($\mu\text{g/L}$) 1970 - 1992 ($n = 211$)

Figure 3

Distribution of mean hair Hg ($\mu\text{g/g}$) at 10 years of age with respect to childhood fish consumption: $\geq$ several times a month ($n = 76$) and less ($n = 42$). Note: The fitted line represents second order polynomial (quadratic), with a shaded confidence band.

Figure 4

Graphic outputs for cluster decision 4a. Plot of the aggregation levels of symptom clusters 4b. Plot of stability of the partitions of clusters 4c. Plot of dispersion of the adjusted Rand index

Figure 5

SEM path diagram linking retrospective latent Hg exposure and covariates to the latent symptoms cluster variable for Extrapyramidal impairment.

Figure 6

SEM path diagram linking retrospective Hg exposure parameters (prenatal and childhood Hg exposure, and having had ≥ 5µg/g hair Hg at least once between 1970 and 1997) and covariates to the latent symptoms cluster variable for Extrapyramidal impairment.

Figure 7

SEM standardized path coefficients for direct and indirect contributions of latent Hg and covariates on the symptom latent variable for each cluster

Figure 8

SEM standardized path coefficients for direct and indirect contributions of prenatal, childhood and $\geq 5\mu\text{g/g}$ hair Hg (1970 – 1997) and covariates on the symptom latent variable for each cluster

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Additionalfile1ENHED2100280.pdf](#)