Appendix A – Supplementary Materials

Contents

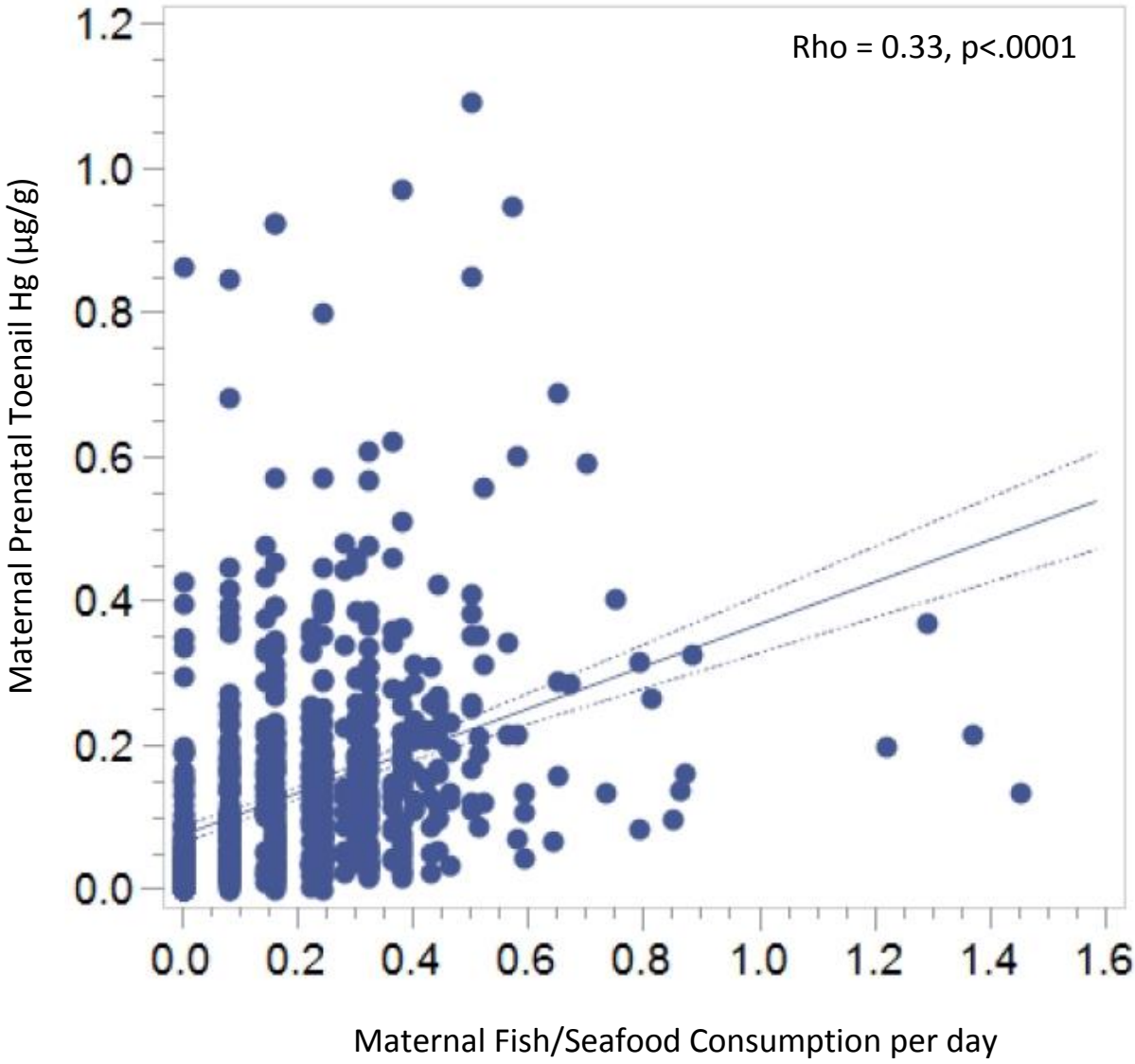

Figure A.1a. Correlation between maternal prenatal toenail Hg and fish/seafood consumption. Spearman correlation coefficient (Rho) and p-value derived from correlations between prenatal toenail Hg and self-reported servings of fish per day (n=1,321).

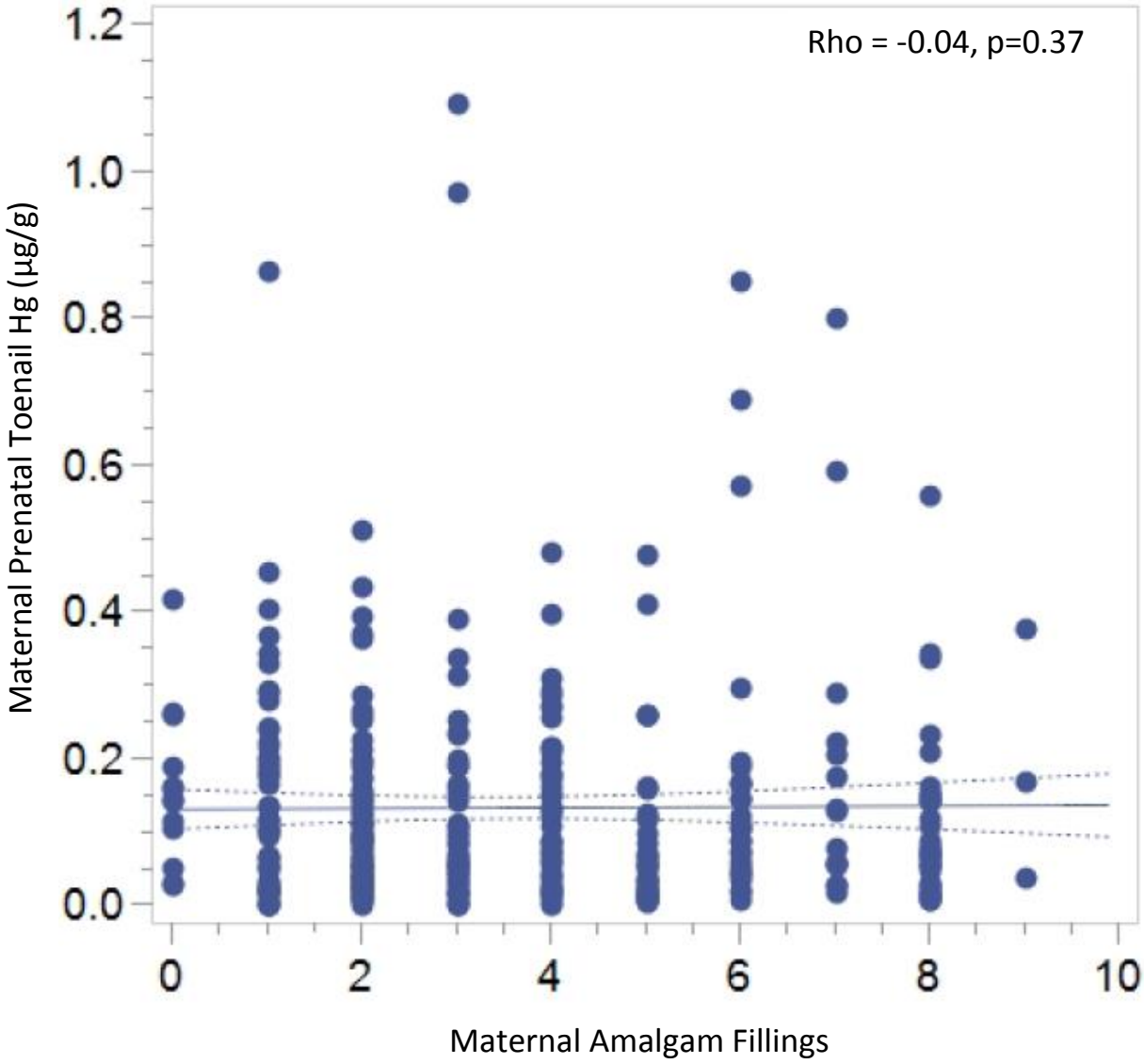

Figure A.1b. Correlation between maternal prenatal toenail Hg and presence of silver-mercury amalgam dental fillings. Spearman correlation coefficient (Rho) and p-value derived from correlations between self-reported number of amalgam fillings and prenatal maternal toenail Hg (n=616).

Table A.1 Sex stratified adjusted relative risks (RR) and 95% confidence intervals (CI) of infant respiratory infections and symptoms requiring a doctor visit, and any allergies confirmed by a doctor in the first year of life in relation to a doubling of prenatal maternal toenail Hg concentrations among mothers who consumed fish or seafood.

Girls												
	0-4 months (n=303)			5-8 months (n=314)			9-12 months (n=313)			Over the year (n=344)		
Outcome	Events (n)	RR	95% CI	Events (n)	RR	95% CI	Events (n)	RR	95% CI	Events (n)	RR	95% CI
Upper Respiratory Infection	74	1.0	(0.9-1.2)	131	1.0	(0.9-1.1)	183	1.0	(0.9-1.2)	388	1.0	(1.0-1.1)
Lower Respiratory Infection	6	0.5	(0.3-1.0)	20	0.9	(0.7-1.0)	10	1.3	(0.9-1.9)	36	0.9	(0.7-1.2)
Respiratory Symptoms	37	1.0	(0.8-1.4)	77	1.0	(0.9-1.3)	50	1.3	(1.0-1.6)	164	1.1	(1.0-1.3)
Eczema	5	1.0	(0.5-2.2)	13	0.7	(0.5-1.1)	7	0.8	(0.5-1.4)	25	0.8	(0.7-1.0)
Wheeze	6	1.0	(0.6-1.9)	15	0.9	(0.6-1.2)	7	1.4	(0.7-2.7)	28	1.0	(0.8-1.3)
Allergy	3	1.7	(0.7-4.4)	7	0.9	(0.5-1.7)	5	1.6	(0.7-3.5)	15	1.2	(0.9-1.6)
Boys												
	0-4 months (n=336)			5-8 months (n=331)			9-12 months (n=319)			Over the year (n=362)		
Outcome	Events (n)	RR	95% CI	Events (n)	RR	95% CI	Events (n)	RR	95% CI	Events (n)	RR	95% CI
Upper Respiratory Infection	84	1.0	(0.8-1.1)	174	1.0	(0.9-1.1)	197	1.0	(0.9-1.1)	455	1.0	(0.9-1.1)
Lower Respiratory Infection	24	0.8	(0.6-1.0)	39	1.0	(0.7-1.3)	14	**1.6**	**(1.2-2.4)****	77	0.9	(0.8-1.2)
Respiratory Symptoms	56	0.9	(0.7-1.1)	101	1.0	(0.9-1.2)	95	1.1	(0.9-1.4)	252	1.0	(0.9-1.2)
Eczema	10	1.1	(0.6-2.0)	29	1.3	(0.9-1.9)	18	0.9	(0.6-1.4)	57	1.1	(0.8-1.5)
Wheeze	13	0.8	(0.5-1.2)	18	1.1	(0.8-1.7)	19	1.1	(0.7-1.6)	50	1.0	(0.8-1.2)
Allergy*	3	1.3	(0.4-4.0)	10	1.3	(0.7-2.4)	15	0.7	(0.5-1.2)	28	0.9	(0.7-1.2)

Models were adjusted for maternal age, parity, smoking, education, dietary index and fish intake, infant sex, gestational age, birthweight, feeding at month 4, 8, 12 and day care attendance at month 4,8,12. Sample sizes for eczema were n=288/321 at 0-4 months for girls/boys, n=310/n=330 at 5-8 months for girls/boys, n=359 for boys at 9-12 months due to missing data. Sample size for allergy confirmed by doctor for girls/boys n=301/335 due to missing data. *In girls, models for allergy were only adjusted for maternal and gestational age whereas in boys models for allergy were run without daycare and feeding covariates in order to achieve model convergence.

**$p < 0.01$

Table A.2. Prenatal exposure to *in utero* Hg exposure and immune markers in offspring.

Reference	Hg exposure: (source, time, level)	Study, Design (n)	Results	Potential confounders considered in the analysis, other comments
			Immune Biomarkers in Newborns	
Cardenas et al 2015 [1]	Maternal toenail, postpartum, median μg/g: 0.07 (range: 0.001-1.44)	New Hampshire Birth Cohort Study, (n=138 maternal/infant pairs)	% change of white blood cell proportion inferred from DNA methylation: -2.5% (95%CI: -5.0, -1.0) monocytes 3.5% (95%CI: 1.0, 7.0) B cells in girls only	Maternal age, infant sex, other white blood cell proportions
Nyland et al 2011 [2]	Umbilical cord blood, geometric mean μg/g: 9.63 (range: 0.08 to 77.80)	Amazon region, Cross-sectional study (n=61 maternal/infant pairs)	Serum IgG at birth: *r*=0.61 (95%CI: 0.21, 1.02) Total serum antinuclear antibodies at birth: OR_{adj} 0.67 (95%CI: 0.41, 1.10) and IgG OR_{adj} 0.62 (95%CI: 0.37, 1.05) Serum cytokines (IL-1β, IL-6, TNFα) at birth: no association with cord blood mercury levels.	Maternal age, education level and residence and child sex

Reference	Hg exposure: (source, time, level)	Study, Design (n)	Results	Potential confounders considered in the analysis, other comments
Belles-Isles et al. 2002 [3]	Umbilical cord blood, geometric mean nmol/L, 95%CI: Exposed group: 9.1 (7.2, 11.3) Reference group:4.5 (3.9, 5.2)	Quebec, Canada cross sectional study (n=108, 48 from remote subsistence fishing population, 60 from urban center)	Newborn naïve T helper cells (CD4+CD45RA+): r = -0.27; p = 0.008 Newborn Natural Killer cell cytolytic activity: no difference between groups Newborn plasma IgM: r = -0.27; p = 0.008 Decreased *in vitro* lymphocyte proliferation ($p < 0.05$) in cord blood samples from subsistence fishing populations compared to urban population	Smoking during pregnancy
Bilrha et al. 2003[4]	Umbilical cord blood	Quebec, Canada cross sectional study (n=112, 47 from remote subsistence fishing population, 65 from urban center)	*In vitro* expression of activation markers following mitogen stimulation of lymphocytes similar between the two groups. TNF-α but not IL-10 secretion following lymphocyte activation was suppressed in subsistence fishing group.	Effect may be due to organochlorines rather than mercury
Immune Biomarkers in Children, Age 5 to 16 years				

Reference	Hg exposure: (source, time, level)	Study, Design (n)	Results	Potential confounders considered in the analysis, other comments
Oulhote et al. 2017[5]	Maternal Blood, geometric (mean, range) ug/L: 3.1, 0.53-20.0, maternal Hair: 0.7, 0.1-3.9, and cord-blood: 4.6, 0.8-21.1	Faroe Islands cohort 2007-2009 (n=54).	White blood cell counts at age 5; a one standard deviation (SD) increase in Hg was associated with a decrease by 23% SD (95%CI: −43, −4) in lymphocytes (including T cells, NK cells, and B cells) No associations between prenatal mercury and neutrophils, eosinophils, basophils, and monocytes were observed.	Age at examination (months), sex, parity (no older sibling vs. 1 or more older siblings), maternal smoking during pregnancy, birth weight (g) and duration of breastfeeding (< or ≥ 6 months).
Hui et al. 2016[6]	Umbilical cord blood, median (interquartile range) 46.1 (33.1-65.1) nmol/L	Chinese mercury birth cohort, prospective (n=407)	Serum cytokines at age 6-9 Regression coefficient (95%CI): IL-4: 0.03 (-0.09, 0.16) IL-5: -0.04 (-0.12, 0.03) IL-6: -0.17 (-0.31, -0.03) IL-8: -0.10 (-0.26, 0.06) IL-10: -0.01 (-0.15, 0.13) IL-13: -0.00 (-0.09, 0.08) TNFα: -0.09 (-0.20, 0.02)	Sex, age, parental education and fish consumption during pregnancy

Reference	Hg exposure: (source, time, level)	Study, Design (n)	Results	Potential confounders considered in the analysis, other comments
Grandjean et al. 2010 [7]	Umbilical cord blood, geometric mean (interquartile range) total mercury μg/L: 11.3 (7.4–21.0)	Faroe Islands birth cohort study, (n=464)	Total serum IgE at 7 years: r_s <0.2; p =0.06 Grass specific serum IgE at 7 years: r_s= -0.17; p <0.001	Sex, age season of birth, preterm birth, low birth weight, maternal age, parity, maternal fish intake and smoking during pregnancy, parental smoking at home, daycare attendance, and the child's body mass index (covariates did not affect unadjusted results).
Osuna et al. 2014 [8]	Umbilical cord blood, geometric mean (interquartile range) total mercury μg/L: 31 (11, 64)	Faroe Islands birth cohort (n=38)	Serum IgM at age 7 years: 30-53% increase (p <0.05) in IgM concentrations- specific to neural antigens (neurofilaments and astrocyte glial fibrillary acidic protein) per 2 fold increase in blood Hg Serum IgG at age 7 years: 60% reduction (p <.05) in anti-keratin IgG concentrations per 2 fold increase in blood Hg No associations were observed between other neural (cholineacetyltransferase, myelin basic protein) or non-neural (actin, desmin) antigens.	Child's sex
Clinical Outcomes in Early Childhood Ages 2 to 4 years				

Reference	Hg exposure: (source, time, level)	Study, Design (n)	Results	Potential confounders considered in the analysis, other comments
Miyake et al. 2011 [9]	Maternal hair, 29-39 months postpartum*, median ug/g:1.52 (range:0.26-6.05)	4th survey of the Osaka Maternal and Child Health Study, cross-sectional (n=582 mother/child pairs)	Wheeze at 29-39 months: OR_{adj} = 0.77 (95%CI: 0.41, 1.44) associated with continuous measures of maternal hair mercury levels Eczema at 29-39 months: OR_{adj} =0.92 (95%CI: 0.69, 1.21) associated with measures levels of maternal hair mercury levels	Maternal age, residential municipality, parental education and history of allergic disorders, maternal energy-adjusted fish intake during pregnancy, maternal smoking during pregnancy, parity, child's sex, household smoking in same room as child, breastfeeding duration, children's fish intake
Shaheen et al. 2004 [10]	Umbilical cord blood geometric mean (GM) parts per billion; Phase 1 (n=1755) =0.0127 Phase 2 (n=817) = 0.0018 20th % method GM=0.54 80th Percentile method GM=1.83 Interpercentile ratio= 3:38	Avon Longitudinal Study of Parents and Children (ALSPAC) study, (n=2,044 for wheeze and n=2,173 for eczema)	Wheeze at 30-42 months: OR_{adj}=0.99 (95%CI: 0.89-1.11) Eczema at 18-30 months: OR_{adj}= 1.03 (95%CI: 0.95-1.12)	Maternal age, parity, smoking during pregnancy, education, ethnic origin, housing status and pre-pregnancy BMI; child's sex, weight, head circumference and crown-heel length at birth; breast feeding and day care use in the first 6 months & other condition (e.g., other symptom or atopic diagnosis (wheeze or eczema)).

Reference	Hg exposure: (source, time, level)	Study, Design (n)	Results	Potential confounders considered in the analysis, other comments
Clinical Outcomes in Children Ages 5 to 16 years				
Grandjean et al. 2010 [7]	Umbilical cord blood geometric mean (interquartile range) total mercury μg/L: 11.3 (7.4–21.0)	Faroese Birth cohort, (n=464)	Asthma or atopic eczema at 5 or 7 years [average Hg (interquartile range)] : No allergy 12.8 (7.2 – 21.1) Asthma 14.7 (7.6 – 29.2), p =0.35 (vs no allergy) Atopic dermatitis 13.2 (7.4 – 24.2), p = 0.80 (vs no allergy)	Sex, age season of birth, preterm birth, low birth weight, maternal age, parity, maternal fish intake and smoking during pregnancy, parental smoking at home, daycare attendance, and the child's body mass index (covariates did not affect unadjusted results).
Jedrychowski et al. 2011[11]	Umbilical cord blood, 2nd trimester, mean 0.88 μg/dL, 95%CI: 0.81, 0.95) Maternal blood at delivery (mean 0.60 μg/dL 95%CI: 0.54, 0.67)	Prospective cohort from Krawkow (n=224)	Atopic status (pin prick test to common allergens at 5 years): Cord blood RR_{adj}=1.11 (95%CI:0.68, 1.80) Maternal blood RR_{adj}= 0.64 (95%CI:0.28, 1.45)	Maternal characteristics (age, education, atopy), child's gender, number of older siblings, prenatal (measured via cord blood cotinine) and postnatal environmental tobacco smoke together with exposure to polycyclic aromatic hydrocarbons (PAH).

$OR_{ad=}$ adjusted odds ratio, RR_{adj} = adjusted RR, r = correlation coefficient, $r_{s=}$ Spearman correlation coefficient*Maternal hair postpartum is surrogate for prenatal concentrations.

References

1. Cardenas A, Koestler DC, Houseman EA, et al. Differential DNA methylation in umbilical cord blood of infants exposed to mercury and arsenic in utero. Epigenetics 2015;10:508-15.

2. Nyland JF, Fillion M, Barbosa F, Jr., et al. Biomarkers of methylmercury exposure immunotoxicity among fish consumers in Amazonian Brazil. Environ Health Perspect 2011;119:1733-8.

3. Belles-Isles M, Ayotte P, Dewailly E, Weber JP, Roy R. Cord blood lymphocyte functions in newborns from a remote maritime population exposed to organochlorines and methylmercury. J Toxicol Environ Health A 2002;65:165-82.

4. Bilrha H, Roy R, Moreau B, Belles-Isles M, Dewailly E, Ayotte P. In vitro activation of cord blood mononuclear cells and cytokine production in a remote coastal population exposed to organochlorines and methyl mercury. Environ Health Perspect 2003;111:1952-7.

5. Oulhote Y, Shamim Z, Kielsen K, et al. Children's white blood cell counts in relation to developmental exposures to methylmercury and persistent organic pollutants. Reproductive Toxicology 2017;68:207-14.

6. Hui LL, Chan MHM, Lam HS, et al. Impact of fetal and childhood mercury exposure on immune status in children. Environmental Research 2016;144:66-72.

7. Grandjean P, Poulsen LK, Heilmann C, Steuerwald U, Weihe P. Allergy and sensitization during childhood associated with prenatal and lactational exposure to marine pollutants. Environ Health Perspect 2010;118:1429-33.

8. Osuna CE, Grandjean P, Weihe P, El-Fawal HA. Autoantibodies associated with prenatal and childhood exposure to environmental chemicals in Faroese children. Toxicol Sci 2014;142:158-66.

9. Miyake Y, Tanaka K, Yasutake A, Sasaki S, Hirota Y. Lack of association of mercury with risk of wheeze and eczema in Japanese children: The Osaka Maternal and Child Health Study. Environmental Research 2011;111:1180-4.

10. Shaheen SO, Newson RB, Henderson AJ, Emmett PM, Sherriff A, Cooke M. Umbilical cord trace elements and minerals and risk of early childhood wheezing and eczema. European Respiratory Journal 2004;24:292-7.

11. Jedrychowski W, Perera F, Maugeri U, et al. Intrauterine exposure to lead may enhance sensitization to common inhalant allergens in early childhood. A prospective prebirth cohort study Environmental research 2011;111:119-24.