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Canada

Individual, independent, and combined effects of toxic metals and manganese on hypertensive disorders of pregnancy

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Health Canada

YOUR HEALTH AND SAFETY... OUR PRIORITY.

Outline

Environmental chemicals – the mixtures problem

(an epidemiologists' perspective…)

Case Study: *Individual, independent, and combined associations of toxic metals and manganese with hypertensive disorders of pregnancy*

- "traditional" regression analysis
- a mixture method (quantile g-computation)

Discussion – how should we evaluate dose-response in the (epidemiological) context of chemical mixtures?

Environmental chemicals - the mixtures problem (the epidemiologists' perspective…)

We live in a chemical soup

Humans are exposed to many (!) environmental chemicals

Some are toxic. Some may not be toxic*. Some may be beneficial within an optimal dose range, but with effects outside of this range (e.g., manganese)

We have insufficient knowledge of the health effects of most chemicals

Environmental epidemiologists have traditionally examined one chemical at a time

Environmental Chemicals - The mixtures problem

This one-chemical-at-a-time approach:

- Provides robust, “unbiased” effect estimates (e.g., relative risks, regression coefficients)
- Are easy to interpret, both statistically and pragmatically
- Is easy to adapt for simple dose (exposure)-response approaches (e.g., threshold effects, ceiling/floor effects, splines, quantiles)

But!

- Does not reflect reality (unique soups!)
- Ignores interaction/non-additivity
- Difficult to isolate exposures in regulatory/public health initiatives
- Ignores the potential for **cumulative effects**

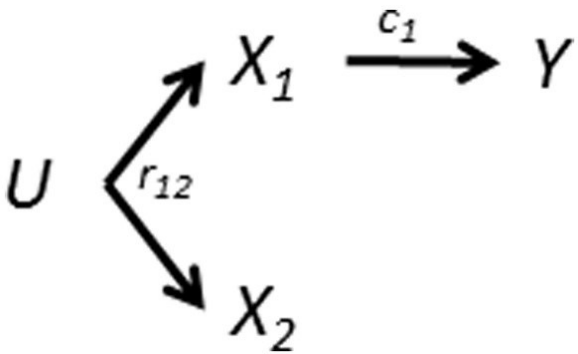
The mixtures problem - dimensionality/collinearity

One approach is to force-entering all of your exposures into the same model

- Issues with collinearity (exposures are often correlated, sometimes highly) because of shared sources, exposure routes, metabolic pathways
- Issues with high-dimensionality

Could use to variable selection methods (ANN, machine learning)

- May not perform well with very high correlations (>0.95) among exposures
- Bias amplification with co-pollutant confounding
- Inconsistent selection across samples
- Matrix/measurement effects



Weisskopf et al. Bias Amplification in Epidemiologic Analysis of Exposure to Mixtures. EHP 2018; 126(4)

Could use variable shrinkage methods (elastic net, LASSO)

- Issue with interpreting coefficients
- May not perform well with very high correlations (>0.95) among exposures

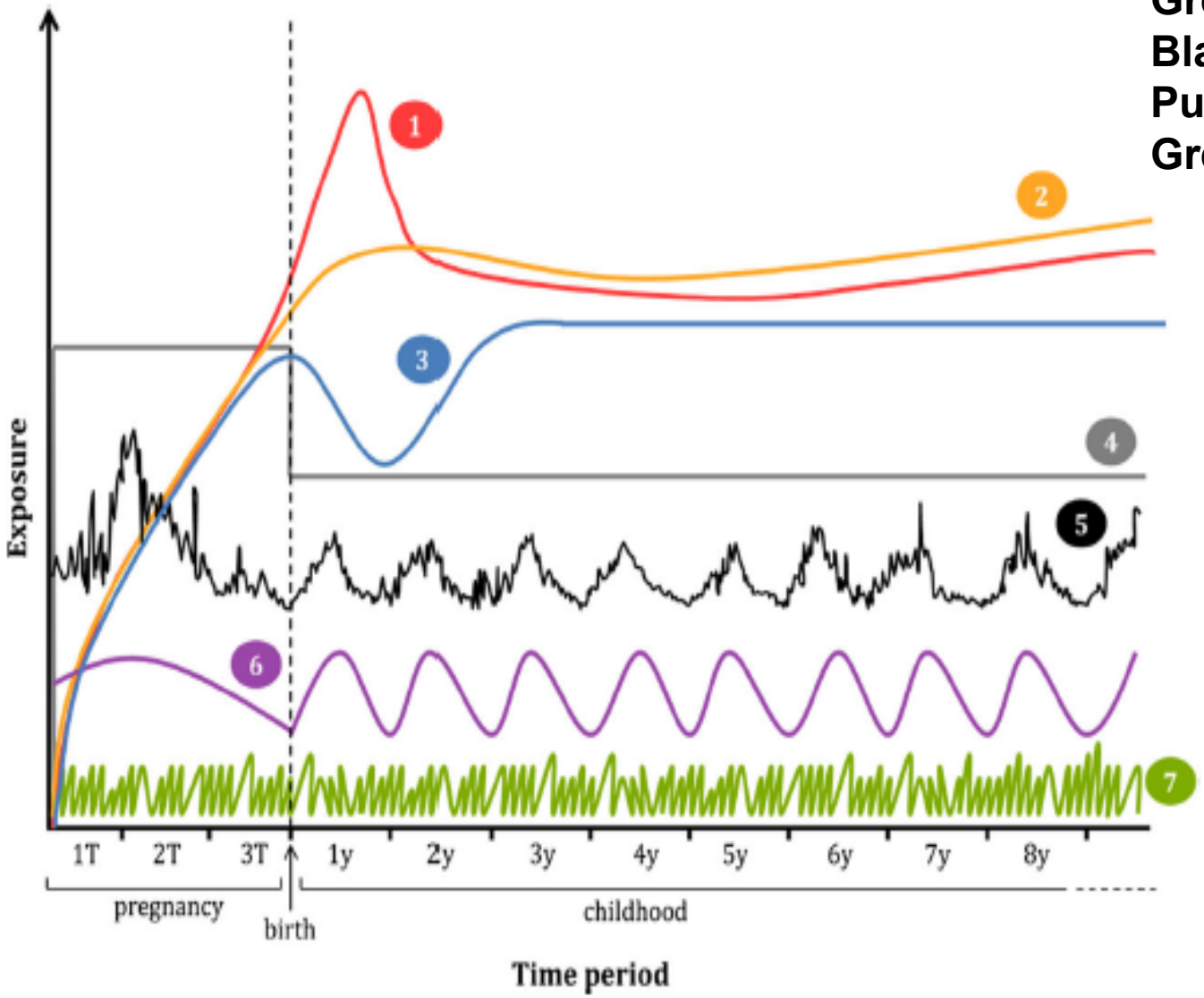
What is a mixture?

Features of a mixture may share:

- common sources (drinking water or air pollution)
- common routes of exposure (ingestion, inhalation)
- metabolic processes (excretion pathways)
- mechanistic outcome pathways (oxidative stress)
- Relevant time windows of exposure (early pregnancy)

What is a mixture?
(it's a bit more complicated than soup…)

Red = POPs
Orange = Mercury and lead
Blue = Arsenic
Grey = tobacco smoke
Black = Air Pollution
Purple = UV radiation
Green = Non-persistents



The composition of our unique "soup" is always changing

Robinson & Vrijheid, 2015

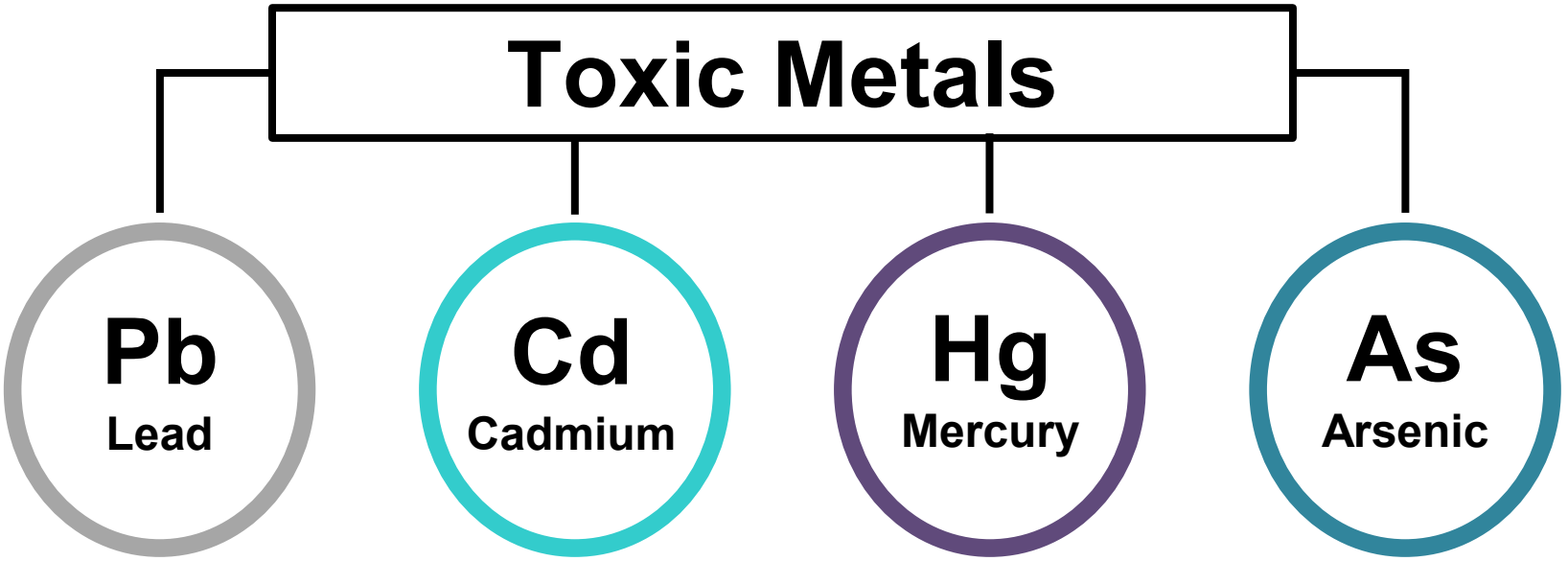
The mixtures problem

Environmental epidemiologists are increasingly interested in the health effects of cumulative exposure to mixtures of chemicals.

Perhaps unsurprisingly, there is no one superior method (solution) for determining the cumulative health effects of a chemical mixture.

It is not yet clear how we should think about dose-response in the context of an (epidemiological) chemical mixture.

The case study



Canadians are routinely exposed to low levels of toxic metals

No known physiological function

Linked with a range of health outcomes

Pregnancy-related changes in physiology, behaviours, and dietary needs can exacerbate low levels of exposure

Toxic metals and hypertensive disorders of pregnancy

Toxic metals are associated with a range of perinatal health/birth outcomes

Thought to be the leading (environmental) cause of hypertensive disorders of pregnancy (HDP) at higher levels of exposure

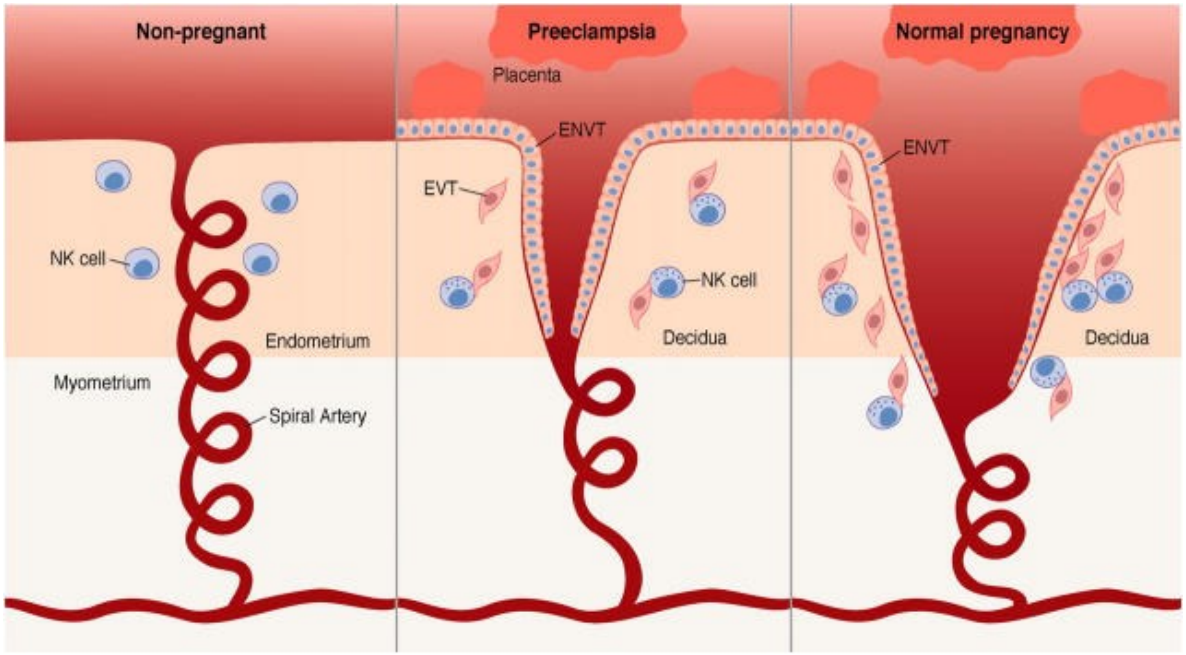
Not clear if associations persist at low levels of exposure typically experienced by Canadian women

Gestational hypertension

Preeclampsia

Toxic metals and preeclampsia

During normal pregnancy, uterine spiral arteries are remodeled into large vessels to vascularize the placenta. In preeclampsia the spiral arteries remain narrow, reducing placental perfusion.



First (placental) stage – defects in placental cytotrophoblast invasion and spiral artery remodelling

Second (maternal) stage – systemic endothelial dysfunction

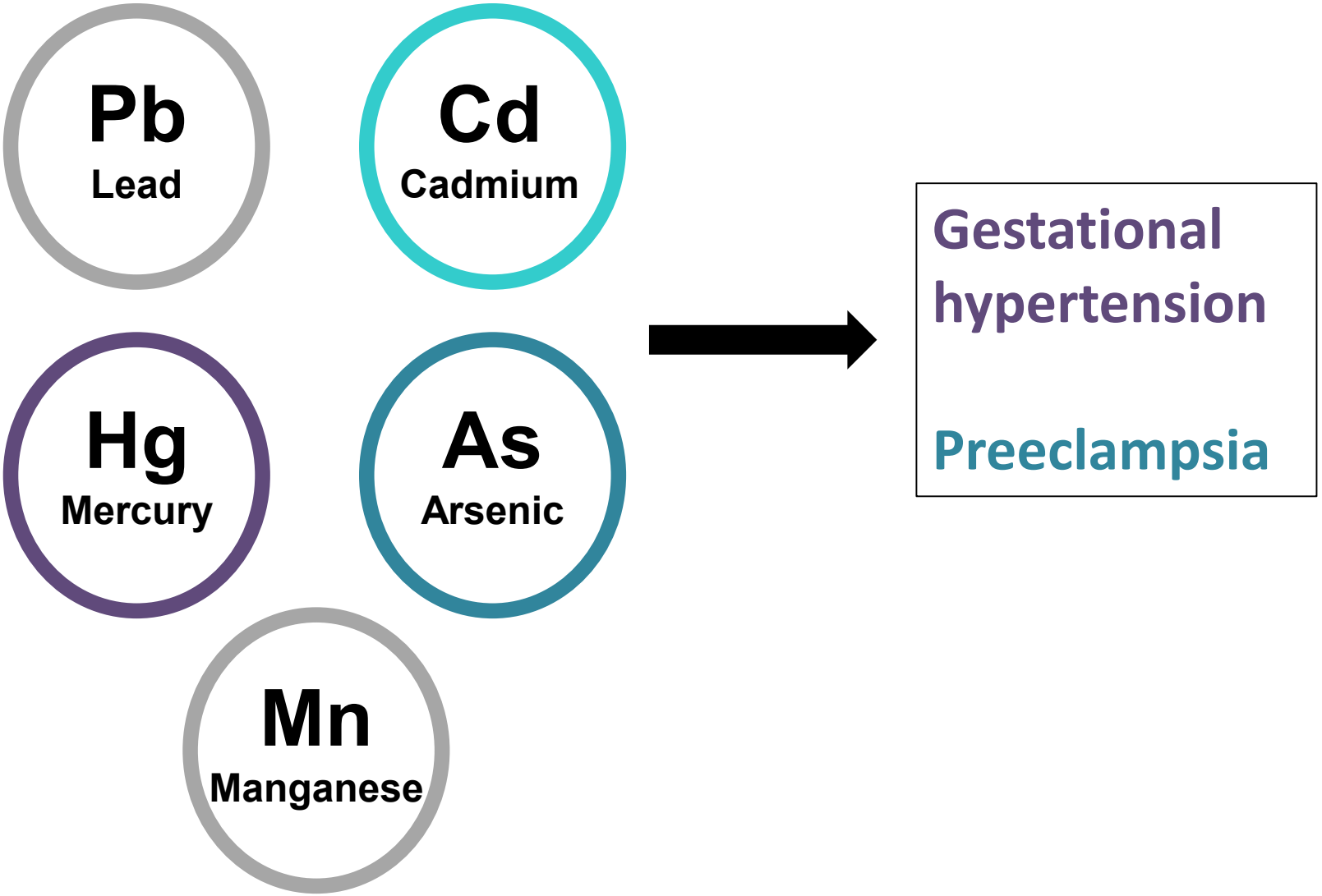
Oxidative stress contributes to this disease process

Toxic metals have been shown to induce oxidative stress

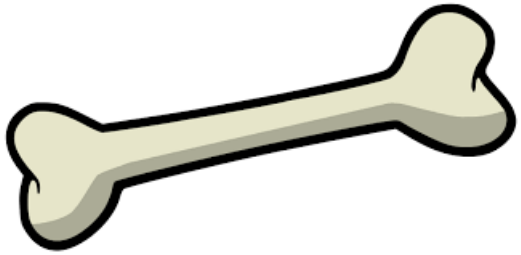
Manganese may play a protective role as a component of the anti-oxidant enzyme superoxide dismutase

Figure reference: Parham (2004). J Exp Med, 200(8):951-955

Objective


Pb
Lead
Cd
Cadmium
Hg
Mercury
As
Arsenic
Mn
Manganese
Gestational hypertension
Preeclampsia

Pb
Lead

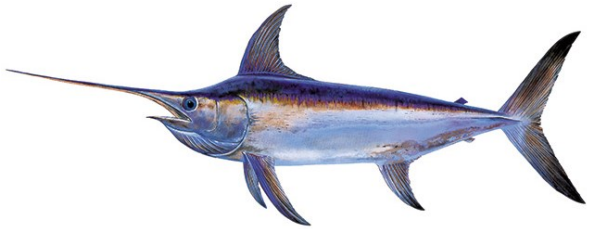


Cd
Cadmium

These metals may act individually or independently – they have some unique, or at least more prominent, sources of exposure

As
Arsenic

Hg
Mercury



Toxic metals can also be thought of a mixture

They share common sources (e.g., drinking water and air pollution)

They share common routes of exposure (ingestion, inhalation)

They may operate on similar mechanistic outcome pathways (e.g., oxidative stress)

What would be the effect of modifying exposure to a **mixture** of toxic metals?

Research Questions

1) How does exposure to **individual** metals impact the risk of developing HDP?

2) What are the **independent** effects of these metals on the risk of developing HDP?

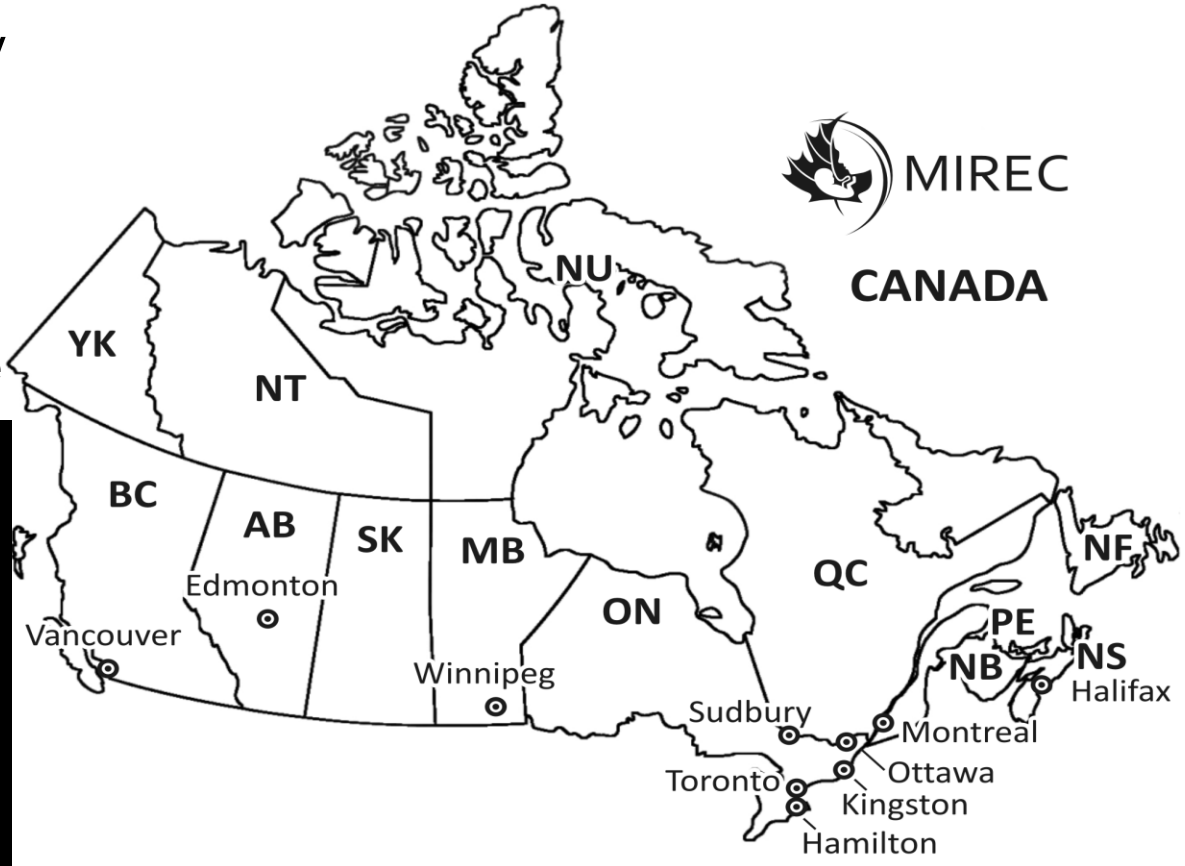
3) What is the **combined** (cumulative) effect of modifying exposure to a mixture of these metals?

Maternal Infant Research on Environmental Chemicals (MIREC) pregnancy cohort

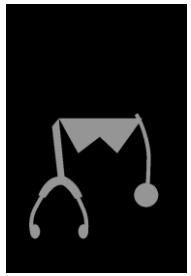
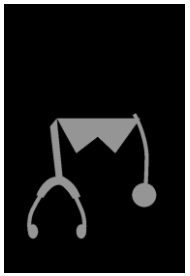
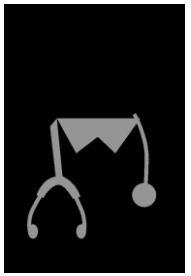
2,001 pregnant women from 10 sites were enrolled between 2008-2011

Recruited in early pregnancy (≈14 weeks) and followed until post-delivery

1,560 were included in this analysis (complete exposure



Data Collection

1st trimester	2nd trimester	3rd trimester
		
		
		

Most previous research has relied on a single measure of exposure during pregnancy. Having both T1 and T3 blood samples is an opportunity to assess different windows of exposure

Defining hypertensive disorders of pregnancy

Society of Obstetricians and Gynaecologists of Canada (SOGC) guidelines

Gestational hypertension

SBP ≥ 140 mmHg and/or
DBP ≥ 90 mmHg, at ≥ 20 weeks gestation

Preeclampsia

Gestational hypertension +
Proteinuria or maternal complications

- Disseminated intravascular coagulation
- Pulmonary edema
- Convulsions (eclampsia)
- Blood transfusion
- Elevated liver enzyme levels
- Platelet count < 50 x 10^9/L

Participants categorized into 3 outcome groups:

1) **Normotensive (reference group)**
2) **Gestational hypertension without preeclampsia**
3) **Preeclampsia**

Statistical analysis

1) Individual effects

Poisson regression with robust variance – relative risks (RR) and 95% CIs of developing outcomes for each blood metal

- Log_2-transformed metal levels (i.e., per doubling of concentration)
- Tertiles
- Sensitivity and model specification analyses

2) Independent effects

All blood metals were mutually adjusted in the same model

3) Combined effects

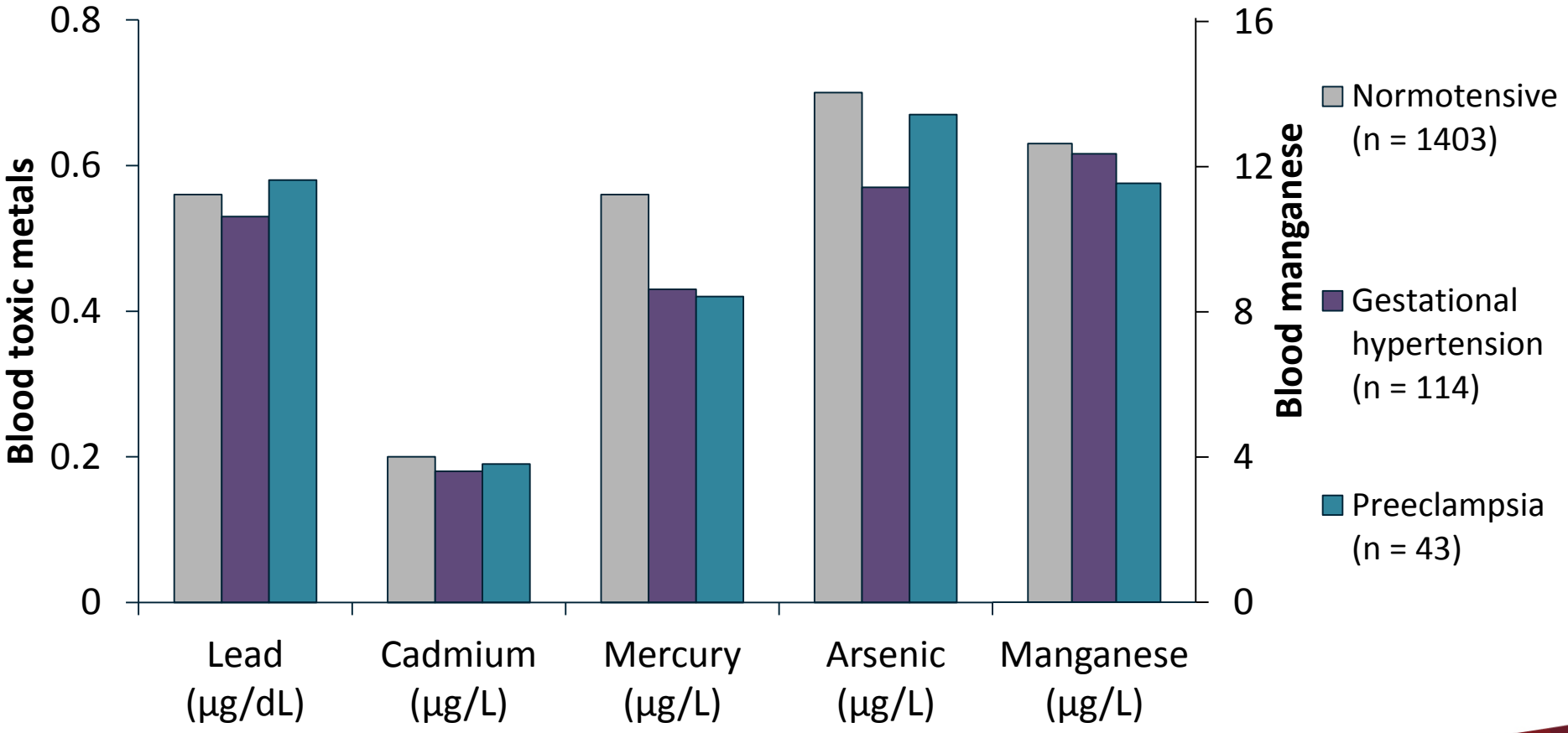
Novel mixture approach, quantile g-computation

Covariates: maternal age, education, smoking status, pre-pregnancy BMI, parity, country of birth, and reported consumption of fish high in mercury (Hg models)

Missing covariate data were multiply imputed (m = 5)

Descriptive Results

Median 3rd trimester blood toxic metals (left axis) and manganese (right axis) concentrations for normotensive, gestational hypertension, and preeclampsia outcome groups



Correlations between and within 1st and 3rd trimester blood metals concentrations (n=1560)

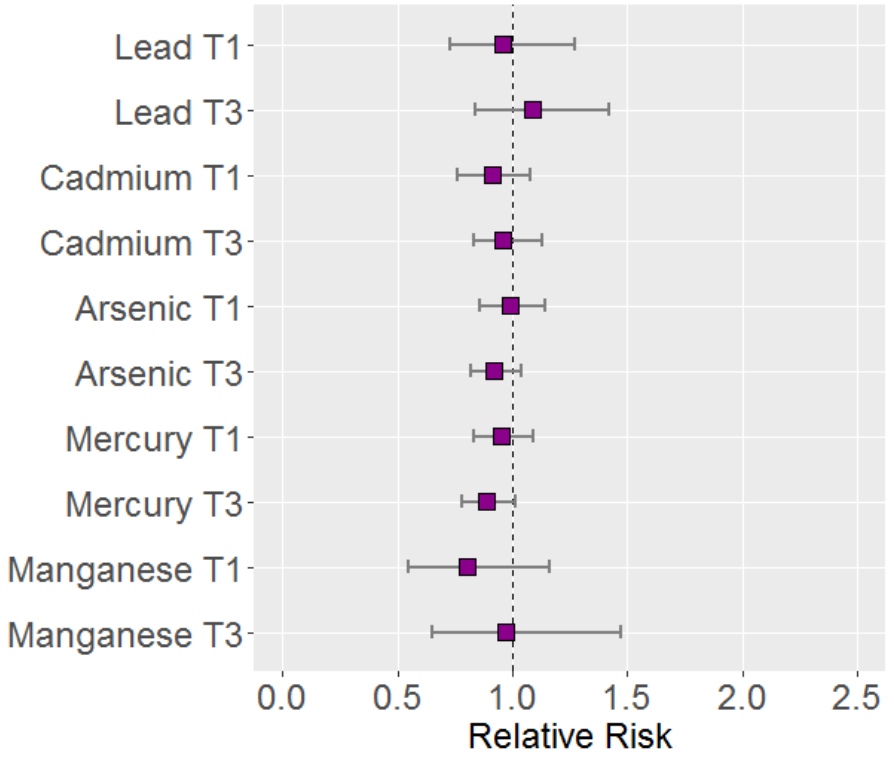
	Lead (µg/dL)	Cadmium (µg/L)	Arsenic (µg/L)	Mercury (µg/L)	Manganese (µg/L)
Lead (µg/dL)	0.74**	0.22**	0.17**	0.26**	0.11**
Cadmium (µg/L)	0.27**	0.61**	-0.05*	0.05	0.13**
Arsenic (µg/L)	0.17**	0.02	0.35**	0.38**	0.05
Mercury (µg/L)	0.28**	0.10**	0.38**	0.76**	0.03
Manganese (µg/L)	0.20**	0.23**	0.06*	0.11**	0.63**

Red cells represent Spearman correlations within the 1st trimester
Blue cells represent Spearman correlations within the 3rd trimester
White cells represent intra-class correlations for the same metal between 1st and 3rd trimester
* $p < 0.05$
** $p < 0.0001$

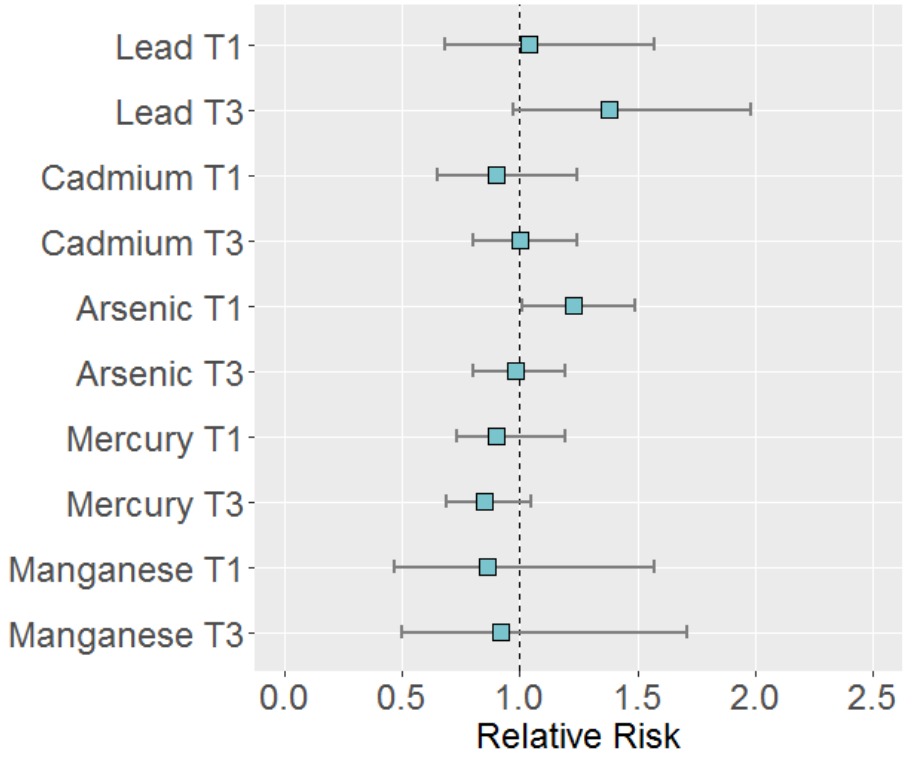
Individual effects – linear models

Adjusted relative risks (95% CI) for each doubling (Log_2) of individual blood metal concentrations (reference = normotensive)

Gestational hypertension



Preeclampsia

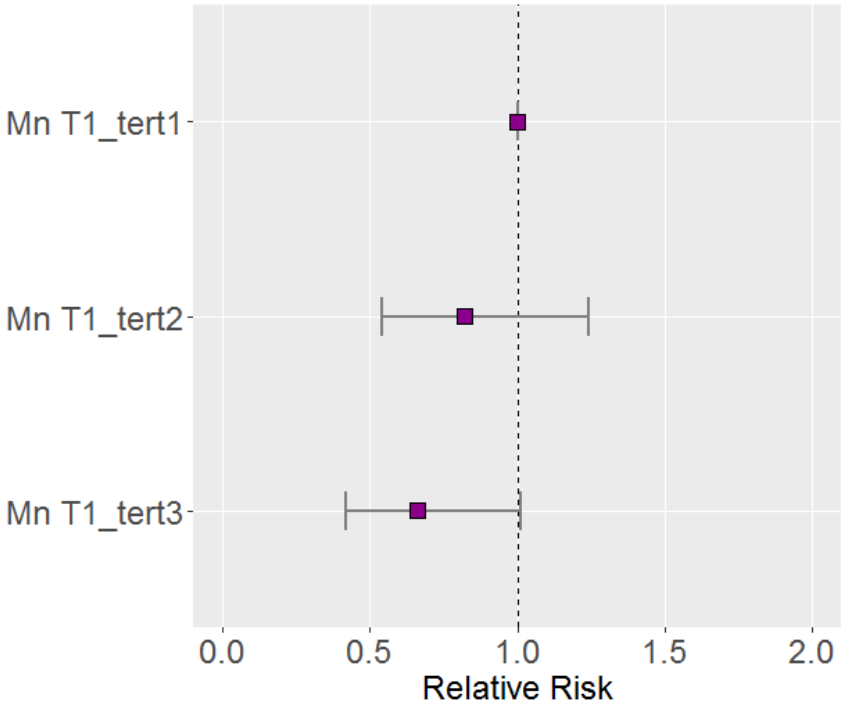


Individual effects – by tertile and sex

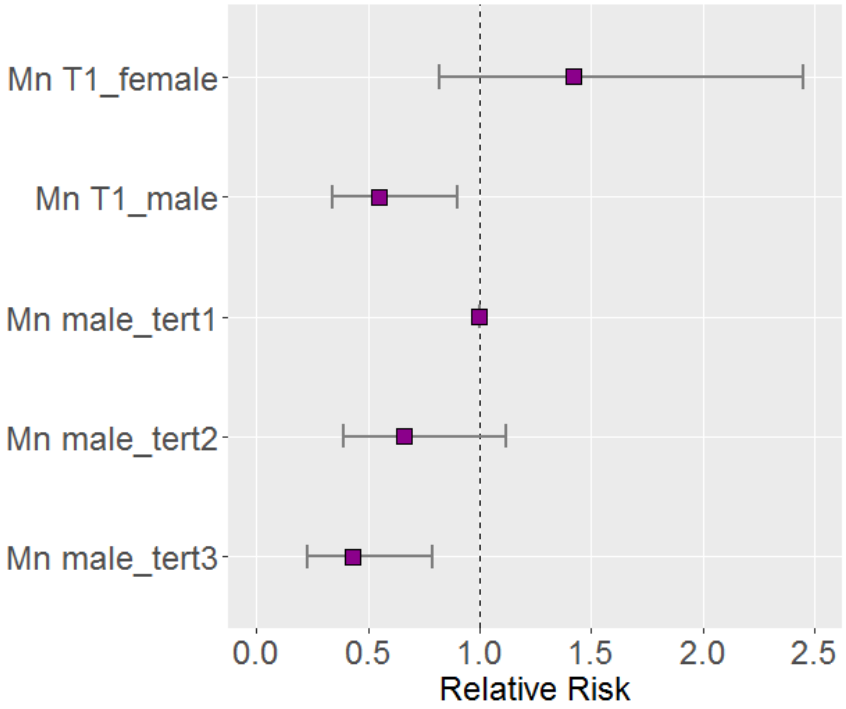
Tertile effects for lead, cadmium, arsenic, and mercury were small and all p-trend crossed the null

Gestational hypertension

T1 Mn tertiles – full sample



T1 Mn by fetal sex



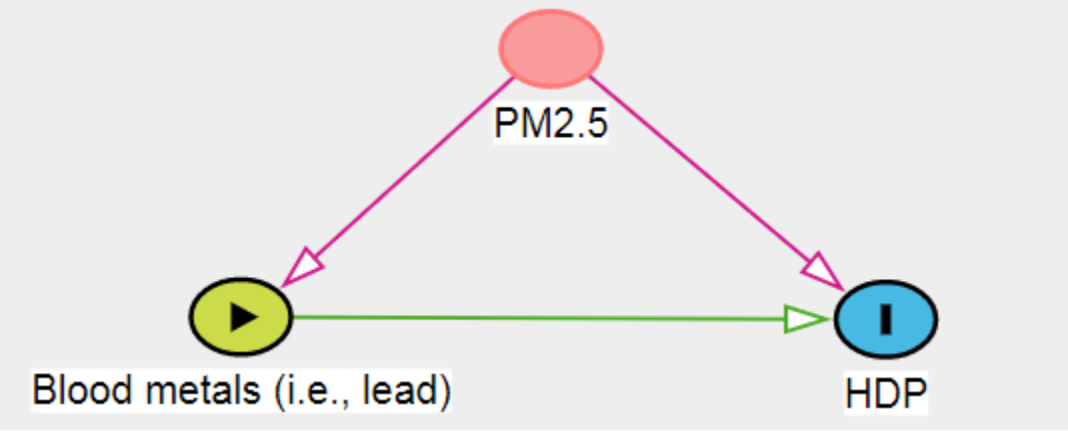
Individual effects – sensitivity analysis with air pollution

Air pollution is associated with risk of developing HDP. $PM_{2.5}$ is one prominent measure of air pollution and represents a complex mixture of particles, including metals.

$PM_{2.5}$ surfaces developed using satellite-based methods combined with chemical transport models. Daily values were averaged across the 1st and 3rd trimester.

Using MIREC data, we recently showed that an IQR-increase in $PM_{2.5}$ was associated with up to 25% higher C-reactive protein levels

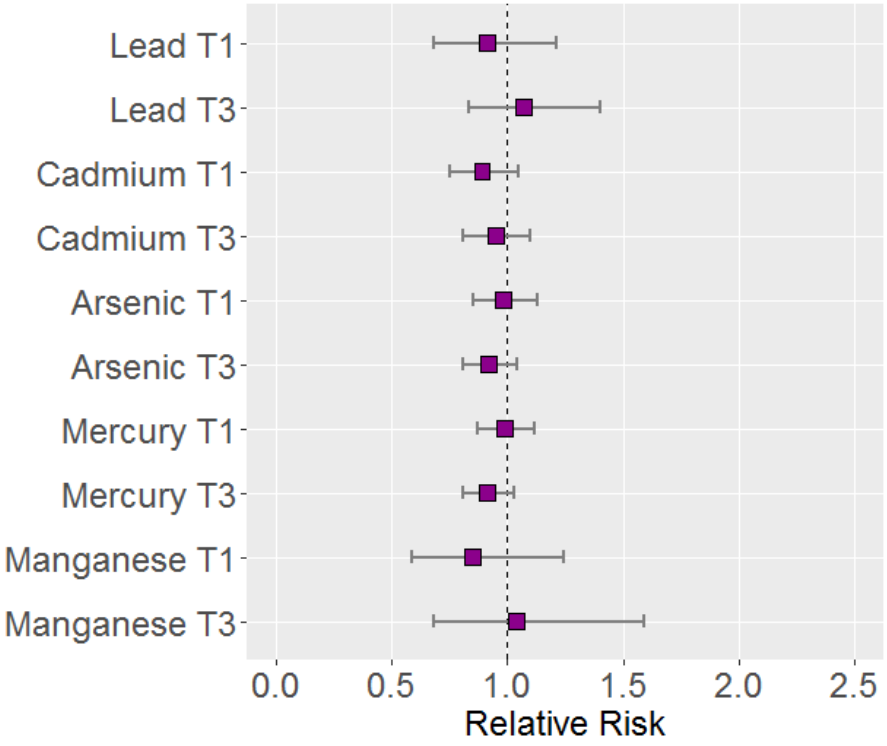
(Gogna et al., Environmental Epidemiology, in press)



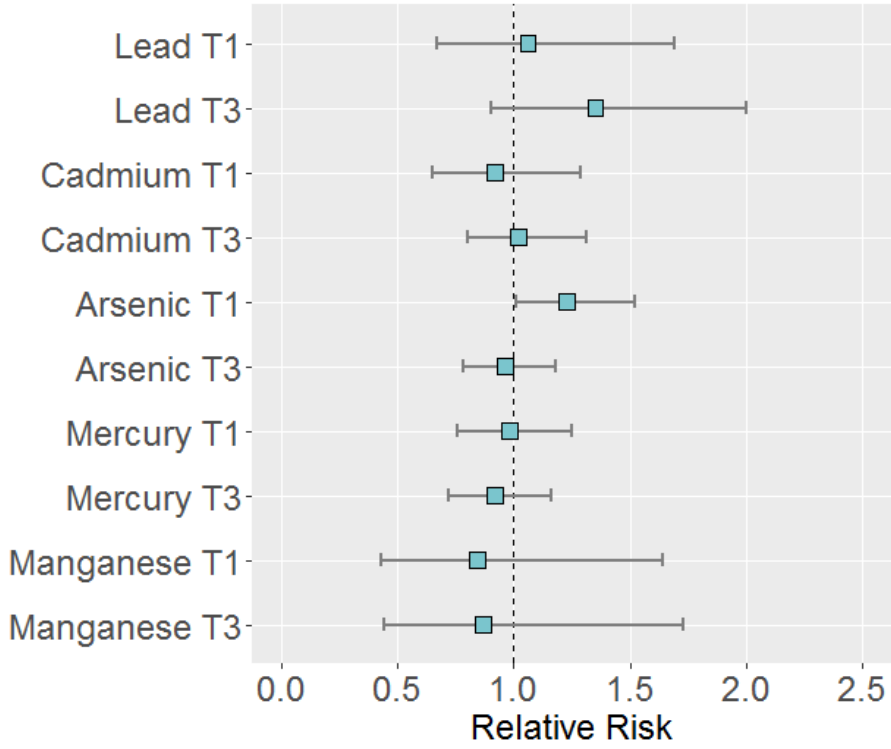
Individual effects – sensitivity analysis with air pollution

Additionally adjusting for trimester-specific $PM_{2.5}$ did not appreciably impact the results

Gestational hypertension



Preeclampsia



Individual effects – additional analyses

Cd
Cadmium

Explored adjusting for trimester-specific urinary cotinine (marker of nicotine exposure) instead of (and with) smoking status.

Smoking status alone resulted in better model fit, and was also better predictive of blood Cd levels (≈50% of the variance)

Hg
Mercury

Explored adjusting for overall fish consumption vs. only fish high in Hg.

Model fit was similar. Each was similarly predictive. Used fish in Hg in the final models.

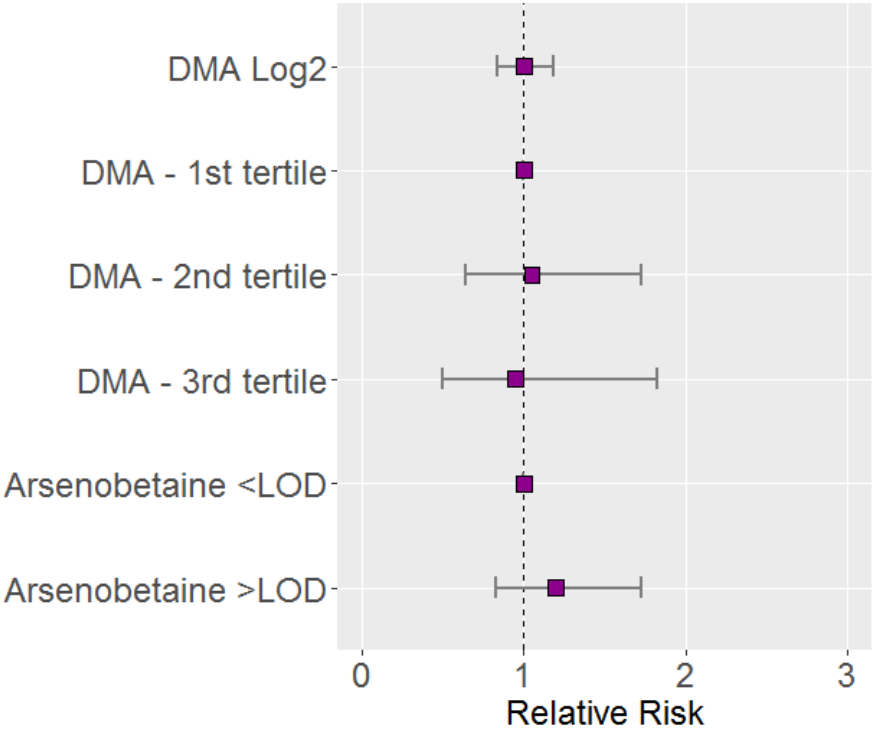
Individual effects – additional analyses

As
Arsenic

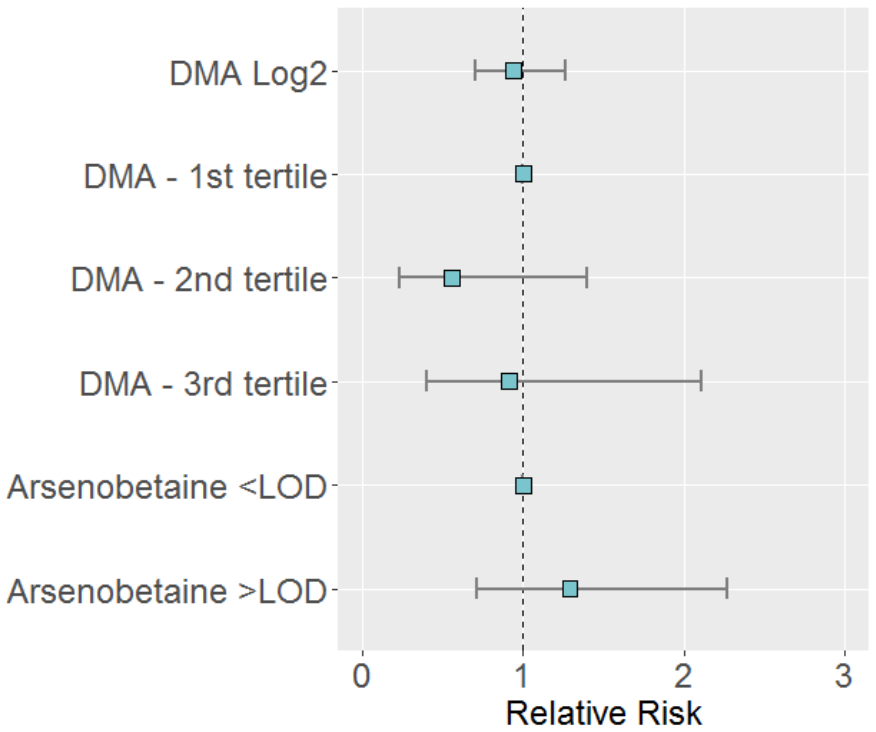
Urine, rather than blood, as an alternative (better) matrix to estimate exposure via metabolites

Analyzed DMA (Log_2 and tertiles) and arsenobetaine (</≥ LOD) in 1st trimester urine samples

Gestational hypertension



Preeclampsia

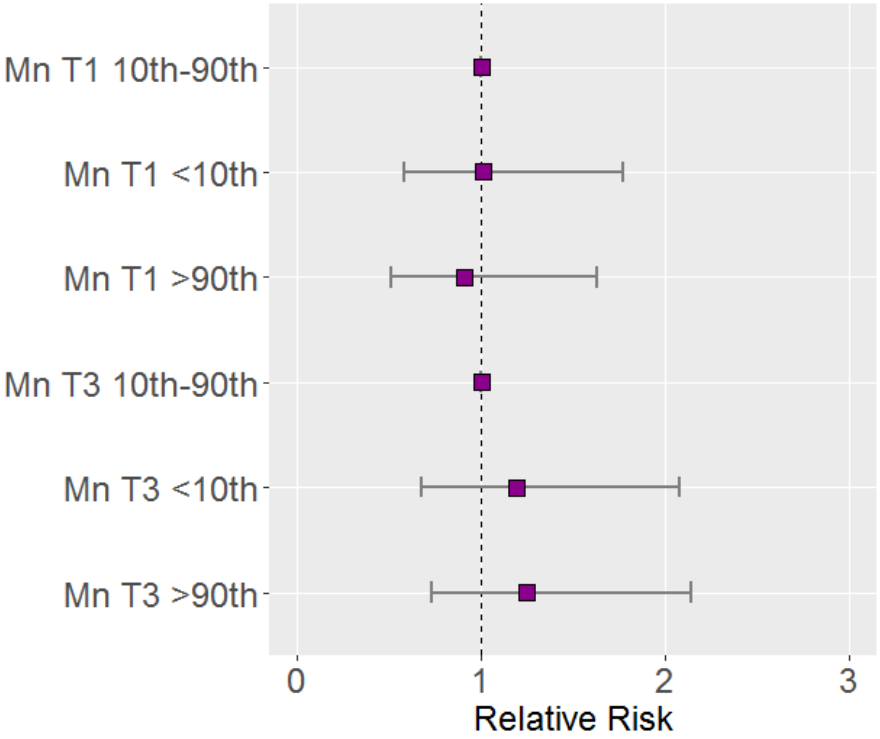


Individual effects – additional analyses

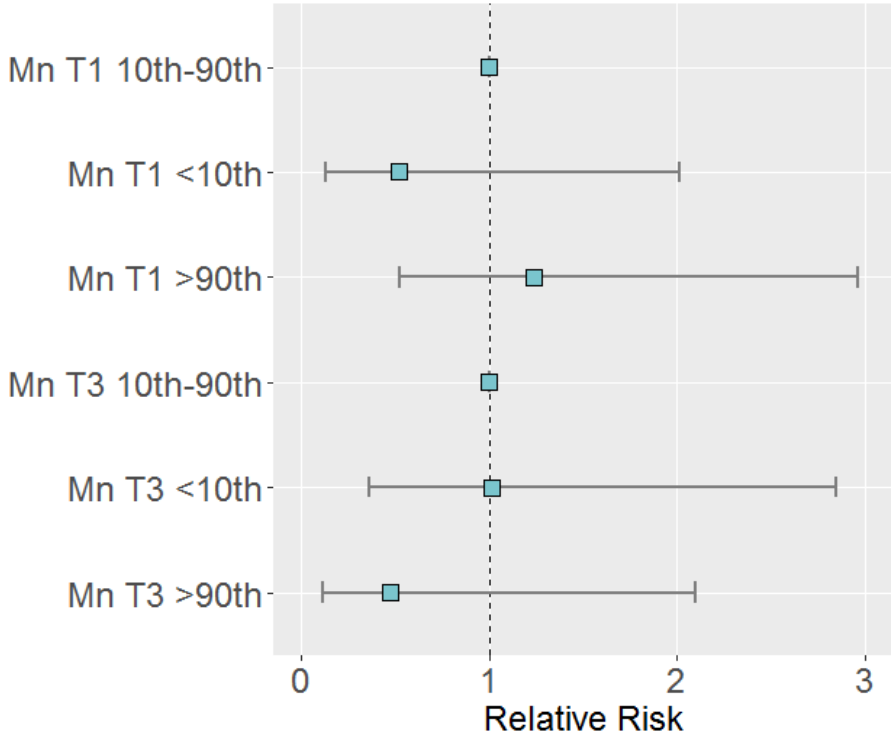
Mn
Manganese

Both a nutrient and a toxin. Well regulated in blood at normal levels.
Health effects tend to be observed at extreme high and low levels of exposure. Tertiles may not adequately capture these effects. Examined <10th and >90th percentiles.

Gestational hypertension



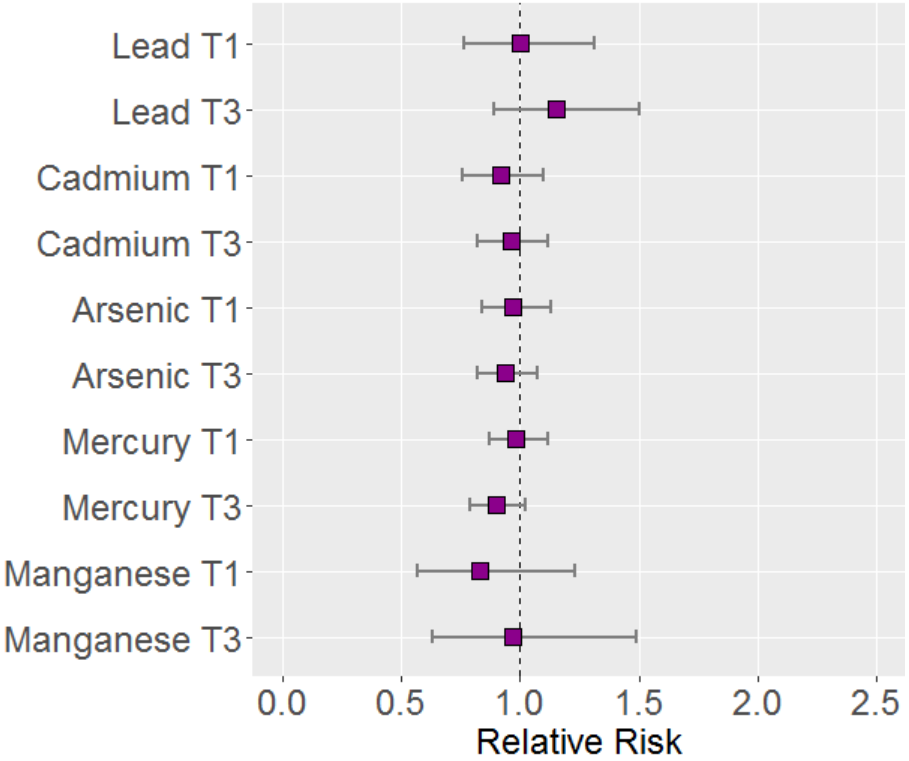
Preeclampsia



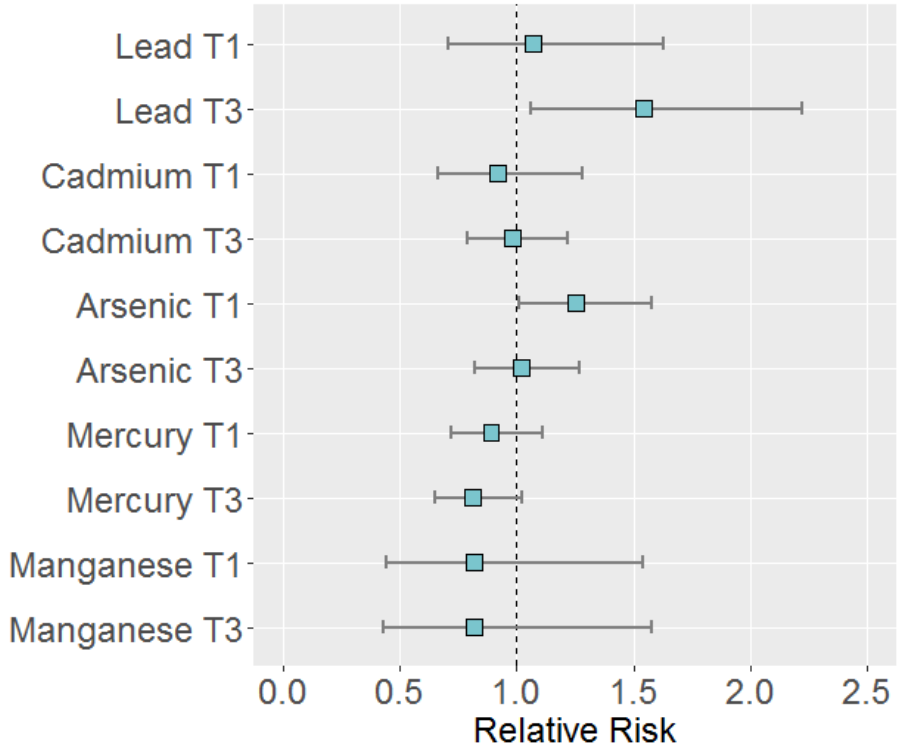
Independent effects – multi-exposure models

Adjusted relative risks (95% CI) for associations with each blood metal adjusting for all other trimester-specific blood metals (reference = normotensive)

Gestational hypertension



Preeclampsia



Combined (mixture) effect – quantile g-computation

A Quantile-Based g-Computation Approach to Addressing the Effects of Exposure Mixtures

Alexander P. Keil,[1,2] Jessie P. Buckley,[3,4] Katie M. O'Brien,[2] Kelly K. Ferguson,[2] Shanshan Zhao,[5] and Alexandra J. White[2]

'How can the mixture as a whole influence the health of the populations exposed to the multitude of components in the mixture?'

Resources:

- Qgcomp package info
- https://cran.r-project.org/web/packages/qgcomp/vignettes/qgcomp-vignette.html
- https://cran.r-project.org/web/packages/qgcomp/qgcomp.pdf

Combined (mixture) effect – quantile g-computation

Quantile g-computation (qgComp) estimates the parameters of a marginal structural model that characterizes the change in the expected potential outcome given a joint intervention on all exposures, possibly conditional on confounders.

Provides an estimate of the effect of increasing all exposures by one quantile simultaneously.

Often thought of as the potential real-world intervention effect on a single source of exposure (e.g., filtering tap water, reducing emissions).

Outputs an overall effect estimate, as well as weights, denoting how much each individual exposure contributes to the overall effect.

- Assigns a positive or negative weight to each exposure
- Weights do not sum to 1 when directional homogeneity is not met

Combined (mixture) effect – quantile g-computation

In contrast to Weighted-Quantile Sum regression (WQS):

- No directional homogeneity – direction of co-adjusted effects does not have to be the determined a priori (can include both harmful and beneficial effects)
- Does not assume linear and additive effects of individual exposures
- Does not require sample splitting into training/validation set

In contrast to Bayesian Kernel Machine regression (BKMR):

- Much less computationally intensive (100K x faster?)
- Can handle interactions, but in practice this seems difficult to implement since the weights are not well-defined
- Nonlinearities are modeled using polynomials only. Can be an issue with dose-response relationships that are stepped or have sharp inflection points.

qgComp – weights

The “weights” in qgComp correspond to the relative contribution of each exposure on the overall effect when all of the exposures have effects in the same direction

If they have different directions, they correspond to the proportion of the effect *in that particular direction*

- NOTE: the left and right sides of the plot should not be compared with each other because the length of the bars corresponds to the effect size only relative to other effects in the same direction

The darkness of the bars corresponds to the overall effect size - if the bars on the right (positive) side of the plot are darker, the overall “mixture” effect is positive

- The shading allows one to make informal comparisons across the left and right sides: a large, darkly shaded bar indicates a larger contribution to the overall effect than a large, lightly shaded bar

Combined (mixture) effect – quantile g-computation

Question:

What is the effect of simultaneously increasing all 5 blood metal levels by one quantile on the risk of developing gestational hypertension or preeclampsia, adjusting for relevant confounders?

All metals are $\log_2$ transformed, standardized to a Z-score beforehand, and are then quantized

Covariates are the same as in the other regression models

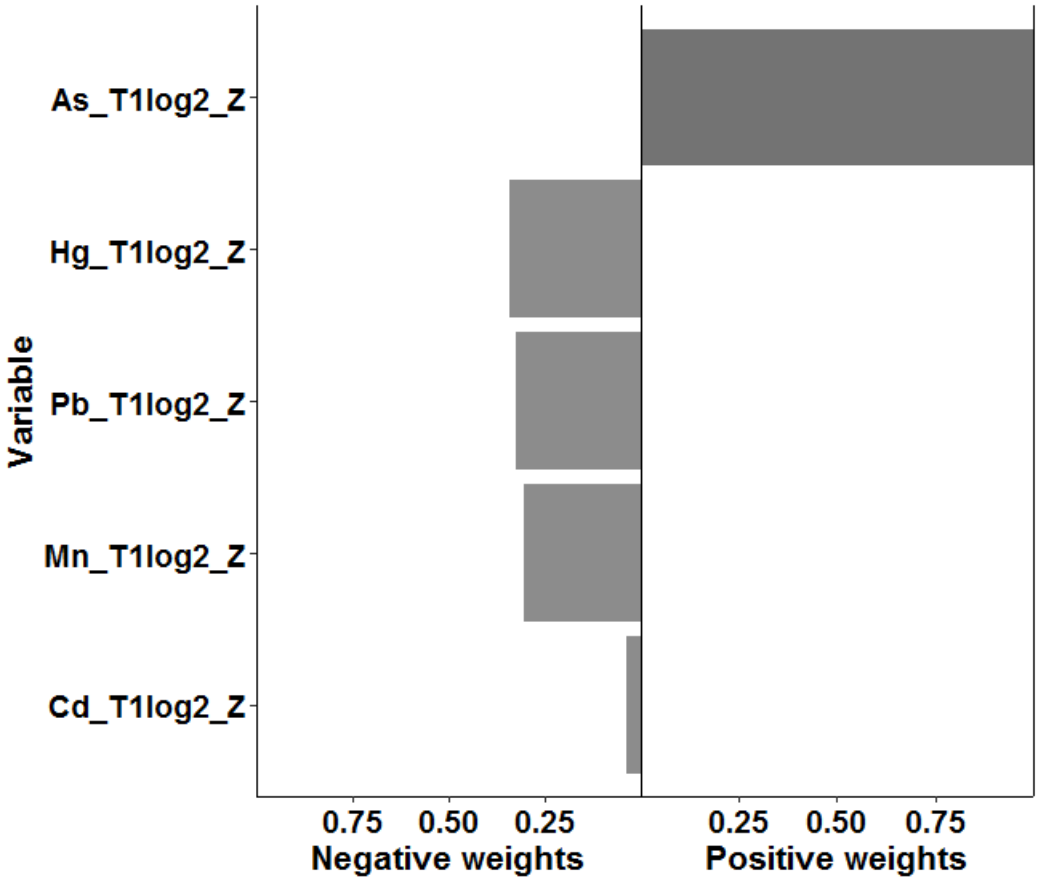
Combined (mixture) effect – quantile g-computation

	RR (95% CI)	p-value	Mixture weights [a]				
			Pb	Cd	As	Hg	Mn
1st trimester							
Gestational hypertension	0.80 (0.57, 1.11)	0.19	-0.322	-0.179	-0.185	1.000	-0.314
Preeclampsia	1.04 (0.62, 1.75)	0.88	-0.324	-0.034	**1.000**	-0.339	-0.302
3rd trimester							
Gestational hypertension	0.86 (0.63, 1.18)	0.35	0.822	-0.220	-0.177	-0.604	0.178
Preeclampsia	0.96 (0.60, 1.55)	0.88	**0.947**	-0.434	-0.046	-0.520	0.053

RR – Relative Risk; 95% CI – 95% confidence intervals
Relative risks for continuous variables represent a doubling (per $\log_2$ increase) in plasma concentration.

[a] Positive mixture weights indicate contributions to increased risk of conditions and sum to positive one; negative weights contribute to decreased risk of conditions and sum to negative one

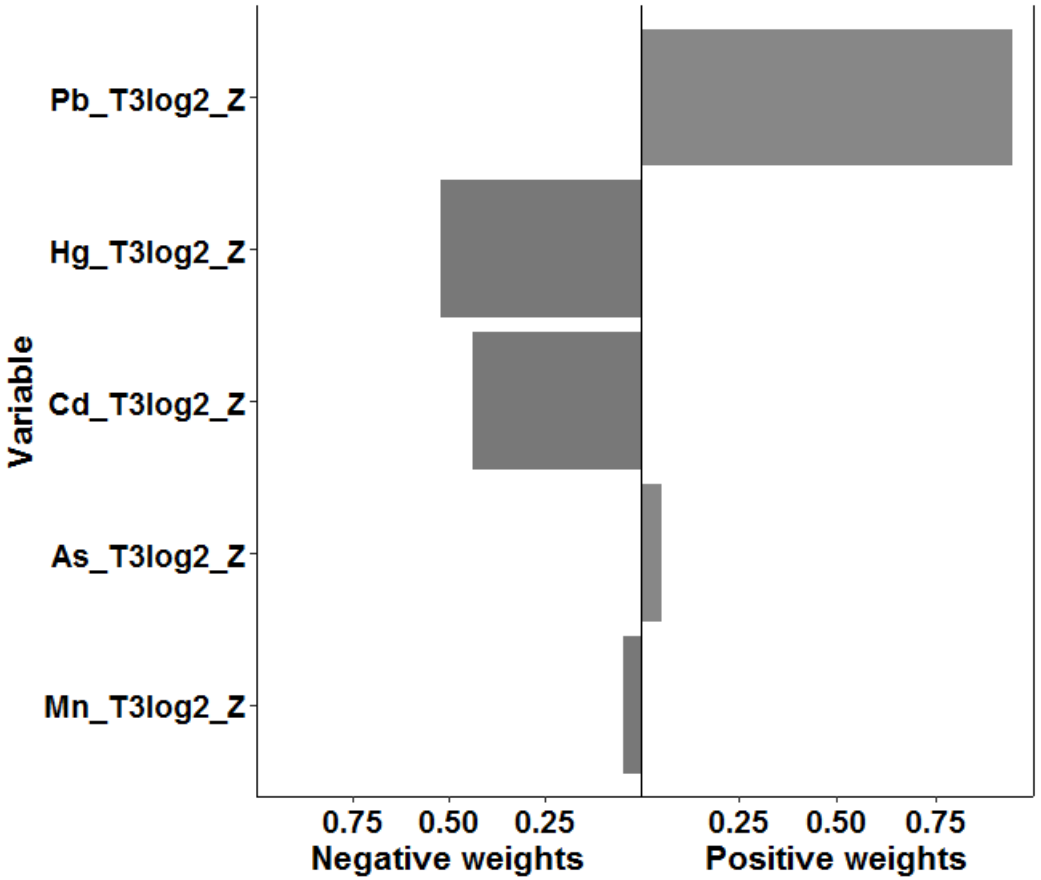
Quantile g-computation – 1st trimester and preeclampsia



Mixture RR = 1.04 (0.62, 1.75)

p = 0.88

Quantile g-computation – 3rd trimester and preeclampsia



Mixture RR = 0.96 (0.60, 1.55)

p = 0.88

Summary of findings

In this prospective study of 1,560 women with low levels of exposure to toxic metals:

Individual effects

Both 1st trimester blood [As] and 3rd trimester blood [Pb] were associated with the risk of developing preeclampsia

Independent effects

Adjusting for co-exposure to other trimester-specific metals resulted in slightly stronger associations for As (RR=1.25) and Pb (RR=1.54)

Combined (mixture) effect

The combination (i.e., mixture) of metals in either 1st or 3rd trimester was not associated with the risk of developing gestational hypertension or preeclampsia

Mixture weighs corroborated the independent effects results

Discussion

Blood Pb concentrations were low (nearly all < 2 µg/dL). Somewhat surprising to see any effect at all.

3rd trimester blood Pb levels might not reflect exogenous exposure during this time window (≈30 days prior to blood draw). Mobilization from bone and plasma volume expansion will impact concentrations.

Normal blood pressure

↓ Pb 10%

Preeclampsia

↑ Pb 6%

Sowers (2002). Arch Env Health; 57(5)

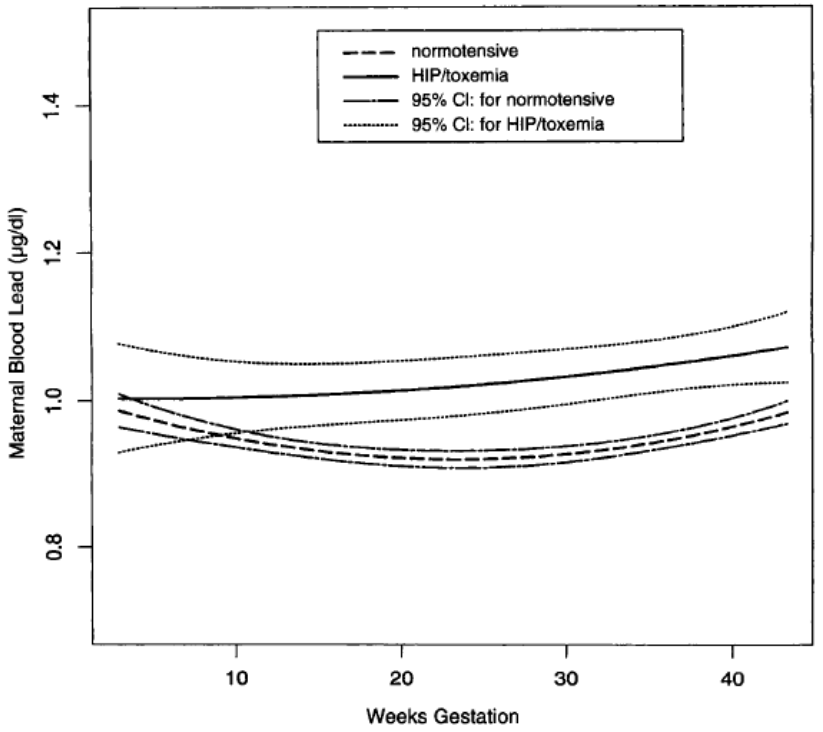


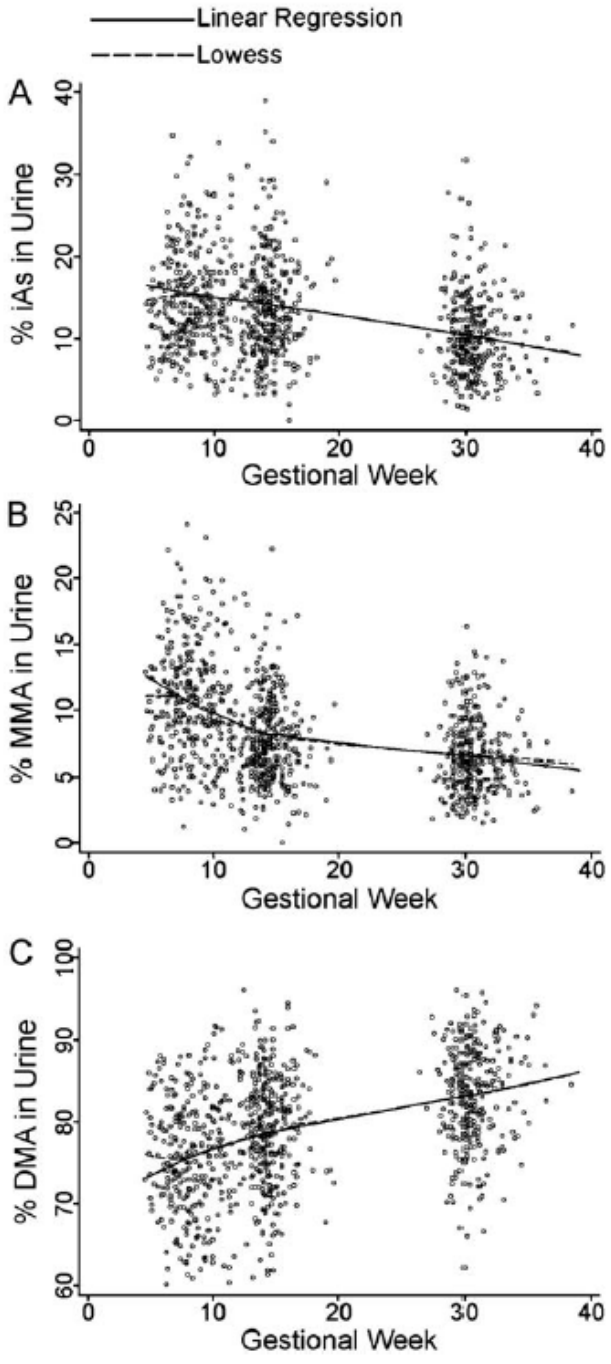
Fig. 1. Maternal blood lead concentrations in pregnancy and delivery among normotensive women, compared with women with hypertension in pregnancy (HIP)/toxemia.

Discussion

1st trimester, but not 3rd, concentrations of As were associated with preeclampsia.

Early pregnancy as the critical window of exposure?

Could be explained by increasing efficiency in As methylation throughout pregnancy →



Gardner (2011). Rep Tox; 31

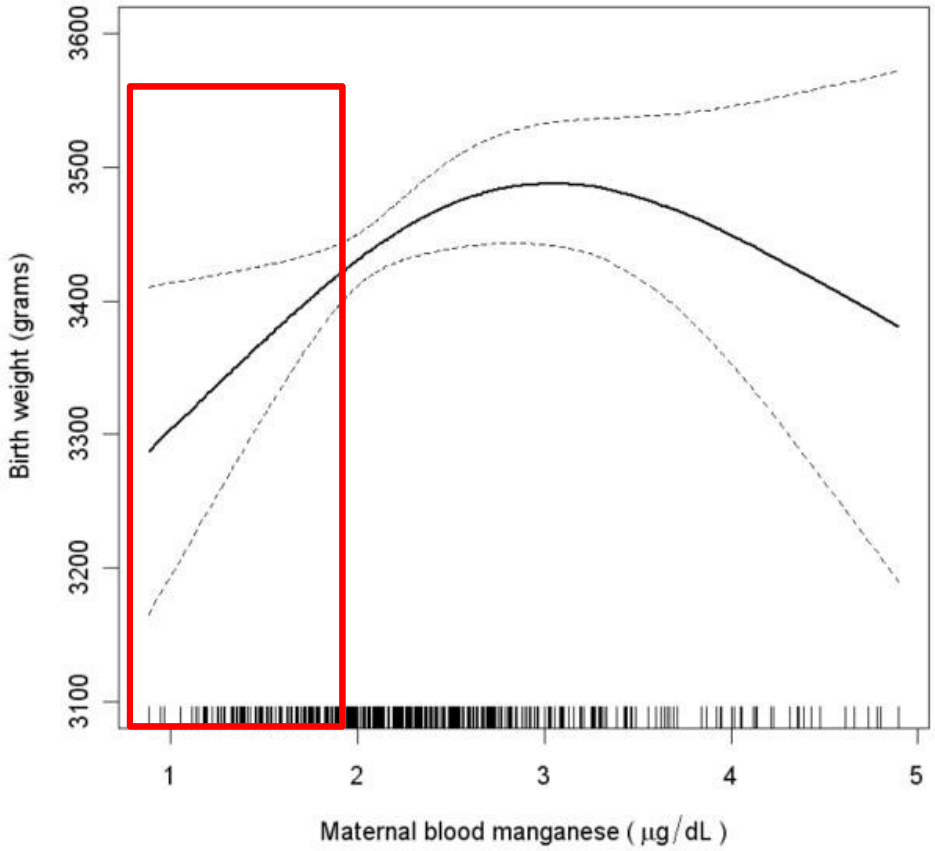
Discussion

Blood is not the best matrix for Mn. Well regulated in blood. We don't have data from other matrices for maternal exposure.

Within this context of this exposure range…

Trend towards lower risk of GH with highest tertile

Among women carrying male fetuses, higher concentrations of Mn were associated with a lower risk of developing gestational hypertension



Zota (2009) Epidemiology; 20(3)

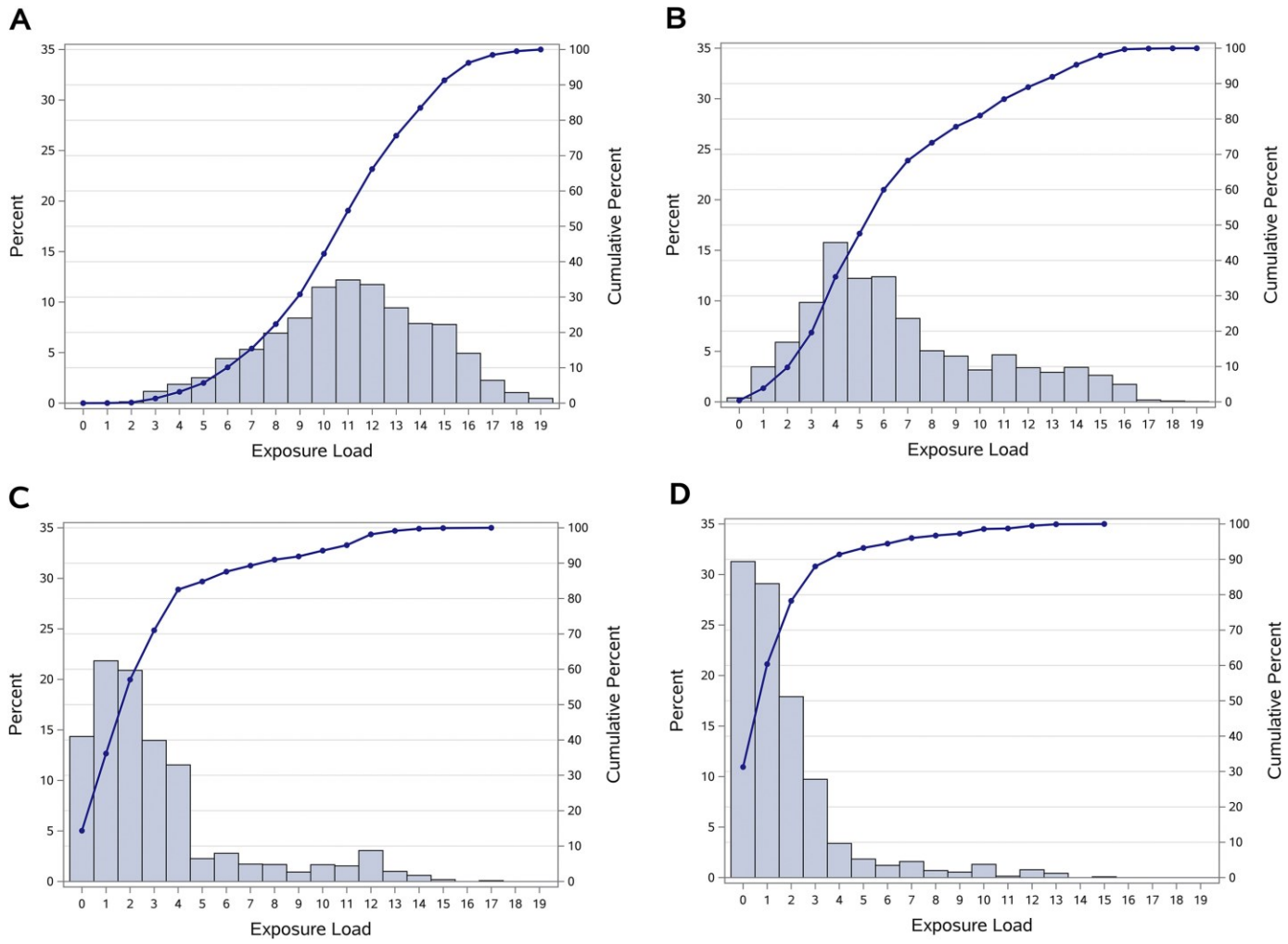
Discussion – how should we evaluate dose-response in the (epidemiological) context of chemical mixtures?

There is no shortage of complicating factors…

Potency
Interactions/non-additivity
Non-linearity
Threshold effects
Low dose effects

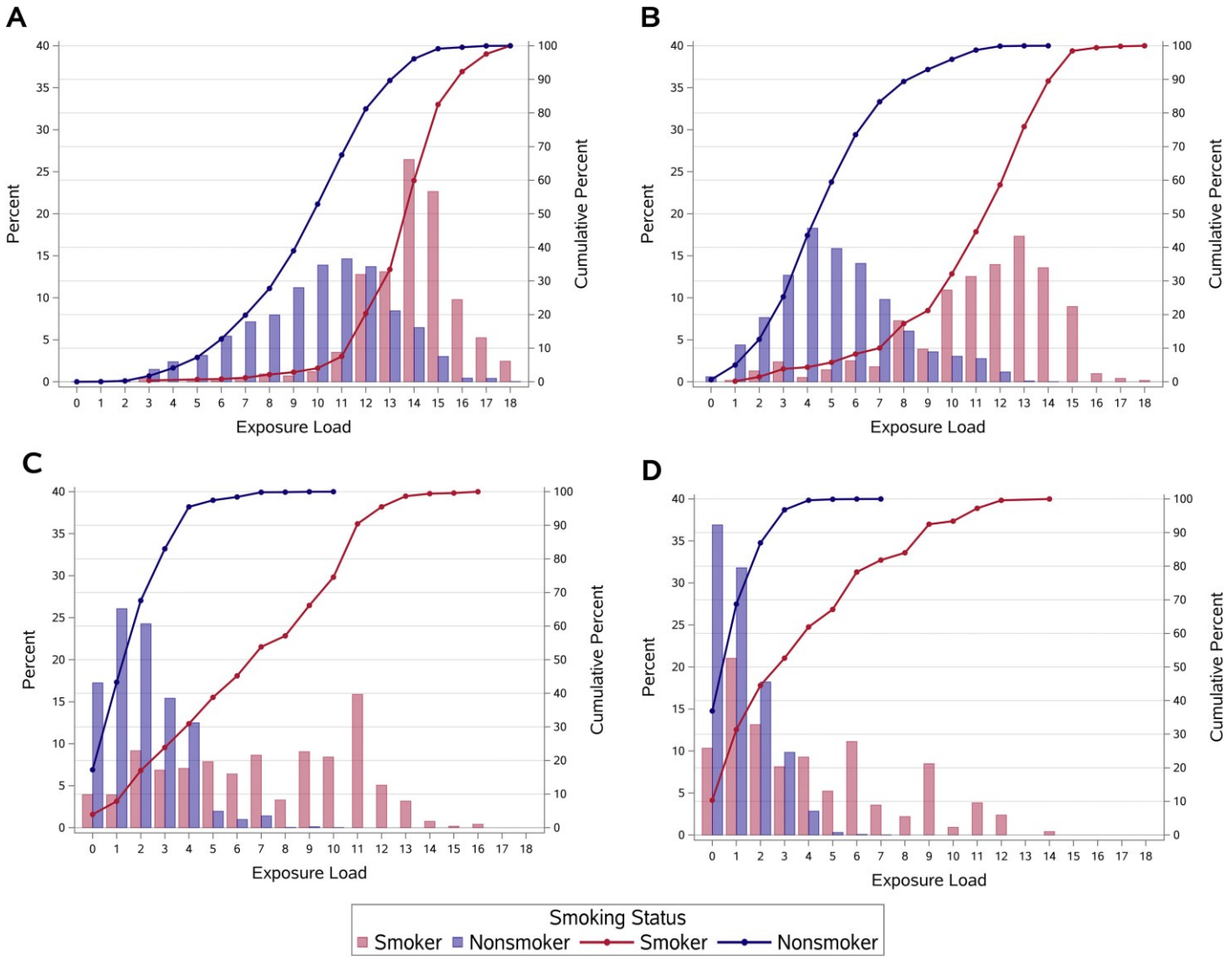
…

Exposure Load



Willey et al. (2021). IJHEH; 234

Exposure Load as population percentage using the 50th (A), 75th (B), 90th (C) or 95th (D) percentile as the exposure threshold.



Willey et al. (2021). IJHEH; 234

Exposure Load as population percentage by smoking status using the 50th (A), 75th (B), 90th (C) or 95th (D) percentile as the exposure threshold.

Discussion – how should we evaluate dose-response in the (epidemiological) context of chemical mixtures?