Assessing our multi-pollutant burden:
environmental chemical exposures and reproductive and child health

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Beoordeling van onze blootstellingslast van een complex van vervuilende stoffen: chemische milieublootstelling en reproductieve en kindergezondheid

(met een samenvatting in het Nederlands)

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Chapter 1

General introduction

Manufactured chemicals are pervasive in modern society. Pesticides, adhesives, sealants, coatings, plastic additives, pharmaceuticals, food additives, detergents, fragrances, and many others are used in large volumes. Global production has increased dramatically since the 1940s. There are around 85 000 chemicals registered on the US market[1] and more than 120 000 registered in the European Union.[2] At least 4500 of these are high production volume chemicals, with more than 1000 tons/year being produced or imported by an Organisation for Economic Co-operation and Development (OECD) member country.[3] Global sales are projected to increase 3% annually until 2050, with higher growth projected in low- and middle-income countries.[4] Many environmental chemicals—defined herein to include both metals and synthetic chemicals—are emitted during chemical-industrial processes, food and energy production, or are released during different phases of the life cycle of building materials and consumer products. Human exposure is essentially universal; we are simultaneously and chronically exposed to low doses of a multitude of chemicals during sensitive periods of *in utero* and early-life development, and in adulthood.[5,6]

There is growing evidence that present-day, ambient exposure levels of numerous chemicals are associated with various adverse health effects, including reproductive and respiratory health outcomes, neurodevelopmental disorders, immune dysfunction, obesity, and certain cancers, and also with perturbations of molecular pathways of sometimes poorly characterised clinical relevance.[7-12] However, environmental chemicals are routinely screened for only a limited set of toxicity outcomes, and risk assessments are fraught with methodological challenges due to the complex nature of human exposure to chemicals. This dissertation focuses on the epidemiological evaluation of multiple chemical classes of concern and their potential impact on fetal, child, and adult health. We applied and evaluated statistical strategies to simultaneously assess the independent exposure–outcome associations for multiple chemicals.

Shift to assessing the risks of chemical mixtures

The majority of studies which have investigated the potential risks of chemicals have evaluated only one or a handful of chemicals at a time. For instance, of the 273 studies published up until 2014 based on the National Health and Nutrition Examination Survey (NHANES) data—publicly available biomonitoring data from the United States—only 8% analysed multiple chemical groups.[13] Chemical exposures, and other environmental risk factors, exhibit complex correlation patterns. An analysis of 575 chemical, lifestyle and demographic factors assessed in NHANES[14] and another analysis of 81 chemical and non-chemical environmental exposures in a Spanish birth cohort[15] demonstrated that although the median pairwise correlation levels were low, within-group and a substantial proportion of between-group pairwise correlations were moderate to high. This implies that single-exposure–outcome associations may be confounded by correlated co-exposures, leading to less accurate health effect estimates.

Data-driven exploratory research is warranted to generate hypotheses. However, conducting fully agnostic and even semi-informed iterative testing of individual chemicals or chemical classes, rather than screening a broad array of the chemical space, leads to increased false positive discoveries that pass conventional statistical significance thresholds but may represent chance findings that fail to be replicated. This phenomenon is related to issues of selective inference (assessing the strength of associations) and selective reporting, and feeds criticisms that biomedical research suffers from a reproducibility crisis.[16,17] Moreover, possible interactions, non-additive effects, and cumulative effects are not captured in single-exposure assessments.

The importance of analysing multiple-exposures in concert, i.e., chemical mixtures, has long been recognised by the environmental epidemiology community[18] and by risk assessment agencies.[19-21] However, quantitative risk assessment approaches and protocols for evaluating mixtures are underdeveloped. Combinations of chemicals can be tested in *in vivo* and *in vitro* experimental models, although these tests are generally restricted to a low number of combinations; the cumulative effect of a single complex mixture can be tested, although usually without isolating the causative or most potent chemicals; and the utility of high-throughput assays for assessing mixtures is unclear as they are mostly based on single-receptor assays.[22-24] Human health risk assessments have historically underutilised epidemiological data, e.g., relying exclusively on toxicological data for pre-market toxicity testing under European Union chemical safety legislation,[25] and relying largely on data from animal models for post-market risk assessments.[26,27] Importantly, environmental epidemiological studies can sometimes uncover effects not observed in experimental toxicological studies, and better capture real-world low-dose exposure mixtures and population variability.[28] Refining methods for assessing multiple exposure–outcome associations would further increase the quality of epidemiological evidence and its utility for risk assessments.

The traditional approach of assessing chemicals in isolation may in part be attributed to the prohibitive financial cost of measuring multiple classes of chemical exposure biomarkers in epidemiological studies, and to a type of observational bias, the 'street light effect', in which researchers are more likely to focus on known or suspected risk factors of concern, or exposures for which exposure assessment methods are already developed and thus more readily applied.[29] The nascent exposome concept has promoted a shift towards evaluations of larger sets of exposures in relation to a single or multiple outcomes.

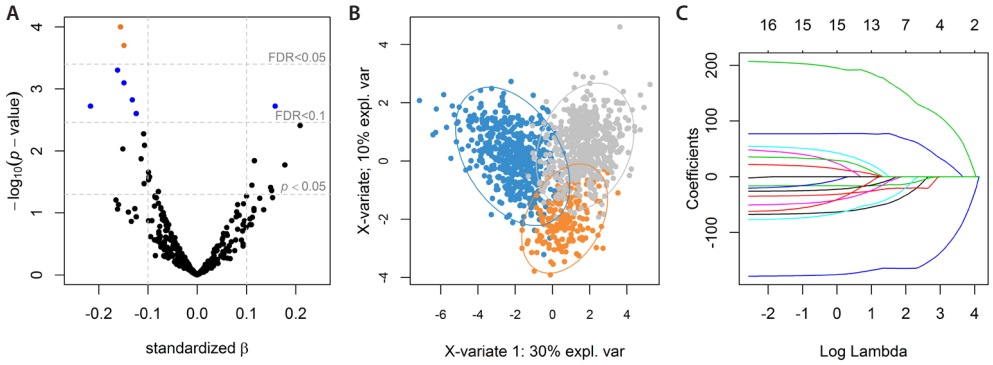
Coined in 2005 as a complement to the genome, the exposome is defined as the totality of environmental exposures from conception onwards.[30,31] The exposome concept was in part spurred by recognition that genetics (our inherited genomes) alone explain little to moderate risk for most chronic diseases, and that exposures act in concert with genetic susceptibility, as analyses of heritability in twin studies have shown.[32,33] Since 2005, more than 2500 genome-wide association studies (GWAS) have been published,[34] whereas investments in research related to environmental causes of disease have been relatively modest. Exposome

studies have the potential to be more useful for shaping public health than GWAS given that many exposures are modifiable through individual decision-making or policies, whereas the genome is largely static. The exposome encompasses both external exposures (the external exposome, e.g., chemicals, air and water pollutants, the built environment, diet, infections, medications, social capital, and psychosocial stress) and internal or endogenous processes (the internal exposome, e.g., metabolites, inflammation, hormones, and microbiota). Characterising the dynamic exposome is daunting and presently infeasible. However, technological developments such as high-throughput analytical methods have enabled exposome-type studies, and concomitantly, the exposome framework and investments in proof-of-concept studies have encouraged advances in exposure science (passive samplers, sensors, smartphone apps, etc.)[35-37] and untargeted mass spectrometry biomonitoring analytical methods.[38,39] The exposome paradigm shift has also highlighted methodological challenges, notably limitations of conventional statistical approaches.

In 2010, Patel and colleagues systematically assessed associations between 266 environmental exposures and type 2 diabetes using NHANES data, and identified several associations with pesticides, polychlorinated biphenyls (PCBs), and micronutrients. This approach was introduced as an environment-wide association study (EWAS), analogous methodologically to the established GWAS approach.[40] Several EWASs (or equivalently, exposome-wide association studies[41]) have since been published based on 100s of exposures measured in repeated NHANES surveys[42-45] and other cohort data.[46-48] EWAS studies have generally used standard single-exposure regression models, sometimes adjusting for confounding factors, and have accounted for multiplicity (using Bonferroni or false discovery rate corrections of significance thresholds). While correcting for multiple comparisons may reduce the number of false positive discoveries, this does not prevent associations with correlated, non-causative exposures from being identified. Furthermore, risk estimates may often be confounded by co-exposures and thus biased and inaccurately estimated.

Conventional methods for controlling for confounding by co-exposures include stratification, which is often not possible to implement for more than a couple of variables due to insufficient power with too fine stratification; stepwise regression, which may suffer from locally optimal models; and multivariable regression, which may yield unstable estimates if exposures are (multi)collinear. Variables are collinear when they are exact or near linear combinations of each other, and collinearity will lead to an ill-conditioned model with inflated variances and possibly regression coefficients with an inverted sign due to the potential singularity of the Hessian matrix.[49] These limitations hamper inference and risk assessment. In the past several years, a growing number of studies have used alternative statistical approaches to evaluate the independent associations of multiple, potentially correlated, exposures in exposome–health studies.[29,50] This is often referred to as multi-pollutant modelling.

Exposure biomarker data was available for 15–26 chemicals in the two cohort studies included in this dissertation. The size of the exposure sets is not particularly large relative



to higher-dimensional exposome studies which are underway[36,37] or are expected in the

Figure 1. (A) A volcano plot visualizing 330 single-exposure–outcome associations between chemical exposures and male reproductive health outcomes (this environment-wide association study is presented in Chapter 3). The joint distribution of p-values (–log10 transformed *p*-values) and effect estimates (regression coefficients standardised to 1 standard deviation) are plotted, where each point represents an association, and coloured points associations that exceeded FDR-adjusted levels of statistical significance. **(B)** A plot of a dimension reduction and discriminant analysis of the chemical exposure data (assessed in Chapter 4), demonstrating clustering by study population (Greenland, Poland, and Ukraine). Points represent sPLS-DA scores, and circles the 95% confidence intervals. **(C)** A coefficient profile plot of a multiple-exposure elastic net penalised regression analysis of prenatal chemical exposures and term birth weight (this analysis is presented in Chapter 4). Each coloured line represents a chemical exposure. The model yields increasingly sparser solutions (the numbers at the top of the plot indicate the number of non-zero regression coefficients) with increasing penalisation (λ penalty values are shown on the x-axis, and the magnitude of the penalised regression coefficients is shown on the y-axis).

future; however, these lower dimensional sets are representative of the typical number in current cohorts, and present a useful starting point for advancing current modelling practices. To assess exposure patterns and to estimate exposure–outcome associations, we applied statistical modelling approaches that fall under dimension reduction and penalisation methods (Figure 1). Principal components analysis (PCA) is an unsupervised dimension reduction approach that extracts a smaller number of constructs from a multi-dimensional dataset (e.g., an exposure matrix), which are often orthogonally rotated to be uncorrelated. Partial least squares (PLS) is a supervised dimension reduction approach that incorporates information from dependent variable(s) in that latent constructs maximise the covariance between exposures and outcome(s). Penalised regression, with the inclusion of a penalty term, shrinks coefficients towards or exactly to zero; this shrinkage introduces bias but improves precision, and allows for correlated exposures to be modelled in one model without suffering from instability in estimates and variance inflation. Penalisation can be integrated into dimension reduction methods to achieve variable selection [e.g., sparse partial least-squares (sPLS)-regression or discriminant analysis (sPLS-DA)]. These methods have frequently been applied in analyses of higher-dimensional data with 100s–1000s of exposures (and frequently more exposures than participants, e.g., in genomics and other '-omics' research), but have been un-

derexploited in environmental and occupational (external exposome) epidemiology, which often seeks to evaluate 10s of exposures and less frequently >100s of exposures. There have been few empirical comparisons of statistical strategies for analysis of multiple environmental-exposure–outcome associations.[51,52] Therefore, we also conducted a simulation study to evaluate how several conventional and modern variable selection methods performed in typical environmental and occupational data analysis scenarios.

Exposure to environmental chemicals

Ample evidence has been collected demonstrating that humans are exposed to numerous persistent chemicals and more rapidly metabolised chemicals.[5,6,53,54] Many, such as persistent organic pollutants (POPs), are synthetic, whereas others, such as metals, are naturally occurring but are released and globally distributed due to human activities. In the epidemiological literature, the terms environmental chemicals, environmental contaminants, pollutants, toxicants, xenobiotics, and endocrine-disrupting chemicals are generally used interchangeably. Exposure is often assessed using biomonitoring of exposure biomarkers in human tissue matrices, commonly serum, plasma, or breast milk for persistent chemicals, and urine for non-persistent chemicals. Maternal levels during or around the period of pregnancy are often used as a proxy of a child's prenatal or perinatal exposures.

POPs have long elimination half-lives and accumulate in the body; lipophilic compounds like PCBs and polybrominated diphenyl ethers (PBDEs) in fat stores, and predominantly protein-bound per- and polyfluoroalkyl substances (PFASs) in blood and the liver. POPs resist degradation, persist in the environment, and biomagnify in the food chain. They can undergo long-range transport to other regions of the globe, travelling via the atmosphere (e.g., mercury,[55] PCBs, PBDEs[56]), via ocean currents (e.g., for the more polar PFASs[57]), and via transport of discarded electronic and electrical equipment (e-waste, e.g., PCBs and PBDEs to major recipient countries such as China, India, and several West African countries[58]). Production of 30 POPs has been restricted globally since the United Nations Environment Program's Stockholm Convention on Persistent Organic Pollutants,[59] a global treaty signed by 160 countries and parties, entered into force in 2004. Year of birth in relation to peak production and emissions is the most important predictor of the body burden for chemicals with longer half-lives.[60,61]

There are a myriad of daily sources of exposure to chemicals, including food, drinking water, food packaging materials, clothing, cookware, furniture, building materials, electronic products, toys, medical devices, cleaning products, and personal care products. Exposure can occur via dietary intake, dust ingestion, inhalation, and dermal absorption following direct skin contact, and it has recently been recognised that direct transdermal uptake from ambient air is an important route for some (semi-)volatile organic compounds such as phthalates[83,84]; the relative contribution of these routes varies depending on the chemical. Table 1 provides an overview of the chemical classes investigated in this dissertation.

Table 1. Overview of chemical classes investigated in this dissertation.

Class	Uses and exposure pathways[a]		Half-life[b]
Metals	Non-essential metals with natural and anthropogenic sources.		
	Cd	Used in batteries, pigments, coatings, plastics, and emitted from fossil-fuel combustion, smelting, municipal incinerators, rock phosphate fertiliser, etc. Exposure via cigarette smoking and diet (primarily from plant-based staple foods).	3 months; small proportion, 10 years
	Pb	Historically used in paints and gasoline; restricted, but still used in batteries, ammunition, building materials, metal soldering, ceramic glazes, electronic products, etc. Exposure via household dust in older homes with lead-based paints, via contaminated soil, and via some toys and jewellery.	30 days
	Hg	Coal combustion and mining are the largest sources of emissions. Exposure primarily via diet, especially fish, and dental amalgam.	60 days
OCPs	Highly persistent in the environment; exposure primarily via diet (fatty foods of animal origin foods, such as fish, dairy products, and meat).		
	DDE	Primary metabolite of DDT, an insecticide widely used from the 1940s–70s; still used for malaria vector control in parts of the world.	8 years
	HCB	Fungicide used from the 1950s–80s; still produced as a by-product during industrial chemical and combustion processes.	6 years
	β-HCH	Isomer of HCH with the longest half-life; an insecticide used from the 1940s–70s.	7 years
	Oxychl.	Major metabolite of chlordane, an insecticide; used from the 1950s–80s.	>3 months
BFRs	BB-153	Flame retardant produced from the 1970s–90s; restricted in some countries in the 1970s/80s.	13–29 years
	PBDEs	Flame retardants produced since the 1970s; restricted in 2000/2010s. Used in upholstered furniture, carpets, textiles, electronics, and plastics. Exposure via diet (including fish and fatty foods) and household dust.	1–7 years[c]
PCBs	Used as industrial insulators, coolants, lubricants, and in building materials, including caulk and other sealants. Produced 1930s–70s. PCB-153, 180 and 138 are the most abundant congeners. Exposure primarily via diet (especially fatty foods or animal origin); inhalation of contaminated indoor air also represents an exposure route for especially lower-chlorinated congeners.		<1–30 years (PCB-153, 15 years)
PFASs	Surfactants produced since the 1950s; long-chain PFASs (e.g., PFOA, PFOS) are being phased out and replaced with shorter-chain PFASs. Used as industrial surfactants, and in water, stain and grease-resistant coatings in consumer goods (e.g., non-stick pans, raincoats, fire-fighting foams). Exposure primarily via diet, and also via drinking water (due to widespread surface and groundwater contamination).		2–8 years
Phthalates	Plasticisers produced since the 1920s; some restricted since 2009. Heavy-molecular weight phthalates (e.g., DEHP and DiNP) are mainly used in plastics, including PVC, toys, food packaging, and medical tubing; exposure primarily via diet (especially fatty foods and grains), and is dependent on storage and processing in plastics.		12–48 hours

BB-153, 2,2′,4,4′,5,5′-hexabromobiphenyl; BFRs, brominated flame retardants; Cd, cadmium; DEHP, di(2-ethylhexyl) phthalate; DiNP, di-iso-nonyl phthalate; HCB, hexachlorobenzene; Hg, mercury; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; OCPs, organochlorine pesticides; Oxychl., oxychlordane; Pb, lead; PFASs, per- and polyfluoroalkyl substances, also referred to as perfluorinated compounds (PFCs); PBDEs, polybrominated diphenyl ethers; PCBs, polychlorinated biphenyls; PVC, poly(vinyl chloride).

[a] Exposure sources and regulations focused on the European and North American scenarios, and non-occupational sources. References[62-70]

[b] Half-life estimates presented for blood levels for the congeners assessed in this dissertation; for phthalates, estimates of urinary half-lives of secondary metabolites are presented as half-lives for blood levels are imprecise. References[71-82]

[c] Half-lives for lower brominated PBDEs (<7 bromine atoms, the most abundant congeners considered in this dissertation) were presented. Highly brominated congeners (7–10 bromine atoms) have considerably shorter half-lives of 11–120 days.

There are other important classes of chemicals of concern that are not addressed in this dissertation, including phenols (e.g., bisphenols and triclosan), alternative flame retardants [e.g., 2-ethylhexyl-2,3,4,5- tetrabromobenzoate (EH-TBB) and organophosphate flame retardants such as tris(2-chloroethyl) phosphate (TCEP)], and non-persistent pesticides (e.g., organophosphates, carbamates, and pyrethroids).

Exposure to environmental chemicals begins *in utero* as many chemicals cross the placenta.[85] Babies are born 'pre-polluted', as was evocatively stated in a US National Cancer Institute report.[86] The ratio of maternal levels to fetal and breast milk levels is determined by chemical and physical properties including lipophilicity, protein binding, and active transport; generally chemicals with a smaller molar volume, lower number of halogen substitutes, and those which are more hydrophilic transfer more readily.[87,88] Breastfed infants receive a substantial dose of persistent chemicals via breast milk. For infants exclusively breastfed for 12 months, internal exposure levels have been simulated to reach a median of 6-times their mothers' levels for chemicals with half-lives longer than 5 years.[89] Young children are also exposed to higher doses of chemicals for their body weight than adults. Their food, water, and air intake is higher per body weight, and young children have increased exposure to contaminated dust due to frequent hand-to-mouth behaviour and because they often spend more time close to the ground.

Environmental chemicals and reproductive and child health

Rachel Carson's 1962 book, Silent Spring,[90] which cautioned that unrestricted use of DDT (dichlorodiphenyltrichloroethane) and other synthetic pesticides was harming birds, other wildlife, and possibly humans, has been credited with bringing the risks of chemical exposures to a wider public consciousness and with mobilising the environmental movement. Several industrial accidents and heavily polluted sites also captured public attention and shaped modern chemical safety efforts. In areas around Minamata Bay, Japan, it was recognised in the 1950s that consumption of fish highly contaminated with methylmercury emitted from a nearby chemical factory lead to severe neurological disorders, labelled Minamata disease.[91] In 1976, an explosion at a chemical manufacturing plant in Seveso, Italy, led to high dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin or TCDD) exposure which was associated with numerous health effects, including excess cancer.[92,93] In the 1970s, discovery of a massive chemical waste disposal site in Love Canal in Niagara Falls, New York, and high rates of health disorders in surrounding neighbourhoods, galvanised federal legislative action in the United States, resulting in the Superfund Act, regulating clean-up of hazardous waste sites, and soon after the National Toxicology Program.[94,95]

Evidence for the prominent role of environmental risk factors in the etiology of reproductive and child health has come from observed geographic differences, temporal trends, and migration studies of disease incidences. For example, atopic diseases have become more common in Finnish Karelian compared with nearby Russian Karelian children, populations

that have a shared ancestry but have had different economic growth trajectories. There is suggestive evidence that in Finland, the loss of household and personal microbial exposures (quantity and diversity) accompanying urbanisation may be an explanatory factor.[96] Testicular cancer, although rare (accounting for <1% of all male cancers), is the most common cancer among young men (ages 15–44) in high-income countries.[97] Incidence rates have increased in the past half-century in most of the 41 countries included in a recent analysis, with indications that rates have recently levelled-off in the highest income countries.[98] Denmark and Norway have the highest age-standardised incidence rates worldwide (>10 per 100 000 men), whereas men in nearby Sweden and Finland have markedly lower rates (half and less than half of this, respectively). Studies of migrants within Nordic countries have shown that young men retain the testicular cancer incidence rate of their country of origin, whereas their sons acquire the incidence rate of the host country.[99,100] As another example, a large study of sperm donors in China reported a decline in semen quality from 2001 to 2015.[101] Some purported temporal trends, such as declining sperm counts,[102,103] remain a matter of debate.[104-106] Nonetheless, it has been argued that temporal trends in many disease incidences have outpaced genetic drift, and are therefore likely attributable to environmental, including lifestyle, factors.[9,107]

In 1979, it was postulated that excess estrogen exposure *in utero* was a risk factor for testicular cancer.[108] In a seminal paper published in 1993, Sharpe and Skakkebaek extended this hypothesis, arguing that increasing levels of estrogen exposure *in utero*, notably from environmental 'estrogenic' chemicals, was related to an increasing incidence of male reproductive disorders.[109] In the past two decades, a broader theory has emerged relating endocrine-disrupting chemicals (EDCs) to adverse health effects. A recent definition of an EDC by the Endocrine Society is "an exogenous chemical, or mixture of chemicals, that interferes with any aspect of hormone action",[110] or more specifically, hormone receptor activation that leads to developmental or physiological effects.[111]

An alarming example of a chemical exhibiting reproductive toxicity is the nematocide DBCP (dibromochloropropane). A high proportion of workers producing this pesticide were found to have severe oligospermia and azoospermia in the late 1970s.[112,113] Another notorious example of an EDC is diethylstilbestrol (DES), a potent synthetic estrogen prescribed from the 1940s to early 1970s to prevent miscarriage and other pregnancy complications. The daughters of women who took DES had a higher risk of clear cell adenocarcinoma of the vagina and cervix, and other adverse reproductive outcomes.[114,115] DES studies provided proof-of-principle for the developmental origins of health and disease (DOHaD) hypothesis that exposure to environmental stressors during sensitive periods of development can have consequences for disease susceptibility later in life; congruent with the life course epidemiology approach.[116,117] To date over 60 chemicals have been studied, most frequently PCBs, organochlorine pesticides, and metals, for the relationships between prenatal and childhood exposures and later-life health outcomes, most frequently neurodevelopmental, cancer, respiratory, and reproductive outcomes.[118]

Fetuses, infants, and children are especially vulnerable to environmental insults: particularly during periods of organogenesis and development (i.e., periods of rapid cell proliferation or differentiation or apoptosis, many of which start *in utero* and continue into childhood and adolescence; e.g., germ cell proliferation in the testes and ovaries, differentiation of lymphocyte and myeloid precursors, alveolar development in the lung, neuronal pruning); and in early-life, when repair mechanisms, and immune and metabolic (including detoxification) systems are immature.[121,122] Transgenerational epigenetic modifications following chemical exposures *in utero* is one documented plausible mechanism for DOHaD (e.g., differential DNA methylation of subsequent generations' sperm in murine models).[119,120] More broadly, the general putative mechanisms for EDCs include oxidative stress, epigenetic alterations, nuclear receptor (notably estrogen receptor and androgen receptor) agonism or antagonism, and altered endogenous hormone metabolism or transport.[9]

The economic burden of EDCs in the European Union was recently estimated to be €157 billion per year in healthcare costs and lost earning potential, or 1.23% of GDP in 2010,[123] although this estimate is arguably highly speculative. While the evidence base for the risks of EDC exposures is growing, data for many specific exposure–outcome associations remains sparse.

This dissertation investigated health outcomes that contribute to substantial disease and economic burden. First, we studied impaired male reproductive health. Around 10 to 15% of couples in high-income countries suffer from infertility, defined as failure to conceive after 12 months of regular unprotected intercourse,[124,125] and male factors contribute in almost half of couples. In addition to risk factors related to secular trends such as postponing parenthood,[126] over 20 years ago it was hypothesised that environmental chemicals may represent a risk factor for impaired spermatogenesis and male fecundity (the capacity to reproduce).[109]

Second, we studied birth weight. Exposure to environmental stressors during the highly sensitive period of embryonic and fetal development can induce later phenotypic variation due to adaptive developmental plasticity, mediated in part by epigenetic changes, and increase the risk of chronic diseases later in life.[127,128] Low birth weight is a non-specific adverse birth outcome which may reflect prematurity or fetal growth restriction related to a suboptimal intrauterine environment, such as impaired placental function, and other causal risk factors.[129,130] Birth weight among term births approximates a Gaussian distribution and is, independent of preterm birth, associated with perinatal risk and later morbidity, including cardiovascular, metabolic, and neurodevelopmental outcomes. These associations have been observed for both the extreme quantiles of low and high birth weight and within the range of normal birth weights, with associations often following a reverse-J-shaped pattern.[116,131,132]

Third, we studied childhood asthma and lower respiratory tract infections (LRTIs). Asthma is characterised by reversible airway obstruction and inflammation. It is the most common chronic disease in children, with higher prevalence rates generally observed in high-income countries, and an estimated 300 million sufferers globally.[133] LRTIs in early-life,

including bronchiolitis and pneumonia due to respiratory syncytial virus, are also a leading cause of pediatric hospitalisation and morbidity.[134]

Role of gut microbiota

In addition to utilizing more modern statistical methods, incorporating potentially modifying or mediating biomarkers of the internal exposome may increase the power of studies to identify chemical exposure and outcome associations, which are often small in magnitude. There has been growing appreciation in recent years for the importance of gut microbiota for health and disease.[135] Gut microbiota can metabolise chemicals, influencing the dose at target tissues, and conversely gut microbiota can be modulated by environmental chemicals, exemplifying the interplay between the external (chemical) exposome, the internal exposome, and health.[136]

Commensal microbes reside on both external and internal surfaces of the body, including the skin, gastrointestinal tract, and respiratory tract. It was recently estimated that a human body hosts 4 x 10^{13} bacterial cells, or 1.3 bacterial cell for every human cell in the body.[137] The largest number of bacteria is found in the colon. There are also a relatively small number of *Archaea*, *Viruses*, and *Eukaryotes* in the gut (<3% of microbiome sequence reads[138]). There has been a proliferation of research in the past decade on the role of these complex communities of microbes (microbiota) and their collective genes (the microbiome) on both health and disease.[135]

To characterise the gut microbiota, usually fecal samples are collected, the DNA is sequenced, and these sequences are often clustered into operational taxonomic units (OTUs) and used to assign taxonomy (e.g., a sequence similarity threshold of 97% as a proxy for species).[139] In addition to taxonomic composition, the within-sample richness (α-diversity) and between sample dissimilarities (β-diversity) can be computed and visualised, the metabolome (including microbial metabolites) can be assessed, and functional profiling may be inferred from libraries.

Humans are exposed to microbiota *in utero*,[140,141] and are rapidly colonised after delivery. The gut microbiota undergoes gradual succession and stabilises to an adult-like profile around 2 to 3 years of age.[142] Composition continues to exhibit considerable temporal variability, although inter-individual temporal variability exceeds intra-individual variability.[143] Mode of delivery (Cesarean section vs. a vaginal delivery), diet (including breastfeeding vs. formula feeding), and medications (notably antibiotics), influence succession of the early-life microbiota, and can rapidly induce transient and sometimes long-lasting changes in the gut microbiota.[144-147]

Gut microbiota play a pivotal role in maturation of the immune system, and emerging evidence suggests that this early-life bacterial imprinting may have lifelong consequences for disease risks, including metabolic and immunological diseases.[148-150] Perturbations in composition (dysbiosis), diversity, and metabolites such as short-chain fatty acids—produced

primarily by fermentation of dietary fibres—have been linked to diseases such as irritable bowel disease, obesity and asthma.[151,152] Gut microbiota are involved in xenobiotic metabolism, for example, of the widely used painkiller acetaminophen[153] and heavy metals such as arsenic.[136] Studies in mice have found that exposure to PCBs,[154] TCDF (2,3,7,8-tetrachlorodibenzofuran),[155] and arsenic[156] can induce substantial changes to the gut microbiota. Thus the possible effects of early-life exposure to chemicals on the rapidly evolving gut microbiota ecosystem, and related implications for development and health, warrant attention.

Aims and outline of the dissertation

The overall aim of this dissertation was to assess the risks of exposure to mixtures of common environmental chemicals on reproductive and child health. The first three chapters are based on a cohort of mother–father–child triads from Greenland, Poland, and Ukraine. In **Chapter 2**, the adult male exposure to brominated flame retardants was characterised, and determinants of exposure and inter-population clustering were explored. In **Chapter 3**, cross-sectional associations between four classes of environmental chemicals and 22 biomarkers of male reproductive function were evaluated. This has been cited as a prototype diseasome-exposome-wide association study.[157] In **Chapter 4**, associations between prenatal exposure to three chemical classes and term birth weight were evaluated. In **Chapter 5**, using data from a birth cohort of mother–child pairs from Norway, associations between early-life exposure to four chemical classes and childhood asthma and lower respiratory tract infections were evaluated, along with independent associations and mediation by gut microbiota diversity and metabolites. Several statistical methods for multi-pollutant modelling were used: dimension reduction (Chapters 2), penalised regression (Chapters 4 and 5), and a combined approach (Chapters 3). In **Chapter 6**, nine statistical approaches for multi-pollutant logistic regression modelling (including dimension reduction and penalised regression) were compared in a simulation study with respect to their selection accuracy and the bias and variance of effect estimates. A summary of the main findings of this dissertation is presented in **Chapter 7**, along with a discussion of the salient methodological issues encountered and perspectives on future research, particularly those pertinent to assessing the health effects of chemical mixtures.

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Chapter 2

Serum concentrations of polybrominated diphenyl ethers (PBDEs) and a polybrominated biphenyl (PBB) in men from Greenland, Poland and Ukraine

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Abstract

Many brominated flame retardants (BFRs)—including polybrominated diphenyl ethers (PBDEs)—have been shown to persist in the environment, and some have been associated with adverse health effects. The aim of the present study was to quantify serum concentrations of common brominated flame retardants in Inuit men from across Greenland, and in men from Warsaw, Poland and Kharkiv, Ukraine. Serum was sampled between 2002 and 2004 from men 19 to 50 years of age. 299 samples were analysed for BDE-28, 47, 99, 100, 153, 154 and 183 and the brominated biphenyl BB-153 using gas chromatography–high resolution mass spectrometry. BDE-47 and BDE-153 were detected in more than 95% of samples from all three populations. Other congeners, except BDE-154 and 183, were detected in more than 70% of samples from Greenland; lower detection frequencies were observed in Polish and Ukrainian samples. Concentrations of individual congeners were 2.7 to 15 fold higher in Greenlandic relative to Polish and Ukrainian men. Geometric mean concentrations of the sum of the most abundant PBDEs of the Penta-BDE commercial mixture (BDE-47, 99, 100, 153 and 154) were 6.1, 1.7 and 0.87 ng/g lipids in the Greenlandic, Polish and Ukrainian men, respectively. Furthermore, significant geographical differences in BFR concentrations were observed within Greenland. Principal component analysis revealed distinct clustering of samples by country of origin. The associations between ΣPBDEs and age were inconsistent, varying from no association in Greenlandic and Polish study populations to a U-shaped relationship in Ukrainians. We report BFR levels for three populations for which sparse biomonitoring data exists.

Introduction

Brominated flame retardants (BFRs) are added to a wide variety of construction and consumer products, such as electronics, furniture and carpets, to delay potential ignition and combustion. Polybrominated biphenyls (PBBs) and polybrominated diphenyl ethers (PBDEs) are important classes of BFRs, first manufactured in the 1960s and 70s. PBDEs largely replaced PBBs after accidental contamination of cattle feed in Michigan, US, in 1973–74, which resulted in acute toxicity in cattle, human exposure via consumption of contaminated food products, and adverse health effects including increases in site-specific cancers.[1-3] There are three main commercial formulations of PBDEs, differing in their bromine content: Penta-BDE, predominantly used in polyurethane foam; Octa-BDE, used in electronics casings and hard plastics; and Deca-BDE, with widespread applications.[4] PBDEs and PBBs are not covalently bound to products, and may be subject to migration from products. They are lipophilic, relatively persistent contaminants, and some congeners have been found to bioaccumulate and biomagnify in the food chain.[5,6]

Several epidemiological studies provide indications that PBDEs may disrupt reproductive and thyroid hormone homeostasis in adult men,[7,8] and there is growing evidence that prenatal or early-life exposure may impair neurodevelopment.[9,10] Smaller studies have also reported associations between serum PBDEs and decreased sperm motility and counts.[11,12]

Commercial Penta- and Octa-BDE were banned in the EU in 2004, and soon after discontinued in the US.[13] Use of Deca-BDE will be restricted in the EU[14] and phased out in the US in 2013.[15] Despite restrictions and bans, human exposure to PBDEs and PBBs will continue as many products will not be replaced for years to come, these BFRs can be released during disposal, and they are persistent. Furthermore, production continues in other regions, and many BFRs undergo long-range transport.[16,17]

Measurement of PBDEs and PBBs in blood and breast milk samples have revealed large geographic differences in exposure levels.[18] Both food and household dust are important sources of exposure. There is some evidence that diet may be the predominant exposure pathway for Europeans,[19,20] whereas it appears ingestion of dust contributes to a larger proportion of body burdens than food in the US, where flammability regulations are generally more stringent than in the EU.[21-23]

In this paper, we report the serum levels of BB-153 and PBDE congeners in men from Greenland, and two European cities: Warsaw, Poland and Kharkiv, Ukraine. We also examined potential determinants of exposure. Given that diet, lifestyle and housing factors differ among these three populations, we expected different exposure profiles. To our knowledge, this is the first description of blood BFR levels in these populations.

Materials and methods

Study design and populations

The study participants include male partners of pregnant women from Greenland, Poland

and Ukraine. The study design and uniform data collection procedures have previously been described in detail.[24,25] Briefly, couples were recruited for a time-to-pregnancy study during routine antenatal care visits from 2002 through 2004 from four settlements and 15 municipalities across Greenland, from a large central hospital in Warsaw, Poland, and from three maternity hospitals and eight antenatal clinics in Kharkiv, Ukraine. Eligible participants were 18 years or older and born in the country. Of the 1710 couples enrolled (45% participation rate), male partners were consecutively invited to participate in a semen study until approximately 200 men at each site were enrolled. Participation rates were 79% in Greenland, 29% in Warsaw, and 33% in Kharkiv.

Participating men filled in a questionnaire on lifestyle factors including smoking and diet (seafood, alcohol and caffeinated beverage consumption) during the period "just before your wife/partner became pregnant", and on reproductive history and occupational exposures. Jobs were classified based on the International Standard Classification of Occupations (ISCO 88).

At the time of interviews, participants had blood samples drawn from a cubital vein into 10 ml vacuum tubes for serum collection without additives (Becton Dickinson, Meylan, France). Samples were immediately centrifuged and processed after clotting, and were shipped to Lund University for storage at −80 °C.[24] Cotinine was analysed in serum samples using liquid chromatography triple quadrupole linear ion trap mass spectrometry.[26]

BFRs were determined in a subset of 300 samples which were of sufficient volume to perform these analyses, stratified by country, of the baseline 602. The mean age in the full cohort did not differ from the subset (29.8 years, SD ± 5.7 vs. 30.0 ± 5.9, $p = 0.62$). Among the included men, blood was collected between August 2002 and February 2004.

Determination of BFRs

Serum samples were analysed for congeners BDE-28, 47, 99, 100, 153, 154 and 183 and BB-153 at the Norwegian Institute of Public Health in 2010–11. There were problems during preparation of one sample, leaving 299 serum samples for analysis. The PBDEs were extracted using automated solid-phase extraction according to the method described in Thomsen et al.[27] In brief, 2 g of serum was extracted using Oasis HLB columns, and the extracts were subsequently cleaned up on sulphuric acid-silica columns with a layer of silica on top. The determination was performed by gas chromatography–high resolution mass spectrometry (GC–HRMS) using isotopically labelled (^{13}C) internal standards as previously described.[28]

BDE-183 (a major constituent of the Octa-BDE commercial mixture) was not detected in any sample. BDE-28 co-eluted with BDE-33 on the GC-column used. The reported concentrations for BDE-28 comprise the total of these two PBDEs. An unidentifiable interfering compound with the same mass fragments eluted closely after BDE-28/33. For samples from Greenland, this compound was present in larger amounts than BDE-28/33. Hence, BDE-28/33 could not be discerned from the interference and the concentrations presented

(in the Supplemental Table S2) comprise the total of these three compounds in the Greenlandic samples. The interference was present in much lower concentrations in samples from Ukraine, which made separate quantification of BDE-28/33 possible. The interference was rarely seen in samples from Poland.

Twenty-four aliquots of an in house quality control sample of human serum were analysed alongside the 299 samples. The variation (relative standard deviation) of the determinations ranged from 6.6 to 11.4% for all PBDEs and was 13.7% for BB-153. The laboratory participated in the third round of the Arctic Monitoring and Assessment Programme (AMAP) Ring Test for Persistent Organic Pollutants in Human Serum in 2010. All results were within ±31% of the consensus value. In addition, three samples from an AMAP ring test arranged previously were analysed. These results were within ±21% of the consensus value.

All together 31 procedural blanks were analysed. BDE-47 was detected in a large proportion of the blanks (> 40%). No increasing or decreasing trend in the blank levels was observed throughout the study, so the median blank contamination level of 1.58 pg/g serum was subtracted from all individual measurements. The limits of quantification (LOQs) were set to the lowest determined concentrations with signal to noise ratios of ~3 (Supplemental Table S1). This corresponded approximately to the lowest level in the calibration curves, ranging from 0.02 to 0.12 ng/g lipids for the individual BFRs. LOQs were lower than those reported from several recent biomonitoring surveys which also used small aliquots of serum.[29,30]

BFR concentrations were lipid adjusted.[5] Serum concentrations of triglycerides and cholesterol were determined by enzymatic methods.[24] The total lipid concentration in serum (g/L) was calculated as total = 0.96 + 1.28 × (triglycerides + cholesterol),[31] and median total lipids were 6.7, 6.5, and 4.6 g/L in Greenlandic, Polish, and Ukrainian samples, respectively. Missing lipids data for two participants were imputed from a linear regression model of total lipids as a function of age, BMI, and study population (R^2 = 0.32).

Statistical analysis

Concentrations of BFRs were natural log-transformed because distributions were skewed. Data below the LOQ were imputed from a log-normal probability distribution via single conditional imputation, dependent on the study population and observed values for the other congeners and allowing the residual variances to vary by study population.[32] The lowest population-specific detected value (LOQ) was used for imputations (Supplemental Table S1). We summed the five tetra–hexa BDE congeners (Σ_5PBDE), converting wet weights to moles, and summing the products of moles and the weighted average molecular weight. Due to the observed interference in determination of BDE-28/33, we summed the congeners excluding this congener. These five congeners represent the most abundant constituents of the commercial Penta-BDE mixture.[33] Summary statistics and exposure–determinant regression analyses were only computed for BFRs with detection frequencies (DF) of > 70%; this cutoff leads to minimally biased parameter and variance estimates with the single imputation method

we used.[32] Analyses of covariance (ANCOVA) were used to test for pairwise differences in age-adjusted BFR concentrations between study populations.

We fitted multiple linear regression models, including the following potential determinants of exposure: age (years), BMI (kg/m^2), serum cotinine levels (ng/mL), consumption of seafood (days/week), 'blue-collar' vs. 'white-collar' job title, and study population. Models included multiplicative interaction terms between each potential determinant and study population. For the Greenlandic study population, we also evaluated the impact of area of residence (mapped in Supplemental Figure S1). As variance inflation factors were < 1.4 for potential determinants in all models, multiple regression models were likely minimally biased by multicollinearity. We tested for non-linear associations with generalised additive models (GAMs),[34] and tested the sensitivity of models to deletion of extreme values. As a *post-hoc* analysis, we evaluated GAMs stratified by study population for age and PBDEs as we expected the emission scenarios for PBDEs to differ across the countries, and because others have reported non-linear relationships with age.[29,30]

We examined Spearman correlations and performed a principal component analysis (PCA) of the BFRs with a DF of > 50% to evaluate patterns of clustering in the BFRs. For the PCA, data were mean-centred and scaled to unit variance. Finally, we examined the relative contribution of each BDE congener to the ΣPBDE. Statistical analyses were performed using R version 2.15.2.[35]

Results and discussion

Serum concentrations of BFRs

Current study

Characteristics of three study populations are presented in Table 1, and varied somewhat across populations. All men were between 18.5 and 49.7 years of age. The detection frequencies and concentrations of the analysed BFRs and summed PBDE congeners are presented in Table 2. At least one BFR (PBDE or PBB) was detected in 298 of the 300 participants' serum samples, while all BFRs were detected in only 35 samples. BDE-47 and 153 were detected in more than 95% of samples in all three study populations. BDE-154 had the lowest DF—less than 37% across all three populations—and BDE-100 and BB-153 were detected in less than 20% of the Polish and Ukrainian samples. Concentrations of BFRs were significantly different between study populations (ANCOVA $p < 0.05$). Much higher concentrations of BFRs were observed in the samples from Greenland compared to the two European study populations for all analysed BFRs. Geometric mean (GM) concentrations per congener were 2.7 to 15 times higher, and lipid adjusted GM Σ_5PBDE concentrations were a factor 3.5 to 7 times higher.

The distributions of both lipid adjusted and wet weight concentrations are reported in Supplemental Table S2. Wet weight GM Σ_5PBDE concentrations were 41.2 pg/g serum [95% confidence interval (CI): 36.6, 46.9] in Greenlandic, 11.3 (9.5, 13.5) in Polish, and

Table 1. Characteristics of the three male study populations.

		Greenland (n=99)		Warsaw, Poland (n=100)		Kharkiv, Ukraine (n=100)		
	N	Mean or %	± SD	Mean or %	± SD	Mean or %	± SD	*p*-value[a]
Age (years)	296	32.0	7.2	30.4	3.8	27.6	5.3	<0.0001
BMI (kg/m^2)	294	25.8	3.0	25.3	2.9	24.0	2.7	<0.0001
Current smoker[b]	299	60%		23%		68%		<0.0001
Smokers' cotinine (ng/mL)[c]	299	239.2	134.7	112.4	139.2	238.4	162.3	0.045
Seafood (days/week)	281	2.2	1.8	1.3	0.9	3.6	1.3	<0.0001
Area: North-West[e]	97	23%						
Mid-West[f]		48%						
South/East[g]		29%						
'White-collar' job title[d]	146	26%		90%		N/A	-	<0.0001

BMI, body mass index; N/A, not available; SD, standard deviation.
[a] Analysis of variance for means and chi-square test for proportions.
[b] Categorised as current smoker if serum cotinine >5.0 ng/mL.[36]
[c] Median cotinine concentrations in smokers.
[d] ISCO-88 code ≤52 (vs. reference ≥61 'blue-collar').
[e] Aasiaat, Ilulissat and Qeqertarsuaq (reference).
[f] Kangaamiut, Maniitsoq, Nuuk, Paamiut, and Sisimiut.
[g] Imaarsivik, Kuummiut, Nanortalik, Narsaq, Qaqortoq, and Tasiilaq.

4.2 (3.5, 4.9) in Ukrainian samples.

This study is the first large scale biomonitoring study of BFR levels in a Greenlandic, Polish, or Ukrainian population. The study populations are representative of the general adult male populations, although no men above 60 years of age or infertile men were included. Somewhat lower concentrations might be expected in the female populations[27,30] due to differing exposure sources and elimination via lactation. The populations were sampled just prior to regulatory restrictions; however, exposure to the analysed BFRs will continue due to their environmental persistence and the long lifetime of impregnated products.

A strength of this study is that blood was sampled from all three study populations according to a standardised protocol,[25] and analysis of BFRs was conducted at one central laboratory. The concentrations observed in this study were comparable to those reported in two small studies (n < 50) which have reported associations between serum PBDEs and adverse effects on semen parameters,[11,12] and a larger study (n = 405) which reported altered hormone levels in men.[8]

Future biomonitoring studies should also quantify other high-production volume flame retardants, such as organophosphorus flame retardants (e.g., triphenyl phosphate), and novel or alternative brominated flame retardants (e.g., ethylene bis-tetrabromo phthalimide and 2,4,6-tribromophenol), some of which have been detected in the Arctic, indicating potential long-range transport.[6,37]

Table 2. Lipid adjusted serum concentrations[a] (ng/g lipids) of BFRs in the three male study populations.

	Greenland (n=99)				Warsaw, Poland (n=100)				Kharkiv, Ukraine (n=100)				ANCOVA *p*-value		
BFR	**% DF**	**P50**	**P95**	**GM (95% CI)**	**% DF**	**P50**	**P95**	**GM (95% CI)**	**% DF**	**P50**	**P95**	**GM (95% CI)**	**GR *vs* PL**	**GR *vs* UA**	**PL *vs* UA**
BDE-47	98	2.0	6.9	1.8 (1.5, 2.1)	100	0.62	5.3	0.66 (0.50, 0.86)	99	0.24	1.1	0.12 (0.07, 0.20)	<0.0001	<0.0001	<0.0001
BDE-99	74	0.54	1.7	0.50 (0.43, 0.59)	23	<LOQ	1.7		56	0.22	0.66				
BDE-100	73	0.55	1.6	0.43 (0.35, 0.51)	19	<LOQ	0.99		19	<LOQ	0.24				
BDE-153	98	2.7	7.8	2.8 (2.5, 3.1)	98	0.52	1.6	0.58 (0.52, 0.65)	95	0.34	0.77	0.32 (0.29, 0.36)	<0.0001	<0.0001	<0.0001
BDE-154	36	0.06	0.54		17	<LOQ	0.23		22	<LOQ	0.14				
Σ_5PBDE[b]		6.1	17.9	6.1 (5.4, 6.8)		1.32	10.3	1.7 (1.4, 2.0)		0.93	2.4	0.87 (0.75, 1.0)	<0.0001	<0.0001	<0.0001
BB-153	93	1.1	4.8	1.2 (0.9, 1.4)	16	<LOQ	0.32		2	<LOQ	0.22				

ANCOVA, analysis of covariance; BB, bromobiphenyl; BDE, brominated diphenyl ether; BFR, brominated flame retardant; CI, confidence interval; DF, detection frequency; GM, geometric mean; GR, Greenland; PL, Poland; P50, 50th percentile; P95, 95th percentile; UA, Ukraine.

[a] Values <LOQ were imputed; GM is not presented if <70% DF or if interference.

[b] Sum of BDE-47, 99, 100, 153, and 154.

Comparisons with reported concentrations

In Figure 1, we compare levels of the most abundant BDEs (47 and 153) and $\Sigma_{tri-hepta}$PBDE observed in the current study with levels reported for other populations (n > 50 individual samples), not occupationally exposed or exposed to site-specific sources (e.g., living nearby an incinerator). The concentrations in Greenlandic men were higher than in European and Asian populations, but generally lower than those reported for North American populations. Relatively high PBDE concentrations in US populations[18] have been attributed to more stringent furniture flammability standards. As with PBDEs, the level of serum BB-153 was higher in the US general population compared to BB-153 the Greenlandic population: GM of 2.8 ng/g lipids (95% CI: 2.2, 3.5) in 988 US men[30] vs. 1.2 (0.9, 1.4) in 99 Greenlandic men.

In pooled plasma samples (n = 3–10) taken in six locations across the Russian Arctic, $\Sigma_{tri-hepta}$PBDE (28, 47, 99, 100, 153, 154, and 183) GM concentrations ranged from 0.12 to 0.93 ng/g lipids, with higher concentrations observed at the westernmost and easternmost areas, closer to European and North American source areas.[44] These concentrations are approximately one order of magnitude lower than those observed in the Greenlandic population.

Spatial trends in Arctic PBDE concentrations, as reviewed by de Wit et al.,[6,45] are also

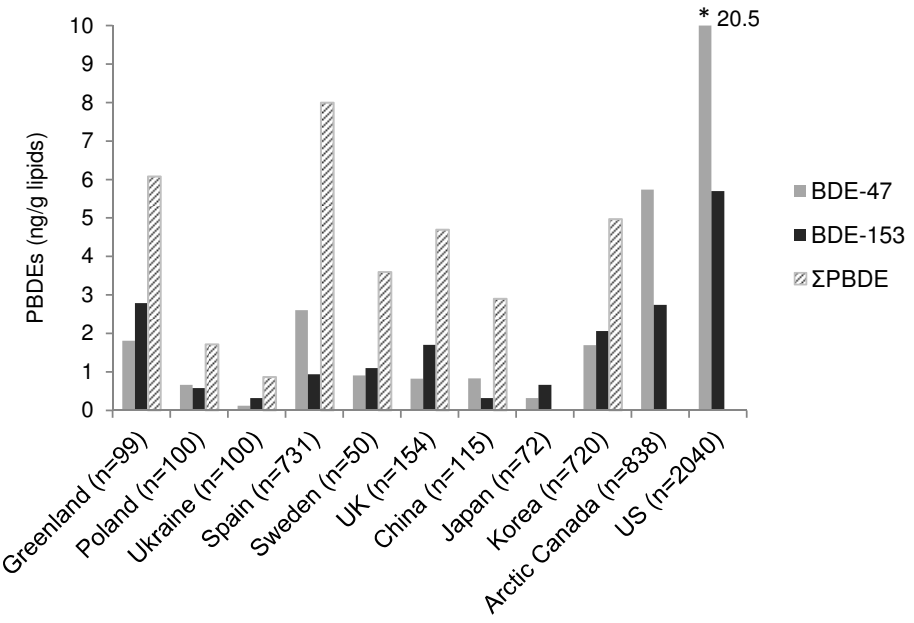

Figure 1. Comparison of geometric mean serum concentrations of BDE-47 and 153 and Σ_5PBDE among the men from Greenland, Poland, and Ukraine (current study), with median or geometric mean serum concentrations ($\Sigma_{tri-hepta}$PBDE) in general populations from Spain,[29] Sweden,[38] the UK,[39] China,[40] Japan,[41] Korea,[42] (plasma samples from Inuit from) Arctic Canada,[43] and the US.[30] European and North American populations were sampled sometime during the years 2000–2004, and Asian populations during 2006–2010.

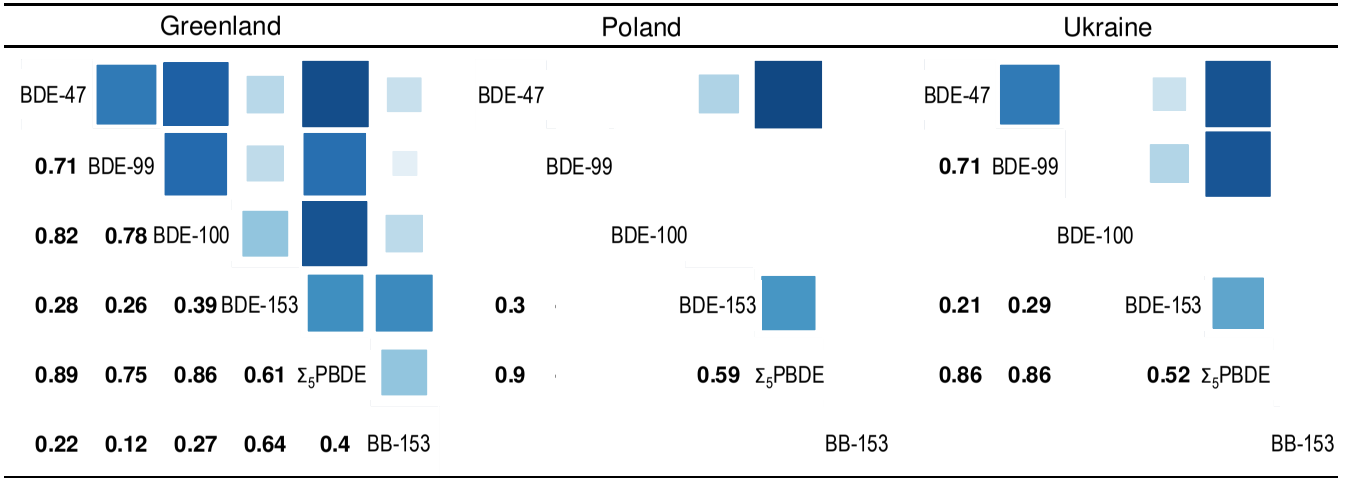

Figure 2. Spearman correlation coefficients between BFRs with > 50% DF within each population. Shading intensity and size of squares are proportional to the level of correlation. Abbreviations: BB, bromobiphenyl; BDE, brominated diphenyl ether.

apparent in seabird eggs and marine mammals. Higher concentrations in East Greenland and Svalbard organisms, compared with Russian and North American Arctic organisms, confirm that Western Europe and eastern North America are likely source areas. While long-range atmospheric transportation may be the primary source of BFRs in the Arctic environment, observations that air concentrations and ΣPBDEs in fish increase with proximity to highly populated settlements indicate that local sources also contribute.[45,46] Open burning and municipal waste incineration are possible local sources.

Greenlanders have very high levels of some legacy persistent organic pollutants, such as PCBs, due to their high intake of contaminated fish and high trophic level mammals (i.e., seal, whale, polar bear).[47] In a 2005–2010 survey among 2742 Inuit from Greenland, traditional food accounted for 21% of energy intake and marine mammals for 12%.[48] In the current study populations, CB-153 was a factor 12 and 5 times higher in Greenlandic men compared to Polish and Ukrainian men, respectively.[24] While PBDEs exhibit a somewhat lower biomagnification potential than PCBs in the Arctic marine food web,[49,50] diet is likely an important determinant of PBDE body burden for the Greenlandic population.[18] However, the relative importance of exposure via locally harvested food versus emissions from imported BFR-impregnated products and their disposal is unknown.

The concentrations in Polish and Ukrainian men of the current study were generally lower than in other populations, presumably because consumption of BFR-impregnated products was relatively low. Two studies assessed PBDE levels in samples of human breast milk from Polish women,[51,52] and levels were comparable yet at the low end of concentrations reported for other European countries.[18] In 2006–2007, serum was sampled from 125 Slovak adults, two-thirds of whom lived in the vicinity of industry or an incinerator.[53] Slovakia borders both Poland and Ukraine. Mean BDE-47 and 153 levels, 0.61 and 0.37 ng/g lipids, respectively, lay between the levels observed in the Polish and Ukrainian populations, and the median $\Sigma_{tri-hepta}$PBDE of 0.86 ng/g lipids was lower.

Congener profiles

PBDEs with a DF of > 50% were moderately correlated within the pooled study populations (r_s = 0.5–0.7), although some pairwise correlations were lower within specific populations, e.g., r_s = 0.2–0.3 between BDE-47 and 153 (Figure 2 and Supplemental Table S3). BB-153 was moderately correlated with Σ_5PBDE (r_s = 0.4). For congeners with higher DFs, correlation patterns were generally consistent across study populations.

The PCA revealed differential clustering by study population, especially distinct for Greenland vs. the European populations: the scores for the three populations were located differently in the three-dimensional Euclidean space representing the first three principal components (Figure 3). This clustering was not due to absolute differences in concentrations, as data were centred and scaled, nor was it an artifact of conditional imputation, as it was still evident in a PCA of only data > LOQ (Supplemental Figure S2).

The relative contribution of congeners, by molar fraction, to Σ_5PBDE followed the same order in all three study populations: BDE-47 (40–58%) > 153 (19–40%) > 99 (9–23%) > 100 (7–9%) > 154 (1–3%). However, by weight, BDE-153 exceeded BDE-47 in the Polish population, and the ratio BDE-47:153 was higher in Polish compared to Greenlandic and Ukrainian samples. Examination of the congener distribution pattern across the continuum of Σ_5PBDE concentrations revealed that the ratio of BDE-47:153 relative concentrations increased with increasing Σ_5PBDE (Supplemental Figure S3). Dallaire et al.[43] also observed this pattern, and suggested that BDE-47 may represent more recent exposure and adoption of Westernised lifestyle (diet and electronics use), whereas BDE-153 is a more persistent and ubiquitous congener.

BDE-47 or BDE-153 was also the dominant congeners in other large serum surveys in which BDE-209 was not measured.[30,38,39] The relative contribution of congeners in serum did not match the (European Bromkal 70-5DE) Penta-BDE technical formulation, in which the order of most abundant congeners is BDE-99 (45% w/w) > 47 (43%) > 100 (8%) > 153 (5%) > 154 (3%).[33] This may be due to differing biomagnification and bioaccumulation potentials, and differing bioconversion and metabolic debromination rates across congeners.[6] Half-lives in humans have been estimated to be in the range of 1–3 years for BDE-47 and 99, and 6–7 years for BDE-153.[20,54]

BDE-209, the major component of the Deca-BDE commercial mixture (> 90%), was

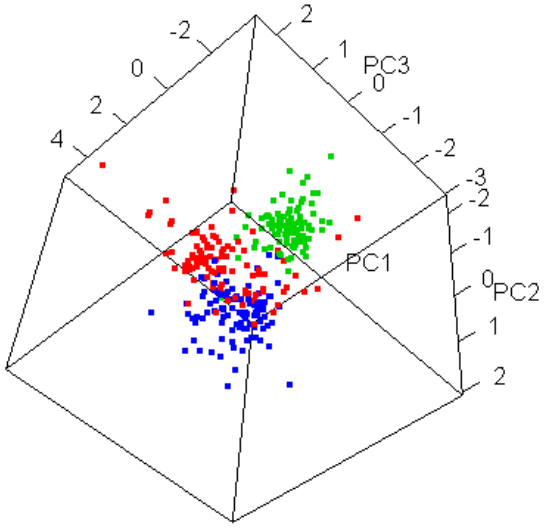

Figure 3. Principal components analysis of BDE-47, 99 and 153, revealing clustering by population (green, Greenland; red, Poland; blue, Ukraine). Scores are projected onto the first three principal components, capturing 73%, 17% and 10% of the explained variance.

not quantified in the current study. BDE-209 has an estimated half-life of 15 days[55]; much shorter than the determined congeners. In a large survey of adults from Catalonia, Spain, BDE-209 was the most abundant congener in serum (the DFs for BDE-47, 153, and 209 were all above 70%). However, in other surveys, BDE-209 has been detected in a small proportion of serum samples (< 35%) and was not the most abundant congener.[39,56]

The distinct congener profiles per study population has implications for analyses of BFR exposure and health outcomes; different mixtures at various exposure ranges may produce different health effects across populations.

Regression analyses

Table 3 summarises the linear regression models of PBDEs as a function of exposure determinants. Study population (Greenland) and area within Greenland were associated with BDE-153 and BDE-47, respectively, and with Σ_5PBDE. No other determinants were significantly associated with PBDEs. However, two interaction terms were significant ($p < 0.05$), and in regression models stratified by study population, seafood consumption was inversely associated with BDE-47, and age was positively associated with BDE-153 in Polish men, but not the other two study populations. BMI was borderline non-significantly associated with BDE-153 in all men; the coefficient was −0.038 (95% CI: −0.078, 0.003), representing a change of 3.7% (95% CI: −7.5, 0.0) per kg/m^2. We also evaluated multiple regression models of lipid weight BFRs and determinants, including total lipids as an independent variable. Results for these models were very similar to those presented in Table 3 (data not shown). Further, we tested linear regression models excluding two extreme values, and GAMs with all potential determinants included in the model. While no additional determinants were discovered, we did identify non-linear relationships for age (elaborated below).

Geographic differences

The majority of the variance was accounted for by only study population (R^2 of 0.30, 0.74, and 0.53 for BDE-47, 153, and Σ_5PBDE, respectively); exposure factors explained little additional variance (Table 3). Adding area of residence to the models for Greenland increased the R^2 from 0.03 to 0.11 and 0.09 for BDE-47 and Σ_5PBDE. Concentrations were higher in men from the South/East than in men from the North-West. GM Σ_5PBDE concentrations were 7.5, 5.6, and 5.3 ng/g lipids for those from the South/East, Mid-West, and North-West, respectively. This East > West pattern was also mirrored in blubber samples from ringed seals, in which median BDE-47 concentrations were one order of magnitude higher in East vs. West Greenland samples.[57,58] Greenlanders from the South/East consume more polar bear and Hooded seal—a large species of seal—which might partially explain the observed East > West difference in serum PBDE levels.

Table 3. Multiple linear regression models of PBDEs (ng/g lipids) and potential exposure determinants.

			Interaction *p*-value[a]	
Potential determinant	***β***	**95% CI**	**GR**	**PL**
Ln BDE-47: *R^2 0.32*				
1) Intercept	-2.983	-6.802, 0.837		
Age (years)	-0.030	-0.099, 0.040	0.34	0.23
BMI (kg/m^2)	0.033	-0.103, 0.170	0.56	0.97
Cotinine (ng/ml)	0.001	-0.001, 0.003	0.25	0.41
Seafood (days/wk)	0.178	-0.147, 0.503	0.25	**0.04**
Population[b]: GR	3.915	-1.362, 9.191		
PL	0.864	-4.819, 6.547		
2) White-collar job[c]	-0.140	-0.964, 0.684		0.55
3) Area[d]: South/East	**0.553**	**0.040, 1.066**		
Mid-West	0.002	-0.457, 0.460		
Ln BDE-153: *R^2 0.77*				
1) Intercept	-0.293	-1.434, 0.848		
Age (years)	-0.002	-0.023, 0.019	0.81	**0.005**
BMI (kg/m^2)	-0.038	-0.078, 0.003	0.80	0.95
Cotinine (ng/ml)	0.0003	-0.0003, 0.001	0.79	0.12
Seafood (days/wk)	0.009	-0.089, 0.106	0.86	0.62
Population: GR	**2.592**	**1.016, 4.168**		
PL	-0.833	-2.531, 0.865		
2) White-collar job	-0.118	-0.465, 0.228		0.90
3) Area: South/East	0.216	-0.107, 0.539		
Mid-West	0.149	-0.140, 0.437		
Ln Σ_5PBDE: *R^2 0.55*				
1) Intercept	-0.751	-2.401, 0.899		
Age (years)	-0.011	-0.042, 0.019	0.43	0.11
BMI (kg/m^2)	0.025	-0.034, 0.083	0.19	0.62
Cotinine (ng/ml)	0.001	0.000, 0.002	0.30	0.13
Seafood (days/wk)	0.059	-0.081, 0.200	0.27	0.26
Population: GR	**3.281**	**1.001, 5.560**		
PL	0.293	-2.162, 2.748		
2) White-collar job	-0.154	-0.675, 0.367		0.64
3) Area: South/East	**0.379**	**0.031, 0.727**		
Mid-West	0.091	-0.219, 0.402		

CI, confidence interval; GR, Greenland; (P)BDE, (poly)brominated diphenyl ether; PL, Poland.
Significant associations are marked in bold. Model 1) included all determinants except job type and area (n=275); Model 2) included all determinants except area, with a reduced complete case set (n=130); Model 3) was fitted for the Greenland study population, and included all determinants except job type (n=91). R^2 reported for Model 1.
[a] *P*-values for the interaction terms between study population and determinant.
Reference categories: [b] Ukraine; [c] Blue-collar job; [d] North-West.

Age dependence

Although linear regression models for age were null, we found associations of an increasing or U-shaped relationship between age and some PBDEs in GAMs stratified by study population. We present GAMs for age, plotted with BMI held at the mean level, as age and BMI were related (r_p = 0.15). BDE-47 showed a clear U-shaped relationship with age in Ukrainian men, with an inflection point around age 30 years (Figure 4). BDE-153 showed an exponential increase with age in Polish men. Upon deletion of two extreme values, this relationship became linear (presented). In Greenlandic men, the relationships were close to flat. The pattern for BDE-47 paralleled the U-shaped relationship between Σ_5PBDE and age in Ukrainian men.

Increasing concentrations of POPs, such as PCBs, with age have often been attributed to bioaccumulation with age. However, Quinn and Wania[59] demonstrated that emissions-related cohort effects and the metabolic half-life are more important predictors of age-trends in body burdens, rather than bioaccumulation. Curvilinear relationships between age and PBDEs have also been observed in two other large general population surveys sampled in the same years as the currently studied populations (between 2002 and 2004). In 2040 men and women aged 12 years and older from the US, BDE-47 and 153 showed a U-shaped relationship, with an inflection point (lowest concentrations) at 40–59 years.[30] In 731 adults aged 18 to 74 years from Catalonia, Spain, higher concentrations were observed in participants younger than 30 years for most congeners and ΣPBDE; ΣPBDE concentrations followed an L-shape (exponential decrease), and levelled off after 30 years.[29] There were positive associations between age and lower-brominated (tri–hepta) but not higher-brominated (octa–deca) PBDEs in whole blood samples obtained in 2007–2008 from 72 Japanese adults aged 15 to 74 years.[41] Similarly, age was associated with the lower-brominated PBDEs in breast milk samples obtained in 2003–2005 from 393 Norwegians aged 16 to 42 years.[60] Some studies of non-occupationally exposed populations (n > 50) sampled 2000–2004 have found no relationship between age and PBDE concentrations.[38,39,61]

BB-153 was phased out in the 1970s yet was detected in 93% of Greenlandic samples, and was positively and linearly associated with age (4.9%, 95% CI: 2.2, 7.8%), seafood consumption (14.7%, 95% CI: 3.4, 27.4%), and living in the South/East of Greenland (89.6%, 95% CI: 11.2, 223.2%). BB-153 was detected in less than 20% of European samples (Table 2). In US adults sampled 2003–2004, BB-153 also increased linearly with age.[30]

BMI and other determinants

We found indications, although inconsistent and only borderline significant, that BDE-153 decreased with increasing BMI, possibly reflecting a dilution effect. This trend was also observed in the large surveys of Catalonians (non-significant) and Nunavik Inuit (for BDE-153 but not 47).[29,43] Seafood consumption was associated with decreased BDE-47 in the Polish population and non-significantly with Σ_5PBDE in all populations. In Nunavik Inuit, marine mammal intake and a biological marker of long-term seafood consumption, n-3 PUFA, was

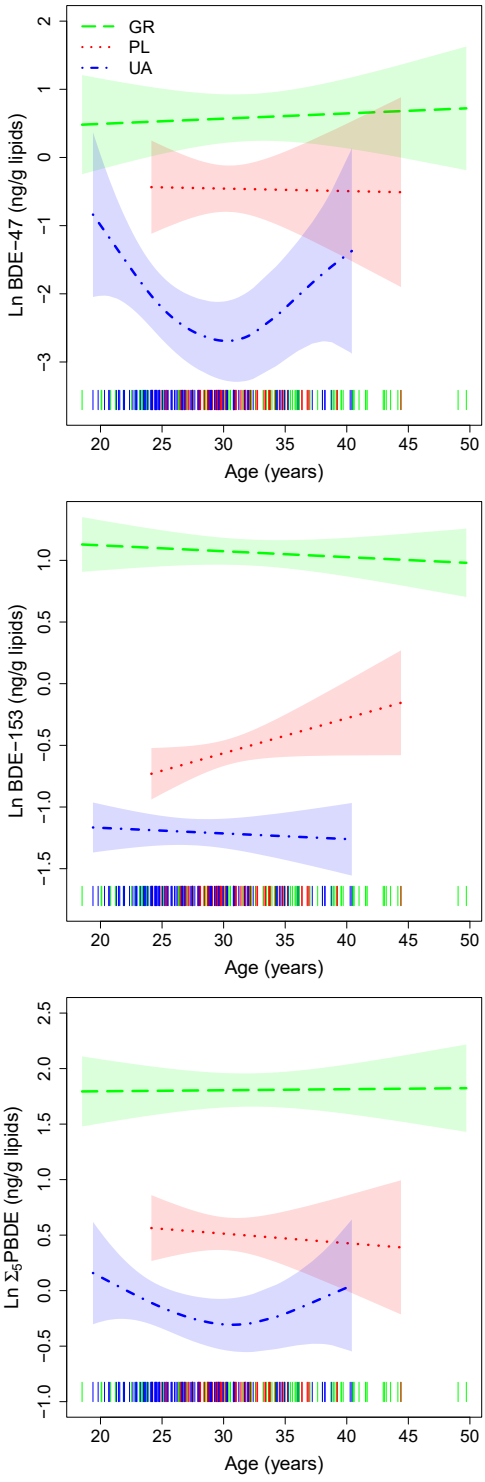

Figure 4. Generalised additive models of age and PBDEs, modelled with BMI fixed at the mean. Population-specific splines and shaded 95% CIs are presented [green, Greenland (GR); red, Warsaw (PL); blue, Kharkiv (UA)]. Two extreme BDE-153 values were excluded to avoid a somewhat irregular curve. Rug plots display the distribution of ages in the study population.

associated with decreased BDE-47, while store bought meat intake was associated with increased BDE-47.[43] In contrast, biological markers of fish consumption were associated with increased blood concentrations of lower (tri–hepta), but not higher, brominated BDEs in Japanese adults.[41] Biomagnification of tri–hepta BDEs has been observed in marine fish in the Baltic Sea and Greenland,[46,62] and fish has been estimated to account for the majority of dietary PBDE intake.[18] Although non-significant, we observed trends of increased PBDEs for smokers compared to non-smokers, and decreased PBDEs for those with a white-collar compared to blue-collar job. Higher PBDEs in smokers,[53] in those with less education,[60] and in those with lower household incomes[63] have been previously reported. Whether these variables are proxies for potentially more important determinants, such as composition of household products and diet, is unknown.

We had data on only a limited set of potential determinants of exposure, not including some exposure factors which have previously been associated with increased PBDE body burdens, such as living with an electrician,[60] and owning a large screen TV,[64] or three or more pieces of stuffed furniture.[65] While this study does not provide new insights into the relative importance of household dust versus food as exposure pathways, the explained variance we modelled within populations (0.02–0.13) was in the range reported by other studies (0.04–0.28).[43,60,64,65]

Conclusions

We report serum concentrations of tri- to hexa-BDEs and BB-153 in adult men from Greenland, Warsaw, and Kharkiv. Body burdens were substantially higher in Greenlandic compared to European men, yet lower than concentrations reported for populations from the United States. While we observed large contrasts in exposure and samples exhibited distinct population-specific congener profiles, data on potential determinants explained little variability. Future biomonitoring efforts should assess body burdens of novel BFRs, in addition to BFRs of the EU-restricted Penta-, Octa-, and Deca-BDE commercial mixtures.

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Supplemental Material

Table S1. Overview of the limits of quantification.

	LOQs[a]					
	All 3 study populations			GR	PL	UA
	DF%	(pg/g serum)	(ng/g lipids)[b]	(pg/g serum)		
BDE-28/33	69.9	0.13	0.02	1.64	0.27	0.13
BDE-47	99.0	0.99[d]	0.16[d]	2.14	1.38	0.99
BDE-99	50.8	0.70	0.12	2.18	1.23	0.70
BDE-100	36.8	0.60	0.10	1.30	1.16	0.60
BDE-153	97.0	0.50	0.08	6.23	1.54	0.50
BDE-154	25.1	0.25	0.04	0.57	0.39	0.25
BB-153	36.8	0.56	0.09	1.35	0.56	1.32

BB, bromobiphenyl; BDE, brominated diphenyl ether; DF, detection frequency; GR, Greenland; LOQ, limit of quantification; PL, Poland; UA, Ukraine.
[a] LOQ based on the lowest measured concentration for samples: S/N>3, corresponding to approximately the lowest level in the calibration curve.
[b] Calculated as the wet weight divided by the population median total lipids (6.080 g/L).
[d] Subtracting the median blank contamination level, the minimum observed value >0 was 0.02 (pg/g serum) and 0.005 (pg/g lipids).

Table S2. Expanded descriptive statistics for the wet weight and lipid adjusted serum concentrations of BFRs.

		Wet weight (pg/g serum)							Lipid adjusted (ng/g lipids)						
	% DF	5th P	25th P	50th P	50th P[a]	75th P	95th P	Max	5th P	25th P	50th P	50th P[a]	75th P	95th P	Max
Greenland (n=99)															
BDE-28/33[b]	99	3.60	10.87	21.27	21.28	40.97	83.89	129.56	0.61	1.49	2.87	2.95	6.26	12.40	15.82
BDE-47	98	3.65	7.33	12.94	12.95	20.99	47.30	81.61	0.55	1.20	1.95	1.96	3.08	6.85	12.48
BDE-99	74	1.13	2.07	3.83	4.48	5.43	12.42	19.58	0.13	0.34	0.54	0.67	0.79	1.71	2.65
BDE-100	73	<LOQ	1.22	3.60	4.58	5.81	11.65	14.13	<LOQ	0.20	0.55	0.67	0.82	1.55	2.32
BDE-153	98	8.66	14.70	19.02	19.29	24.46	40.08	114.10	1.30	2.13	2.73	2.73	3.59	7.84	22.46
BDE-154	36	<LOQ	<LOQ	0.45	1.54	1.15	4.05	4.80	<LOQ	<LOQ	0.06	0.21	0.18	0.54	0.76
Σ_5PBDE[c]	98	13.88	29.14	41.47	40.40	59.68	115.87	139.13	2.26	4.50	6.07	5.85	8.05	17.94	23.16
BB-153	93	1.19	4.58	7.75	8.10	16.27	35.17	94.80	0.18	0.71	1.08	1.14	2.37	4.84	18.20
Warsaw, Poland (n=100)															
BDE-28/33	46	<LOQ	<LOQ	0.23	0.97	0.94	2.17	6.94	<LOQ	<LOQ	0.04	0.13	0.13	0.30	1.20
BDE-47	100	1.08	2.24	4.13	4.13	7.96	41.47	184.44	0.18	0.33	0.62	0.63	1.20	5.33	31.75
BDE-99	23	<LOQ	<LOQ	<LOQ	3.78	1.13	13.76	49.24	<LOQ	<LOQ	<LOQ	0.57	0.16	1.67	8.48
BDE-100	19	<LOQ	<LOQ	<LOQ	3.83	1.08	7.14	38.62	<LOQ	<LOQ	<LOQ	0.52	0.15	0.99	6.65
BDE-153	98	1.84	2.68	3.60	3.63	5.10	9.77	40.32	0.30	0.41	0.52	0.53	0.78	1.55	6.94
BDE-154	17	<LOQ	<LOQ	<LOQ	1.09	<LOQ	1.50	5.98	<LOQ	<LOQ	<LOQ	0.16	<LOQ	0.23	1.03
Σ_5PBDE[c]	100	4.36	6.52	9.13	8.44	17.36	76.43	343.70	0.64	0.96	1.32	1.18	2.50	10.27	59.16
BB-153	16	<LOQ	<LOQ	<LOQ	1.21	<LOQ	1.93	6.02	<LOQ	<LOQ	<LOQ	0.19	<LOQ	0.32	0.90

Table S2. *Continued.*

		Wet weight (pg/g serum)							Lipid adjusted (ng/g lipids)						
	% DF	5th P	25th P	50th P	50th P[a]	75th P	95th P	Max	5th P	25th P	50th P	50th P[a]	75th P	95th P	Max
Kharkiv, Ukraine (n=100)															
BDE-28/33	65	<LOQ	<LOQ	0.22	0.28	0.31	0.57	0.99	<LOQ	<LOQ	0.05	0.06	0.07	0.11	0.14
BDE-47	99	<LOQ	0.53	1.12	1.24	2.16	6.15	27.79	<LOQ	0.12	0.24	0.27	0.45	1.08	2.96
BDE-99	56	<LOQ	<LOQ	0.97	1.80	1.96	3.07	11.65	<LOQ	<LOQ	0.23	0.36	0.38	0.66	1.24
BDE-100	19	<LOQ	<LOQ	<LOQ	1.01	<LOQ	1.26	4.10	<LOQ	<LOQ	<LOQ	0.20	<LOQ	0.25	0.44
BDE-153	95	0.49	1.13	1.59	1.62	2.19	3.60	6.10	0.11	0.25	0.34	0.34	0.44	0.77	1.65
BDE-154	22	<LOQ	<LOQ	<LOQ	0.51	<LOQ	0.67	1.02	<LOQ	<LOQ	<LOQ	0.11	<LOQ	0.14	0.21
Σ_5PBDE[c]	99	0.82	2.57	4.53	3.61	7.33	13.49	51.06	0.19	0.55	0.93	0.83	1.49	2.40	5.44
BB-153	2	<LOQ	<LOQ	<LOQ	1.64	<LOQ	1.02	1.96	<LOQ	<LOQ	<LOQ	0.35	<LOQ	0.22	0.43

BB, bromobiphenyl; BDE, brominated diphenyl ether; BFR, brominated flame retardant; DF, detection frequency; LOQ, limit of quantification; P, percentile.
Values below the sample-specific LOQ were imputed (refer to main text), except for columns [a] for which the median of the detected (>LOQ) values are presented.
[b] Interference by an unknown compound was observed during quantification of BDE-28/33 for most samples from Greenland (refer to main text).
[c] Sum of BDE-47, 99, 100, 153, and 154.

Table S3. Spearman correlation coefficients between BFR concentrations (ng/g lipids) in the three study populations, and for all men. Correlations are presented for BFRs with **(1)** data <LOQ imputed (reported if >50% pairwise complete cases based on detected data); and **(2)** for only data >LOQ with an indication of the number of pairwise complete observations in brackets.

		1) <LOQ imputed r_s (n=99 GR; n=100 PL/UA; n=299 All)							2) Only data >LOQ r_s (n)						
		BDE-47	**BDE-99**	**BDE-100**	**BDE-153**	**BDE-154**	**Σ_5PBDE**	**BB-153**	**BDE-47**	**BDE-99**	**BDE-100**	**BDE-153**	**BDE-154**	**Σ_5PBDE**	**BB-153**
BDE-28/33	GR	**0.33**	0.08	**0.23**	**0.32**	—	**0.32**	**0.75**	**0.28** (97)	-0.02 (73)	0.19 (72)	**0.34** (97)	**0.53** (36)	**0.29** (98)	**0.80** (92)
	PL	—	—	—	—	—	—	—	**0.37** (46)	**0.75** (18)	**0.79** (13)	**0.63** (46)	0.52 (12)	**0.40** (46)	-0.29 (7)
	UA	**0.33**	—	—	-0.08	—	**0.25**	—	**0.43** (62)	**0.52** (38)	**0.58** (16)	0.19 (62)	**0.63** (16)	**0.36** (65)	— (2)
	All	***0.64***	—	—	***0.68***	—	***0.70***	—	***0.73*** *(205)*	***0.46*** *(129)*	***0.49*** *(101)*	***0.87*** *(205)*	***0.65*** *(64)*	***0.79*** *(209)*	***0.85*** *(101)*
BDE-47	GR		**0.71**	**0.82**	**0.28**	—	**0.89**	**0.22**		**0.74** (73)	**0.90** (72)	**0.26** (96)	**0.63** (36)	**0.86** (97)	**0.22** (92)
	PL		—	—	**0.30**	—	**0.90**	—		**0.91** (23)	**0.92** (19)	**0.31** (97)	**0.60** (17)	**0.91** (99)	0.19 (16)
	UA		**0.71**	—	**0.21**	—	**0.86**	—		**0.66** (55)	**0.77** (19)	0.13 (86)	0.28 (20)	**0.82** (90)	— (1)
	All		***0.69***	—	***0.69***	—	***0.94***	—		***0.83*** *(151)*	***0.94*** *(110)*	***0.68*** *(279)*	***0.68*** *(73)*	***0.93*** *(286)*	***0.35*** *(109)*
BDE-99	GR			**0.78**	**0.26**	—	**0.75**	0.12			**0.68** (69)	0.10 (73)	**0.45** (33)	**0.69** (73)	-0.03 (70)
	PL			—	—	—	—	—			**0.96** (14)	**0.63** (23)	**0.68** (15)	**0.96** (23)	0.26 (6)
	UA			—	**0.29**	—	**0.86**	—			**0.69** (18)	0.17 (54)	0.42 (18)	**0.73** (56)	— (1)
	All			—	***0.53***	—	***0.78***	—			***0.75*** *(101)*	***0.53*** *(150)*	***0.57*** *(66)*	***0.82*** *(152)*	*-0.03 (77)*
BDE-100	GR				**0.39**	—	**0.86**	**0.27**				**0.37** (72)	**0.62** (34)	**0.88** (72)	0.23 (69)
	PL				—	—	—	—				**0.69** (19)	**0.66** (13)	**0.94** (19)	0.14 (7)
	UA				—	—	—	—				0.07 (19)	-0.43 (7)	**0.72** (19)	— (0)
	All				—	—	—	—				***0.64*** *(110)*	***0.68*** *(54)*	***0.93*** *(110)*	*0.21 (76)*
BDE-153	GR					—	**0.61**	**0.64**					0.31 (36)	**0.59** (97)	**0.62** (92)
	PL					—	**0.59**	—					**0.77** (17)	**0.59** (98)	0.24 (16)
	UA					—	**0.52**	—					**0.68** (22)	**0.50** (95)	— (2)
	All					—	***0.84***	—					***0.68*** *(75)*	***0.84*** *(290)*	***0.71*** *(110)*

Table S3. *Continued.*

		1) <LOQ imputed r_s (n=99 GR; n=100 PL/UA; n=299 All)							2) Only data >LOQ r_s (n)						
		BDE-47	**BDE-99**	**BDE-100**	**BDE-153**	**BDE-154**	**Σ_5PBDE**	**BB-153**	**BDE-47**	**BDE-99**	**BDE-100**	**BDE-153**	**BDE-154**	**Σ_5PBDE**	**BB-153**
BDE-154	GR						—	—						**0.57** (36)	**0.54** (36)
	PL						—	—						**0.73** (17)	0.31 (6)
	UA						—	—						**0.54** (22)	— (1)
	All						—	—						***0.75*** *(75)*	***0.50*** *(43)*
Σ_5PBDE	GR							**0.40**							**0.37** (92)
	PL							—							0.20 (16)
	UA							—							— (2)
	All							—							***0.49*** *(110)*

Correlation coefficient bolded if $p < 0.05$.
— Too few pairwise complete cases [majority of values imputed (1); or too few data to compute a correlation, for BB-153 in Ukrainian samples (2)].

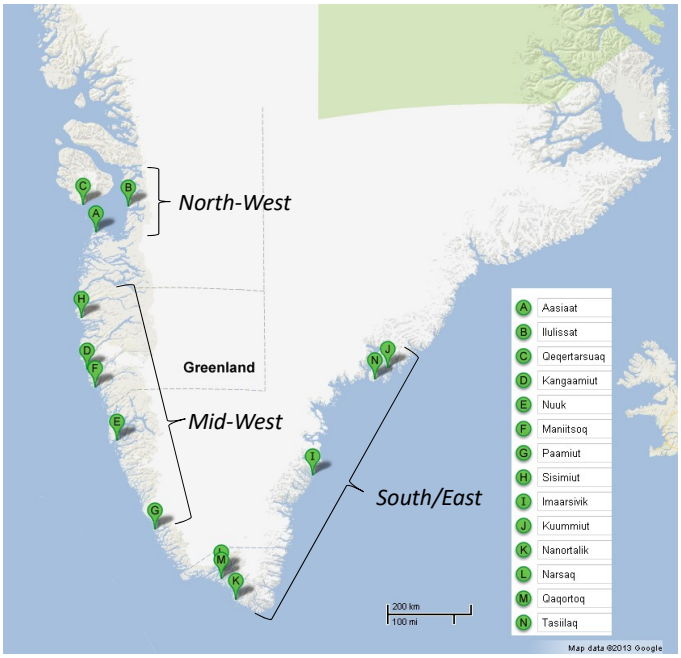

Figure S1. A map of the municipalities and settlements where participants from Greenland lived and were sampled, with the three 'area' categories demarcated.

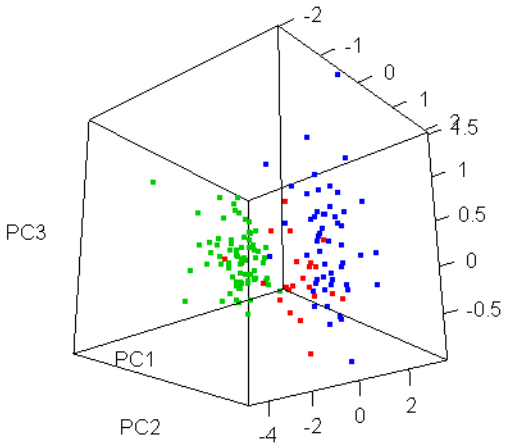

Figure S2. Principal components analysis of detected (>LOQ) values for the 3 BDEs with a detection frequency of >70%: 47, 99, 153, excluding BDE-28/33 for which there was interference (n=149). The proportion of explained variance was 78%, 17%, and 5% for the first three principal components (PC). This sensitivity analysis shows that clustering of samples by population (green, Greenland; red, Poland; blue, Ukraine) was not an artifact of the imputation.

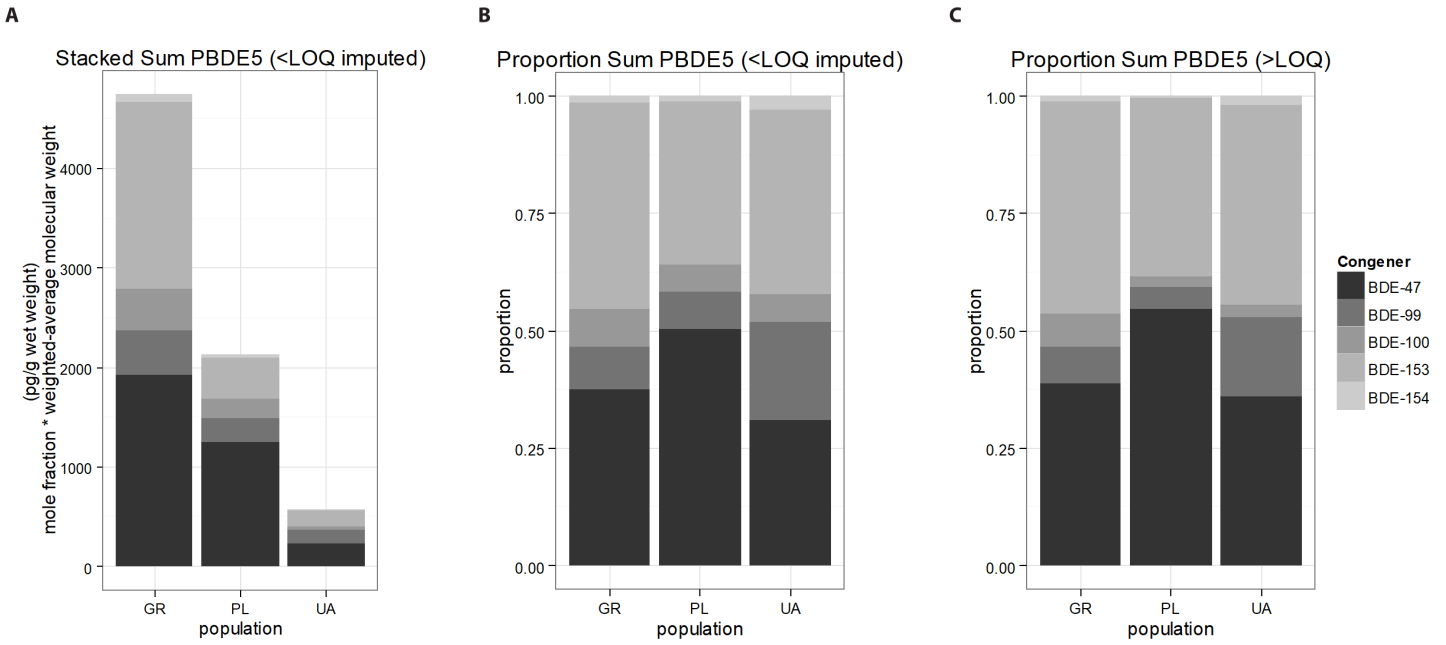

Figure S3. Absolute **(A)** and relative **(B-C)** contributions of PBDE congeners to the sum of congeners (Σ_5PBDE: BDE-47, 99, 100, 153, and 154) per study population; and **(D-F)** loess smoothed relative contributions across continuous Σ_5PBDE values, from the 5th to 95th percentiles of Σ_5PBDE per study population.

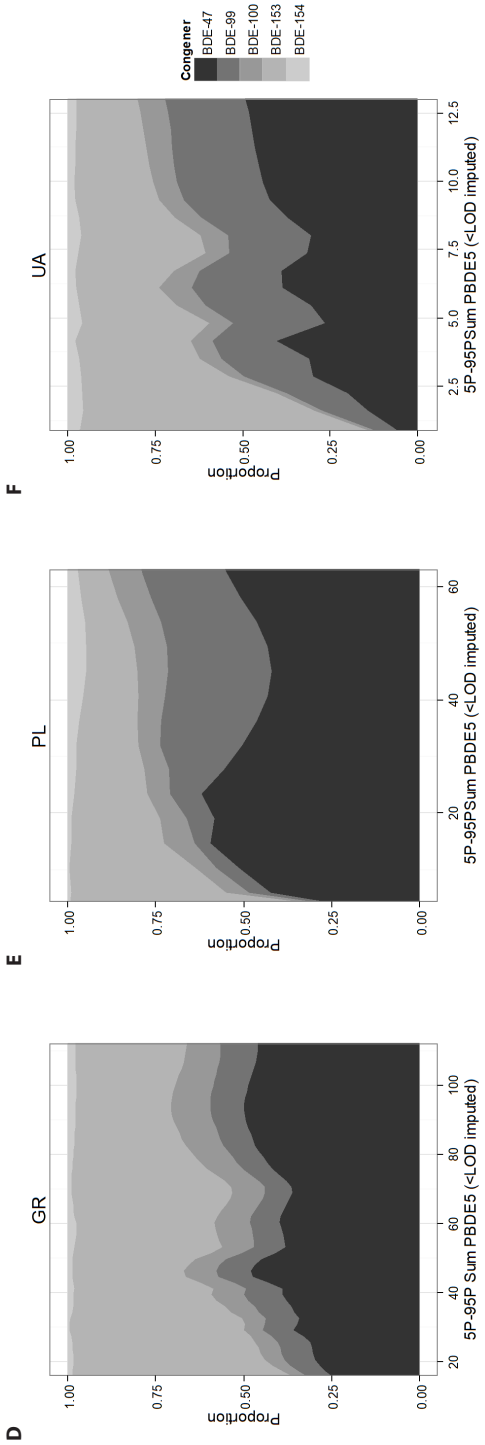

Figure S3. *Continued.*

Chapter 3

Phthalates, perfluoroalkyl acids, metals and organochlorines and reproductive function: a multipollutant assessment in Greenlandic, Polish and Ukrainian men

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Abstract

Objectives: Numerous environmental contaminants have been linked to adverse reproductive health outcomes. However, the complex correlation structure of exposures and multiple testing issues limit the interpretation of existing evidence. Our objective was to identify, from a large set of contaminant exposures, exposure profiles associated with biomarkers of male reproductive function.

Methods: In this cross-sectional study (n = 602), male partners of pregnant women were enrolled between 2002 and 2004 during antenatal care visits in Greenland, Poland, and Ukraine. Fifteen contaminants were detected in more than 70% of blood samples, including metabolites of di(2-ethylhexyl) and diisononyl phthalates (DEHP, DiNP), perfluoroalkyl acids, metals, and organochlorines. Twenty-two reproductive biomarkers were assessed, including serum levels of reproductive hormones, markers of semen quality, sperm chromatin integrity, epididymal and accessory sex gland function, and Y:X chromosome ratio. We evaluated multipollutant models with sparse partial least squares (sPLS) regression, a simultaneous dimension reduction and variable selection approach which accommodates joint modelling of correlated exposures.

Results: Of the over 300 exposure–outcome associations tested in sPLS models, we detected 10 associations encompassing 8 outcomes. Several associations were notably consistent in direction across the three study populations: positive associations between mercury and inhibin B, and between cadmium and testosterone; and inverse associations between DiNP metabolites and testosterone, between polychlorinated biphenyl-153 and progressive sperm motility, and between a DEHP metabolite and neutral α-glucosidase, a marker of epididymal function.

Conclusions: This global assessment of a mixture of environmental contaminants provides further indications that some organochlorines and phthalates adversely affect some parameters of male reproductive health.

Introduction

There has been considerable scientific and public concern, particularly over the past two decades, that exposure to environmental contaminants may impede development *in utero*, perturb hormonal homoeostasis and regulation, and contribute to subfertility in humans. This concern stems in part from evidence of geographic variability in semen quality,[1] secular trends of decreasing testosterone[2] and reports, although inconsistent and contested, of secular declines in sperm concentrations and semen quality.[3,4]

Despite the surge in studies on environmental contaminants and male reproductive health, interpretation of the evidence base is hampered by the piecemeal approach to many investigations. In observational epidemiology, often one class of compounds is correlated with a subset of outcomes, disregarding correlations among background low-level exposures. We chose to simultaneously evaluate a large set of environmental contaminant exposures and biomarkers of male reproductive function in a pregnancy-based study of men from Greenland, Poland, and Ukraine.[5,6] The set of contaminants comprises high priority[7] legacy and emerging contaminants of concern, suspected to interfere with the endocrine system: high-molecular-weight phthalates, plasticisers used in a wide variety of consumer products, including polyvinyl chloride; perfluoroalkyl acids (PFAAs), surfactants used in many applications, such as water and stain-repellent coatings; three non-essential metals; one polychlorinated biphenyl, and two organochlorine pesticides. These leach from products or are deposited directly into the environment, leading to near-ubiquitous human exposure,[8,9] and all, except phthalates, are persistent and bioaccumulate.

We sought an approach to identify exposure–outcome associations between the 19 exposures, representing four classes of xenobiotic compounds, and 22 biomarker outcomes, reflecting various aspects of male reproductive function. Given the relatively weak prior information for the many possible associations, we decided to take a more agnostic, data-driven approach. That the exposures are highly correlated represented a methodological challenge. For this reason, we used sparse partial least squares (sPLS) regression modelling, which relates linear combinations of a subset of the most predictive exposures to an outcome via linear regression.[10] Unlike conventional ordinary least squares (OLS) regression, this dimension reduction approach does not suffer from instability in estimates and failure to converge due to multicollinearity, and enabled a joint assessment of the full set of measured biomarkers of low-level environmental contaminant exposures and reproductive function.

Methods

Study populations

Study design and data collection procedures have been described previously.[6] Briefly, pregnant women and their male partners were recruited during routine antenatal care visits from 2002 through 2004 inclusive at a large central hospital in Warsaw, Poland, at three hospitals and eight antenatal clinics in Kharkiv, Ukraine, and at local hospitals in 19 municipalities and

settlements across Greenland. Of the 1710 couples enrolled (45% participation rate), male partners were consecutively invited to participate in a semen study until approximately 200 men at each of the three locations had agreed (38% participation rate). Participants provided a semen sample, a venous blood sample, and information on lifestyle factors (further details provided in the Supplemental Methods).

Exposure and outcome assessment

Phthalate metabolites and PFAAs were analysed in serum samples by liquid chromatography-tandem mass spectrometry[9] (expanded in the Supplemental Methods). Phthalates included secondary oxidised metabolites of di(2-ethylhexyl) phthalate (DEHP) [mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP, alternatively 5OH-MEHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP, alternatively 5oxo-MEHP) and mono(2-ethyl-5-carboxypentyl) phthalate (MECPP, alternatively 5cx-MEPP)], and of diisononyl phthalate (DiNP) [mono(4-methyl-7-hydroxyoctyl) phthalate (MHiNP, alternatively 7OH-MMeOP), mono(4-methyl-7-oxooctyl) phthalate (MOiNP, alternatively 7oxo-MMeOP) and mono(4-methyl-7-carboxyheptyl) phthalate (MOiCP, alternatively 7cx-MMeHP)]. Analysed PFAAs [a subset of per- and polyfluoroalkyl substances (PFASs), also referred to as perfluorinated compounds (PFCs)] included perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorhexane sulfonic acid (PFHxS), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA) and perfluorododecanoic acid (PFDoDA). Metals were measured in whole blood samples; cadmium (Cd) and lead (Pb) by inductively coupled plasma-mass spectrometry (Thermo X7, Thermo Elemental, Winsford, UK), and mercury (Hg) by cold vapour atom fluorescence spectrophotometry. 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB-153) and the dichlorodiphenyltrichloroethane metabolite, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethylene (*p,p′*-DDE), were analysed by gas chromatography-mass spectrometry,[11] as was hexachlorobenzene (HCB) according to a modified method by Otero et al.[12]

Twenty-two markers of reproductive function (listed in Table 1) were assessed following standardised protocols[13-17]: reproductive hormones, conventional semen characteristics, markers of sperm chromatin integrity and apoptosis, markers of epididymal and accessory sex gland function, and the proportion of Y:X chromosome-bearing sperm. Analytical details are provided in the Supplemental Methods and Table S1.

Statistical analysis

We used sPLS regression modelling to assess associations between the exposure profiles and each outcome. In PLS regression, an X-matrix of exposures and Y outcome vector are simultaneously decomposed into latent variables and regressed in a way that maximises the covariance between X and Y.[18,19] These latent variables, called components, are orthogonal, linear combinations of the full set of input variables. Chun and Keleş[10] proposed sPLS, embedding variable selection into the PLS algorithm by eliminating 'uninformative' X-variables

Table 1. Summary statistics for biomarkers of reproductive function and potential confounders among 602 male partners of pregnant women.

Outcomes and covariates	n[a]	Greenland (n=199) GM (5, 95 P)	Warsaw, Poland (n=197) GM (5, 95 P)	Kharkiv, Ukraine (n=206) GM (5, 95 P)	*p*-value[b]
Male reproductive hormones in serum[c,d,e]					
Follicle-stimulating hormone (IU/L)	456	4.38 (1.70, 9.20)	3.46 (1.40, 7.40)	3.67 (1.60, 9.40)	0.001
Luteinizing hormone (IU/L)	456	4.05 (1.80, 8.50)	3.81 (1.90, 7.10)	3.79 (1.90, 7.60)	0.34
Inhibin B (ng/L)[g]	456	184.21 (83.00, 314.00)	157.94 (71.00, 275.00)	194.74 (98.00, 321.00)	<0.001
Sex hormone-binding globulin (nmol/L)	455	26.90 (15.60, 46.70)	21.61 (9.45, 40.90)	26.19 (15.50, 43.20)	<0.001
Total testosterone (nmol/L)[g]	456	14.81 (7.27, 24.41)	13.08 (6.83, 20.76)	18.01 (10.26, 26.89)	<0.001
Free testosterone (nmol/L)[g]	455	0.31 (0.18, 0.48)	0.29 (0.16, 0.42)	0.39 (0.22, 0.58)	<0.001
Estradiol (pmol/L)	454	63.44 (40.50, 98.70)	71.50 (45.80, 112.60)	80.73 (52.50, 137.50)	<0.001
Conventional semen characteristics					
Semen volume (mL)[f,h]	535	3.21 (1.20, 7.20)	3.37 (1.25, 7.19)	3.29 (1.59, 7.57)	0.68
Sperm concentration (10^6/mL)[f]	600	51.12 (10.75, 199.00)	55.95 (7.15, 257.50)	53.07 (10.15, 185.80)	0.65
Total sperm count (10^6/ejaculate)[f,h]	533	160.15 (31.50, 692.30)	186.68 (18.82, 1061.96)	179.46 (34.20, 804.80)	0.39
Morphologically normal (%)[g]	598	6.97 (2.00, 13.00)	6.66 (2.00, 14.00)	7.37 (1.00, 15.00)	0.20
Progressive motility (% A + B)[f,g,i]	565	55.29 (22.00, 81.00)	60.98 (19.00, 87.00)	55.08 (14.50, 87.00)	0.007
Sperm chromatin integrity[d,f]					
SCSA DNA fragmentation index (%)	546	7.66 (3.32, 20.28)	10.07 (4.16, 31.50)	10.83 (3.54, 31.56)	<0.001
High DNA stainability (%)	546	11.37 (4.37, 29.88)	8.66 (3.60, 25.14)	10.29 (4.35, 26.55)	<0.001
TUNEL DNA fragmentation index (%)	462	2.95 (0.79, 13.37)	11.64 (2.57, 38.28)	6.46 (1.90, 30.18)	<0.001
Apoptotic markers[d,f]					
Fas positivity (%)[g]	454	25.00 (2.22, 70.60)	48.57 (3.13, 96.42)	28.24 (0.00, 90.60)	<0.001
Bcl-xL positivity (%)[g]	286	26.54 (0.44, 90.65)	18.05 (0.81, 69.64)	66.01 (9.54, 99.17)	<0.001
Epididymal and accessory sex gland function[d,f,h]					
Neutral α-glucosidase (mU/ejaculate)	448	16.32 (4.80, 52.00)	21.81 (6.55, 71.52)	17.51 (4.86, 52.56)	0.001

Table 1. *Continued.*

Outcomes and covariates	n[a]	Greenland (n=199) GM (5, 95 P)	Warsaw, Poland (n=197) GM (5, 95 P)	Kharkiv, Ukraine (n=206) GM (5, 95 P)	*p*-value[b]
Prostate-specific antigen (µg/ejaculate)	507	3187.74 (1078.00, 8330.85)	3708.59 (1312.37, 11127.89)	2497.94 (784.08, 7707.70)	<0.001
Zinc (µmol/ejaculate)	497	4.68 (1.06, 12.63)	6.31 (1.50, 25.23)	4.22 (1.11, 16.56)	<0.001
Fructose (µmol/ejaculate)	506	47.00 (12.67, 153.85)	42.24 (6.10, 161.65)	39.36 (10.69, 117.35)	0.18
Y chromosome sperm cells (%)[g]	411	51.26 (48.52, 55.49)	50.30 (48.79, 52.11)	50.76 (48.72, 53.61)	<0.001
Age (years)[g]	597	31.34 (20.83, 43.15)	30.34 (25.36, 36.80)	27.88 (20.67, 38.75)	<0.001
BMI (kg/m^2)[g]	592	25.99 (20.56, 31.77)	25.81 (20.43, 31.93)	24.20 (19.71, 29.41)	<0.001
Abstinence period (days)	557	2.86 (0.50, 7.10)	4.27 (1.00, 30.00)	3.41 (1.50, 7.00)	<0.001
Current smoker, *n* (%)	597	144 (72.7%)	53 (27.2%)	136 (66.7%)	<0.001
Current smokers: cotinine (ng/mL)	589	113.73 (0.96, 465.23)	23.95 (<LOD, 358.42)	33.59 (<LOD, 447.36)	<0.001
Time of blood sampling, <12:00 hr, *n* (%)	504	17 (16.8%)	187 (94.9%)	183 (88.8%)	<0.001

GM, geometric mean; P, percentile.
[a] Number available out of 602.
[b] Test for difference in levels between the three study populations: ANOVA for means and chi-square test for proportions.
In adjusted analyses, models were adjusted for study population and cotinine levels, and additionally for [c] time of blood sampling; [d] age; [e] BMI; and [f] ln-abstinence period.
[g] Outcome was not ln-transformed; arithmetic mean presented.
[h] Samples with spillage were excluded (n=67 for semen volume and count; n=41–52 for the 4 epididymal and accessory sex gland function markers).
[i] Samples with a delay of >1 hr from collection to semen analysis were excluded (n=28).

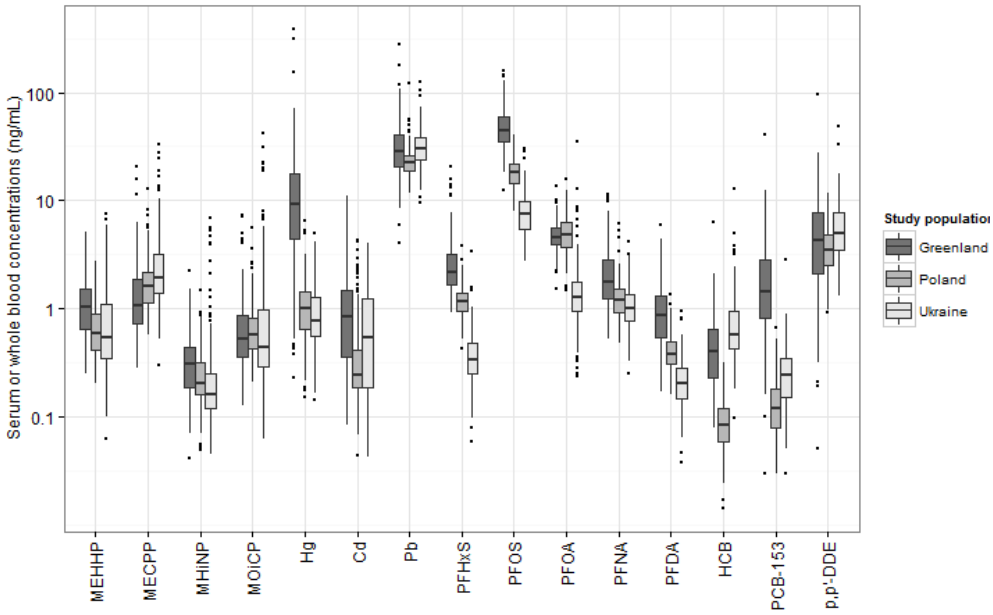

Figure 1. Box plots of the distributions of exposure biomarker concentrations per study population. MEHHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MECPP, mono(2-ethyl-5-carboxypentyl) phthalate; MHiNP, mono(4-methyl-7-hydroxyoctyl) phthalate; MOiCP, mono(4-methyl-7-carboxyheptyl) phthalate; Hg, mercury; PFHxS, perfluorohexane sulfonic acid; PFOS, perfluorooctane sulfonic acid; PFOA, perfluorooctanoic acid; PFNA, perfluorononanoic acid; PFDA, perfluorodecanoic acid; HCB, hexachlorobenzene; PCB, polychlorinated biphenyl; DDE, dichlorodiphenyldichloroethylene.

via penalisation. A penalty term (L_1) is applied during dimension reduction, and regression coefficients are obtained via PLS for the reduced set of X-variables (see Supplemental Methods). Model complexity is a function of the number of components used to construct the model (K) and the degree of sparsity (η). To determine the optimal model (avoid overfitting), we performed Monte Carlo cross-validation,[20] and added a null (intercept only) model in the cross-validation loop to assess significance. We tested models with K values 1–5 and η values between 0.01 and 0.99 (more sparse) in steps of 0.01, using a fivefold cross-validation, repeated (with different random partitions of the data) 500 times. We selected the most parsimonious model within one standard error of the overall minimum mean squared error of prediction (MSEP).[19]

We took several data pretreatment and exploration steps before undertaking sPLS analyses. We imputed exposure data below the limit of detection (LOD) (0–18%) and values missing-at-random in the matrix of exposures (2–16%). Values were imputed from a log-normal probability distribution via single conditional imputation, dependent on the population, and detected values for the other exposures. In the primary analyses, HCB, PCB-153, and *p,p′*-DDE were lipid adjusted (see Supplemental Methods), and four contaminants for which

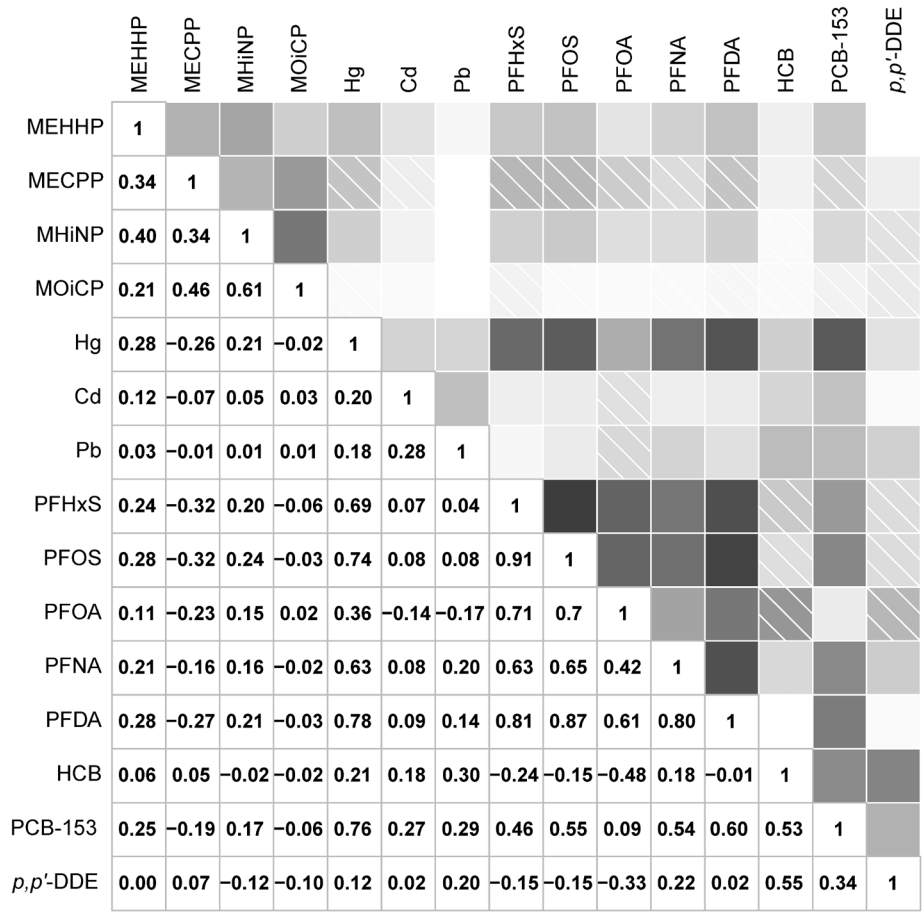

Figure 2. Pairwise Pearson correlation coefficients, also represented as a heat map, of the exposure biomarkers. MEHHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MECPP, mono(2-ethyl-5-carboxypentyl) phthalate; MHiNP, mono(4-methyl-7-hydroxyoctyl) phthalate; MOiCP, mono(4-methyl-7-carboxyheptyl) phthalate; Hg, mercury; PFHxS, perfluorhexane sulfonic acid; PFOS, perfluorooctane sulfonic acid; PFOA, perfluorooctanoic acid; PFNA, perfluorononanoic acid; PFDA, perfluorodecanoic acid; HCB, hexachlorobenzene; PCB, polychlorinated biphenyl; DDE, dichlorodiphenyldichloroethylene.

<70%[21] of the samples had concentrations above the LOD were excluded: MEOHP, MOiNP, PFUnDA and PFDoDA. To approximate normality, we used natural logarithm (ln)-transformed values for all exposure variables and for 14 of the 22 outcome variables.

To address potential confounding, we employed a two-stage regression approach[22]: first, each outcome and each exposure were separately regressed on potential confounders, and second, sPLS regression models were fit inputting the residuals. We *a priori* selected the set of potential confounders,[5] including study population and serum cotinine level, and variably age, body mass index (BMI), abstinence period and time of blood sampling, depending on the outcome (as specified in Table 1). We imputed missing covariate data (0.8–16%; as

Table 2. Exposure–outcome associations and regression coefficients selected from multipollutant sPLS regression modelling and corresponding single-pollutant OLS regression coefficients.

		sPLS		OLS		
Outcome	**Exposure**[a]	***K*, *η***[b]	β_{sPLS}	β_{OLS}	**95% CI**	***p*-value**
LH[a] (IU/L)	*p,p'*-DDE (ng/g)	1, 0.99	0.083	0.083[c,d]	(0.031, 0.135)	0.002[e]
Inhibin B (ng/L)	Hg (ng/mL)	1, 0.99	10.79	10.82	(3.90, 17.73)	0.002[e]
Total testosterone (nmol/L)	MHiNP (ng/mL)	1, 0.90	-1.14	-1.15[c]	(-1.74, -0.57)	0.0001[e]
Free testosterone (nmol/L)	MHiNP (ng/mL)	1, 0.64	-0.0113	-0.019[c]	(-0.032, -0.007)	0.002[e]
	MOiCP (ng/mL)		-0.0091	-0.010[c]	(-0.020, 0.000)	0.043
	Cd (ng/mL)		0.0091	0.012	(0.001, 0.023)	0.029
Semen volume[a] (mL)	MEHHP (ng/mL)	1, 0.99	-0.106	-0.106	(-0.167, -0.045)	0.0008[e]
Progressive sperm (%)	PCB-153 (ng/g)	1, 0.99	-3.37	-3.37[d]	(-5.48, -1.25)	0.002[e]
TUNEL DFI[a] (%)	MHiNP (ng/mL)	1, 0.99	-0.218	-0.218[c]	(-0.332, -0.104)	0.0002[e]
NAG[a] (mU/ejaculate)	MEHHP (ng/mL)	1, 0.77	-0.163	-0.164	(-0.255, -0.073)	0.0005[e]

Regression coefficients (β) are expressed per ln-unit change in exposure. Refer to Supplemental Table S3 for percent changes and coefficients from models only adjusted for study population. sPLS and OLS models were adjusted for study population and for additional potential confounders as specified in Table 1; sPLS models by inputting exposures and outcomes 'pre-standardised' for covariates (residuals).

[a] Variable ln-transformed in statistical analyses.

[b] sPLS tuning parameters: *K*, the number of components used, η, the degree of sparsity.

[c] Interaction *p*-value <0.10 for the cross-product term between exposure and study population (see Supplemental Figure S3 for population-stratified regression plots).

[d] Sensitivity analysis: β_{OLS} (95% CI) for models with organochlorines unadjusted for lipids (ng/mL), and with total lipids (g/L) included as an additional covariate: LH and *p,p'*-DDE, 0.070 (0.017, 0.123); progressive sperm and PCB-153: -3.592 (-5.871, -1.313).

[e] Significant after adjustment for multiple comparisons in single-pollutant OLS regression (FDR <10%).

elaborated in the Supplemental Methods). To assess the potential for overadjustment, in a secondary analysis we evaluated sPLS models with exposure and outcome variables only prestandardised (centred) for study population. This was considered the minimal adjustment, necessary due to the large differences in central tendencies between the populations for the exposures (Figure 1), and to a lesser degree but still conspicuous, for the outcomes. Mean-centring also removed baseline offsets, while scaling by log-transformation reduced the variance differences between X-variables, and thus the relative importance during dimension reduction.[23]

As an additional screening approach, we tested 330 single-pollutant, covariate-adjusted OLS linear regression models for associations between the 15 exposures (with >70% detection frequency) and 22 outcomes. To adjust for multiple comparisons, we computed the false discovery rate (FDR),[24] and set the significance threshold at an FDR <10%. In sensitivity analyses, we tested the four contaminant exposures with <70% detection frequency dichotomised as detect versus non-detect variables, and tested models excluding participants with imputed confounder and (missing-at-random) exposure data. We also assessed models with lipid-unadjusted organochlorines, including total lipids as a covariate. Finally, we tested for heterogeneity in effect estimates between study populations with an interaction term, and by assessing plots of the population-stratified regressions.

sPLS and OLS regression coefficients are presented per ln-unit increase in exposure, and for interpretability, converted into the per cent change in outcome per IQR increase in exposure; for ln-transformed outcomes, the proportional change, and for untransformed outcomes, the absolute change relative to the mean outcome level. Statistical analyses were performed using R version 3.0.1 (R Foundation for Statistical Computing, Vienna, Austria), and specifically the *spls* package for sPLS modelling.[25]

Results

Descriptive characteristics of the study populations are shown in Table 1. Participants from Kharkiv were slightly younger than their counterparts from Greenland and Warsaw, and fewer participants from Warsaw smoked. In exploring the structure of the exposure data, we observed large variations within and between the three study populations (Figure 1). Levels differed across study populations for all contaminant exposures (analysis of variance p <0.05, Supplemental Table S2), except for MOiCP. Levels were higher in samples from Greenland compared to Warsaw and Kharkiv for many exposures, especially for Hg and PCB-153. Correlations within the X-matrix ranged from r_p = −0.48 to 0.91 (Figure 2). We also observed distributional differences for many outcomes (Table 1), although distributions for the three populations were generally more similar for the outcomes than for the exposures. Of the 22 sPLS regression models tested in the primary analysis (n = 286–600 complete cases), the X-matrix significantly improved the prediction error beyond the null model for eight outcomes: luteinising hormone (LH), inhibin B, total testosterone, free testosterone, semen volume,

progressive sperm motility, terminal deoxynucleotidyl transferase dUTP nick end-labelling assay (TUNEL) DNA fragmentation index (DFI), and neutral α-glucosidase (NAG). Within each of these significant sPLS regression models, one to three exposures within the X-matrix contributed to a total of 10 significant exposure–outcome associations (Table 2). The optimal models were all K=1 component models and quite sparse (η between 0.64 and 0.99).

In the secondary analysis with exposure and outcome variables only prestandardised for study population but not for other potential confounders, sex hormone-binding globulin (SHBG) was additionally selected (the inclusion of the X-matrix improved the MSEP beyond the null model), and the LH model was no longer selected (see Supplemental Table S3). Extra exposures were also selected, for a total of 24 associations.

In the covariate-adjusted OLS regression analyses, eight overlapping exposure–outcome associations, comprising the same eight outcomes but fewer exposures (8 of 10) compared to the primary sPLS analyses, were identified as significant (FDR <10%). Two associations, between MHiNP and total testosterone and TUNEL DFI, were significant at an FDR <5% significance threshold (see Supplemental Figure S2). An additional association between MEHHP and TUNEL DFI, not selected in the sPLS model, was significant in OLS analyses (see Supplemental Table S3). Consistent with the sPLS modelling results, a greater number of associations was significant in the secondary analysis with OLS models only adjusted for study population compared to the primary analysis with further adjusted models (15 compared to 9; 14 of which overlapped with those selected in sPLS secondary analysis only prestandardised for study population). No additional associations were detected in the sensitivity analysis testing contaminants with <70% detection frequency inputted as binary detect versus non-detect variables. In a reanalysis using only the complete (non-imputed) exposure and covariate data, two associations—*p,p′*-DDE and LH, and MHiNP and total testosterone—were selected in both the sPLS and OLS modelling (with n = 321–480 and 328–586 complete cases, respectively, except for Bcl-xL positivity models which had 192–252 complete cases). This analysis had reduced power, and while CIs were wider, coefficients for the complete case analysis were remarkably similar to the primary analysis with imputed data (data not shown). Coefficients for lipid unadjusted organochlorines, adjusted for total lipids, were only slightly different in magnitude (see footnotes of Table 2).

For five of the 10 sPLS-selected associations, there was a significant interaction between exposure and study population in the OLS sensitivity analysis (Table 2). It was apparent that eight associations were consistent in slope direction across study populations in regression models stratified by population (see Supplemental Figure S3). Poland exhibited a slope in the opposite direction for LH and *p,p′*-DDE, and for the oxidative phthalates and TUNEL DFI. Associations with consistent slope directions, and their β_{sPLS} corresponding overall per cent change per IQR increase in exposure, included a 11% increase in inhibin B with Hg; a 6% decline in total testosterone with MHiNP; a 3% and 2% decline in free testosterone with DiNP metabolites, and a 5% increase with Cd; a 10% decline in semen volume with MEHHP;

a 11% decline in the fraction of progressively motile sperm with PCB-153; and a 16% decline in NAG with MEHHP.

Discussion

Using a multipollutant modelling approach, we identified 10 associations between biomarkers of environmental contaminant exposure and biomarkers of male reproductive function from several hundred associations tested in (sub)fertile men from Greenland, Warsaw, and Kharkiv. This is the first time several of the relationships have been assessed in an epidemiological study, or simultaneously.

One of the most statistically robust and consistent associations was between DiNP metabolites and decreasing free (bioactive) testosterone and total testosterone. DEHP metabolites did not exceed the significance threshold in confounder-adjusted analyses but exhibited inverse relationships. DiNP exposure is outpacing DEHP exposure in Europe, where DiNP use is less restricted than that of DEHP. These inverse associations are consistent with an antiandrogenic effect, which has more often been observed compared to pro-androgenic effects in experimental studies.[26] Phthalates are generally considered to act by inhibiting Leydig cell synthesis of testosterone. While most experimental studies have focused on *in utero* exposure, DEHP and its primary metabolite MEHP were recently shown to suppress steroidogenesis of adult human testis explants in vitro, without affecting production of the hormone insulin-like factor 3 (INSL3).[27] Several epidemiological studies have found inverse associations between (urinary) MEHP and testosterone in men; in 74 occupationally exposed workers and 63 controls in China,[28] and in a pooled analysis of 425 men of infertile couples and 425 men recruited through a US infertility clinic.[29] No association was found in 234 young Swedish men sampled during routine medical conscript examinations.[30] Joensen et al.[31] considered another exposure metric, the proportion of the summed urinary metabolites excreted as the primary metabolite, as a marker of DEHP and DiNP metabolism; %MEHP and %MiNP were associated (significant at $p < 0.05$ or near-significant) with total testosterone and free androgen index in 881 healthy young men from Denmark around 19 years of age. However, no significant associations were reported for the sum of primary and secondary metabolites. Primary monoester phthalate metabolites were not measured in the current study—only secondary oxidative metabolites—due to the lipase activity of serum and risk of contamination from the diesters from the sampling devices.

We found a clear dose-dependent decrease in serum testosterone levels with increasing levels of MHiNP. We recently discovered, however, that the measurement of MHiNP was possibly confounded by an unknown co-eluting agent. Nevertheless, in an additional analysis using the molar sum of the other two DiNP metabolites versus all three oxidative metabolites tested in relation to total testosterone, the magnitude of the regression coefficients was similar: β_{OLS} −0.713 (p-value=0.0037) for $\Sigma DiNP_{MOiNP+MOiCP}$versus −0.929 (0.0007) for ΣDiNPom. Moreover, that other phthalate metabolites were significantly inversely associated with testos-

terone lends weight to the evidence that one or more phthalates is indeed related to decreasing testosterone levels.

We observed a positive association between cadmium and free testosterone in the sPLS analyses, although this association was not significant in the OLS analyses adjusted for potential confounders, notably cotinine. Disentangling the effect of smoking and cadmium is difficult as smoking is an important source of cadmium exposure. A cadmium–testosterone association has previously been reported in 219 men recruited from two US infertility clinics,[32] and in a representative sample of 1262 US men,[33] although this latter association did not sustain statistical significance after adjustment for smoking. An experimental study of chronic low-dose oral exposure in rats reported cadmium-related elevated testosterone[34]; however, the underlying mechanism remains unknown.

We observed decreasing NAG levels with increasing MEHHP. Sperm undergo maturation in the epididymis, and NAG is a marker of epididymal function. A smaller study (n = 234) of urinary phthalates and NAG in Swedish conscripts was null.[30] In a study of 40 rats administered di-*n*-butyl phthalate, epididymal weight and NAG activity decreased dose dependently, in line with our finding.[35]

We found decreasing sperm DNA damage with increasing levels of the secondary hydroxylated metabolite of DiNP, counter to the hypothesis of a deleterious effect of exposure. However, the exposure–outcome relationship was inconsistent in direction, exhibiting a positive slope in the Polish study population. We detected associations for DNA fragmentation assessed with TUNEL, but not the sperm chromatin structure assay (SCSA). TUNEL represents DNA strand breaks measured directly in the ejaculated spermatozoa, whereas SCSA is a measure of susceptibility to acid denaturation.[36] Sperm DNA damage might be mediated by testicular oxidative stress. In rats, DEHP decreased antioxidant enzymes and concomitantly increased reactive oxygen species.[37] In 379 US men seeking infertility treatment, urinary monoethyl phthalate and MEHP were positively associated with neutral Comet assay parameters.[38] MEHP was more strongly associated when adjusted for oxidative metabolites (MEHHP and MEOHP), and similar to our finding for MEHHP, the metabolites were inversely associated with DNA damage. Hauser et al.[38] suggested that an increased ratio of oxidative metabolites to MEHP could represent interindividual differences in rates of DEHP metabolism to 'less toxic' metabolites, or it may reflect inhibition of phase 1 enzymes involved in xenobiotic metabolism.

Some of the exposure–outcome associations tested in the current analysis have previously been tested in the same or overlapping study populations in analyses considering a single-pollutant or a single class of chemicals (refer to refs.[39-41]; previous investigations of PCB-153 and *p,p'*-DDE were reviewed by Bonde et al.[5]). In addition to identifying several novel associations, we confirmed, using a more conservative significance threshold and joint modelling approach, several previously reported associations, including an unfavourable inverse association between PCB-153 and the proportion of progressively motile sperm.[5,16]

Other smaller studies have reported comparable findings, for instance, for PCB-153 in 305 Swedish men,[42] and for PCB-138 but not PCB-153 and the odds of <50% progressive motility in 212 US men recruited at an infertility clinic (uncorrected for multiple comparisons).[43]

We also confirm a previously reported strong positive association between mercury and inhibin B.[40] As inhibin B, predominantly excreted by Sertoli cells, is correlated with sperm concentration and counts and serves as a marker of spermatogenesis,[44] this positive association is contrary to a deleterious effect of exposure. The authors[40] suggest that this association may be driven by a higher consumption of seafood—particularly relevant for Greenlandic Inuit—and the resultant concomitant exposure to mercury and ω-3 polyunsaturated fatty acids. There are indications that the latter may beneficially impact certain semen quality parameters.[45] This mercury–inhibin B association was not observed in 219 US men recruited from infertility clinics.[32]

We observed an association between MEHHP and a decline in semen volume, as previously reported for this study population.[39] A null finding for MEHP was reported for a study of 234 young Swedish men,[30] and a statistically significant increase in semen volume for the highest versus lowest %MiNP quartile in 881 Danish men was interpreted by the authors as a chance finding.[31]

We also observed a positive, yet inconsistent-across-populations, association between *p,p′*-DDE and increasing LH, as previously reported.[17] As LH regulates testosterone production by Leydig cells, this is in line with an antiandrogenic effect of *p,p′*-DDE. A null finding was reported for 341 men from a US infertility clinic with lower *p,p′*-DDE levels (geometric mean 236 ng/g lipids).[46]

We found indications of associations between phthalates, metals, and organochlorines and SHBG, which did not sustain significance on adjustment for confounders, specifically BMI. These non-significant perturbations in SHBG, together with the other associations with hormones, might be indicative of effects of exposure profiles on hypothalamo–pituitary–gonadal axis regulation. Similar to the potential (over)adjustment for cotinine, adjustment for BMI may introduce overadjustment bias rather than (only) prevent confounding bias, given that some exposures may causally influence BMI.

A limitation of this assessment is the cross-sectional nature of this study, although, besides phthalates, all measured contaminants are relatively stable over time and as such were most likely reasonable proxies of recent past exposure as related to the measured outcomes. However, while blood and semen samples were collected on the same day for nearly all of the participants from Poland and Ukraine, for around 60% of the participants from Greenland, samples were collected more than 3 days apart (elaborated in the Supplemental Methods), contributing to misclassification of exposure, especially for the phthalates. Analyses excluding these participants yielded generally consistent results.

Recent advances in molecular epidemiology (high-throughput screening techniques) and the introduction of the exposome concept[47] imply that epidemiological research-

ers will be confronted more and more with the challenging task of analysing data with high numbers of measured analytes with often complex correlation structures. Single-pollutant modelling may result in a high potential for false positives (type I errors), although multiple testing corrections and the recently proposed environment-wide association study[48] design addresses this. Using multipollutant OLS regression modelling may lead to inflated variances and flipped signs of parameter estimates, thus making it difficult to disentangle the independent effects of these variables. Methods which project data onto a lower dimensional space, like PLS regression, can better cope with collinear variables and, as a corollary, reduce the number of models tested.[10,18] While PLS-based methods are well established in chemometrics and bioinformatics, they are only recently gaining traction in genomics, metabolomics, and epidemiology.[49]

In the current analysis, the associations selected using sPLS rather closely matched those we would have selected using single-pollutant OLS models. However, if we had screened for associations using multipollutant OLS models, accounting for correlations between exposures, these estimates would have suffered from multicollinearity and been unreliable (variance inflation factors exceeded 3 for 8 to 9 of the 15 exposure terms across multipollutant OLS models).

As sPLS captures the complementary contribution of X-variables, it has the power to detect multiple pollutants which might not be detected in single-pollutant modelling. This can be explained by additivity, and/or consistency at large, in that noise from measurement error and biological variation can be averaged out when a larger number of X-variables inform the latent variable. This was evident for the free testosterone model, and for several models in the sensitivity analysis with inputs only prestandardised for study population, in which up to eight exposures were selected. Further, in constructing latent variables, sPLS regression delineates groupings in the X-matrix of exposures, which is a step forward in tackling the long-standing challenge of assessing mixture effects of contaminants.

Some aspects of (s)PLS modelling implementation are underdeveloped, such as quantifying uncertainty (stability of the selection), and explicitly correcting for multiple testing, beyond the implicit sparseness step. We estimated an empirical null distribution using a Monte Carlo approach to establish a significance threshold. A caveat of sPLS is that penalisation methods optimise prediction and will tend to select one of two highly correlated exposures with nearly equal coefficient sizes.[19] Competing multipollutant modelling approaches exist[22] (e.g., tree-based models, elastic net penalised regression); however, simulation and validation assessments, specifically for data structures relevant for environmental epidemiology (often low dimensional), are lacking.

There is no straightforward way to adjust for potential confounders in (s)PLS analyses. Adjusting for confounders in sPLS models by inputting residuals in a two-step approach should, as with OLS modelling,[50] yield unbiased regression coefficients, although with a slight loss of efficiency under certain (sample size, correlation) scenarios. We observed large con-

trasts in exposures across the three study populations, and some differences in outcomes such as TUNEL DFI. To reduce the risk of ecological bias due to potential between-population confounding, in all analyses we prestandardised (mean-centred) the exposure and outcome data by population (sPLS models) or adjusted by population (OLS models). This may have artificially reduced true contrast in exposure and outcome data between study populations, and negatively affected our power to detect some associations.

While (s)PLS modelling has some power to detect non-linear relationships (weighted over components), the PLS and OLS regression coefficients reflect linear relationships. In an exploratory analysis with generalised additive modelling, this assumption of linearity was met for most exposure–outcome relationships (<2% deviated from linearity, with an estimated degree of freedom >1.0). Further research is needed to compare the efficacy of sPLS regression with other multipollutant modelling and variable selection approaches, including non-parametric techniques.

Conclusions

We used a systematic, multipollutant modelling approach to perform a global assessment of biomarkers of contaminant exposure and male reproduction function in one of the largest studies to date. We identified several environmentally perturbed outcomes, including a robust inverse association between DiNP phthalates and circulating testosterone, and between PCB-153 and sperm motility.

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Supplemental Material

Supplemental Methods

Study populations

The present study is based on a previously established cohort, INUENDO,[1] and includes 602 men from Greenland, Warsaw (Poland), and Kharkiv (Ukraine) who filled in a questionnaire on lifestyle factors, provided a semen sample, and provided a blood sample. For the baseline study, 598 (90% participation rate), 472 (68%), and 640 (26%) expectant couples (pregnant women and their male partners) were enrolled from the eligible target populations in Greenland, Warsaw, and Kharkiv, respectively. To be eligible, it was required that both partners were ≥18 years of age and born in the country of study. The baseline study also included Swedish fisherman, whose partners were not necessarily pregnant; this sub-cohort was excluded from the present analysis to achieve a more uniform study population. The age distribution and number of children did not differ between participants, non-respondents and those who declined participation from Greenland and Kharkiv. A non-response analysis was not possible for the Polish subcohort as no data were available for those who did not explicitly accept or decline participation.[1] Of the eligible men, 201 (79% participation rate) from Greenland, 198 (29%) from Warsaw, and 208 (33%) from Kharkiv provided a semen sample.

Collection of blood samples

Blood samples were drawn from a cubital vein into 10 mL EDTA-containing vacuum tubes for collection without additives (Becton Dickinson, Meylan, France). The blood sample was collected on the same day as the semen sample for >97% of Polish and Ukrainian men, and within 3 days for the remaining men. For Greenlanders, 41% of samples were collected within 3 days, and for the rest, within a year (median 18 weeks, IQR 23–44). We did not collect samples in trace metal-free tubes, and therefore cannot exclude that there may have been some contamination in the analysis of metals. After cooling to room temperature the tubes were centrifuged at 4000 g for 15 min. Serum was transferred with ethanol rinsed Pasteur pipettes to ethanol rinsed brown glass bottles (Termometerfabriken, Gothenburgh, Sweden). A piece of aluminum foil was placed on top of the bottles which were then sealed. Samples were stored at −20°C until shipment, but it was accepted to keep it in refrigerator for up to four days (as originally described in Jönsson et al.[2]). Samples were transported on dry ice to the Department of Occupational and Environmental Medicine, Lund University, Sweden, where all chemical analyses were performed. Samples were stored at −80°C until later analysis.

Exposure assessment

PCB-153 and *p,p´*-DDE were analysed as previously described.[2] Additional analytes (phthalates, metals, perfluoroalkyl acids, and hexachlorobenzene) were more recently analysed. Perfluoroalkyl acids were analysed[3] along with phthalates using a triple quadrupole linear

ion trap mass spectrometer equipped with a TurboIonSpray source (QTRAP 5500; AB Sciex, Foster City, CA, USA), coupled to a liquid chromatography system (UFLCXR, Shimadzu Corporation, Kyoto, Japan; LC/MS/MS). Aliquots of 100 µL serum were added with ^{2}H- ^{13}C- or ^{18}O-labelled internal standards for all evaluated compounds. The samples were digested with glucoronidase and the proteins were precipitated with acetonitrile.

Only oxidised metabolites were analysed. Serum has lipase activity, and if the monoesters should be analysed it is necessary to deactivate the lipases with e.g., phospheric acid immediately at sampling collection to avoid contamination from phthalate diesters in the environment.[4,5] While oxy-functional group metabolites were detected in only 40–50% of samples in our study, all phthalates metabolites were measured with relatively high precision; coefficients of variation between 7% and 19% were achieved.

For all analytes, the limits of detection (LOD) were determined as the concentrations corresponding to three times the standard deviation of the responses in chemical blanks.

Lipid assessment and adjustment

HCB, PCB-153 and *p,p´*-DDE were lipid adjusted, with the total lipid concentration in serum (g/L) calculated as total = 0.96 + 1.28 × (triglycerides + cholesterol).[6] Serum concentrations of triglycerides and cholesterol were determined by enzymatic methods using reagents from Roche Diagnostics (Mannheim, Germany). The inter-assay coefficients of variation for cholesterol and triglyceride determinations were 1.5–2.0%. The average molecular weights of triglycerides were assumed to be 807. For cholesterol we used an average molecular weight of 571, assuming that the proportion of free and esterified cholesterol in plasma was 1:2.[2]

Outcome assessment

Reproductive hormones were measured in male serum samples at Malmö University Hospital, Sweden, as previously described in detail.[7] Measurements of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol were made using a competitive binding immunoenzymatic assay (UniCel DxI 800 Beckman Access Immunoassay system, Chaska, MN, USA). Serum total testosterone levels were measured by means of a competitive immunoassay (Access; Beckman Coulter Inc., Fullerton, CA, USA). Sex hormone-binding globulin (SHBG) was measured using a fluoroimmunoassay (Immulite 2000; Diagnostic Products Corporation, Los Angeles, CA, USA). Inhibin B levels were assessed using a specific immunometric method.[8] Free testosterone—the estimated bioactive fraction, unbound to SHBG or albumin—was calculated based on the measured total testosterone and SHBG levels.[9]

Conventional semen characteristics were assessed by centrally trained technicians as previously described.[10,11] Briefly, sperm concentration, motility and morphology were assessed according to WHO 1999 guidelines.[12] Sperm concentration was determined in duplicate using an Improved Neubauer Hemacytometer (Paul Marienfeld, Bad Mergentheim, Ger-

many). Sperm motility was determined by counting the proportion of a) rapid progressive spermatozoa; b) slow progressive spermatozoa; c) non-progressive motile spermatozoa; and d) immotile spermatozoa among 100 spermatozoa within each of two fresh drops of semen, and progressively motile sperm were classified as a) plus b). For motility, samples with a delay of >1 hr from collection to analysis were excluded (n = 28). Sperm morphology was assessed for at least 200 sperms in each sample by two technicians. Abnormalities were classified as head defects, midpiece defects, tail defects, cytoplasma drop and immature spermatozoa. For ejaculate volume and sperm counts, data was excluded if there had been spillage of the sample (n = 67).

Sperm chromatin integrity. We evaluated two indices of DNA fragmentation index (DFI), as assessed by the sperm chromatin structure assay (SCSA) and the terminal deoxinucleotidyl transferase-driven dUTP Nick End Labelling (TUNEL) assay, as previously described.[13,14] SCSA is the more frequently used, standardised method which detects sperms with abnormal chromatin packaging as characterised by susceptibility to acid-induced DNA denaturation *in situ*.[15] The TUNEL assay detects single- and double-strand DNA breaks, specifically free 3'-OH termini, present in spermatozoa. High DNA stainability (HDS), capturing incomplete chromatin condensation and considered a marker of immature sperm, was also determined via SCSA.[15]

Apoptotic markers. Apoptosis plays a crucial role in spermatogenesis. Proapoptotic (Fas) and anti-apoptotic (Bcl-xL) proteins present on ejaculated sperm were detected by means of indirect immunofluorescence.[14] Regarding the analysis of markers of sperm chromatin integrity and apoptotic markers, there were a high number of missing values due to a lost sample shipment from Ukraine and due insufficient number of sperm cells for some samples.[13,14] A minimum of 10,000 sperm cells were measured by flow cytometry (Epics XL flow cytometer, Beckman Coulter-IL, Fullerton, Ca, USA). There were no significant differences in age and seminal parameters between participants with assessed versus missing data.[14]

Epididymal and accessory sex gland function. Motility of sperm is dependent maturation in the epididymis and interaction between prostatic and seminal vesicle secretions following ejaculation. Markers were assessed as previously described in detail[16]: neutral α-glucosidase (NAG) as a marker of epididymal function; prostate specific-antigen (PSA) and zinc as markers of prostatic function, and fructose as a marker of seminal vesicle function. The semen samples were first used to assess conventional semen characteristics, sperm chromatin integrity, apoptotic markers and the proportion of Y chromosome sperm cells.[10,13,14,17] The epididymal and accessory sex gland function markers were subsequently assessed in semen samples with sufficient volume and in samples with no reported spillage (n = 41–52 excluded). Samples were first analysed for PSA, zinc, and fructose, and the remaining amounts of

seminal plasma were used to analyze NAG.[16]

Y chromosome sperm cells. The proportion of Y:X chromosome-bearing sperm was assessed in around 500 sperms per sample (range 253-743) using two-colour fluorescence *in situ* hybridisation analysis (FISH), as previously described in detail.[17] Some samples were excluded from analysis because of low number of cells available or hybridisation failure.

Statistical analysis

Partial least squares regression

To describe the univariate (single outcome) partial least squares (PLS or PLS1) regression model, for n observations, let $\boldsymbol{X}$ denote a matrix of mean-centered p predictors or exposures ($n \times p$), and $\boldsymbol{y}$ a vector of mean-centered continuous outcome data ($n \times q$ with q=1). Matrix or vector transposition is indicated by superscript ⊤, and the inverse of a matrix by superscript -1.

In both ordinary least squares (OLS) and PLS, $\boldsymbol{y}$ and $\boldsymbol{X}$ are related through a linear relationship $\boldsymbol{y} = \alpha + \boldsymbol{X\beta} + \in$ (with α=0 with centered inputs). For OLS, the least squares solution is $\widehat{\boldsymbol{\beta}}_{OLS} = (\boldsymbol{X}^\top \boldsymbol{X})^{-1} \boldsymbol{X}^\top \boldsymbol{y}$, and requires independent $\boldsymbol{X}$-variables (and $n > p$), whereas for PLS, the least squares solution is obtained via data compression into $\boldsymbol{K}$ latent components (latent variables; where $p \leq \boldsymbol{K}$, thus allowing for $n < p$), and PLS can accommodate multicollinear $\boldsymbol{X}$-variables. PLS decomposition is generalised (in matrix form) as[18,19]:

$$\boldsymbol{y} = \boldsymbol{T}\boldsymbol{q}^\top + \boldsymbol{f} \;\; = \boldsymbol{X}\boldsymbol{W}\boldsymbol{q}^\top + \boldsymbol{f} \;\; = \boldsymbol{X}\boldsymbol{\beta}_{\boldsymbol{PLS}} + \boldsymbol{f}$$

$$\boldsymbol{X} = \boldsymbol{T}\boldsymbol{P}^\top + \boldsymbol{E}$$

where $\boldsymbol{T}$ represents the matrix ($n \times \boldsymbol{K}$) of latent components or 'scores' of orthogonal, linear combinations of $\boldsymbol{X}$ for the $\boldsymbol{K}$ number of model components; $\boldsymbol{q}$ and $\boldsymbol{P}$ represent the vector of $\boldsymbol{y}$- and matrix of $\boldsymbol{X}$-loading coefficients or 'loadings'; and $\boldsymbol{f}$ and $\boldsymbol{E}$ the random errors. $\boldsymbol{W}$ is a matrix ($p \times \boldsymbol{K}$) of direction vectors or 'loading weights' ($\boldsymbol{W}_{\boldsymbol{K}}$). Latent components are derived via successive optimisations (depending on the PLS1 algorithm), such that $\widehat{\boldsymbol{T}} = \boldsymbol{X}\widehat{\boldsymbol{W}}_{\boldsymbol{K}}$ or $\widehat{\boldsymbol{T}} = \boldsymbol{X}\widehat{\boldsymbol{W}}(\widehat{\boldsymbol{P}}^\top\widehat{\boldsymbol{W}})^{-1}$. (s)PLS models were fitted with the SIMPLS algorithm, described in detail elsewhere.[18,20]

As such, latent components are constructed ordered by the amount of explained variance in $\boldsymbol{y}$, so that the first component has the largest covariance with the outcome, the second component, the second largest covariance, and so on. PLS regression coefficients are computed as $\widehat{\boldsymbol{\beta}}_{\boldsymbol{PLS}} = \widehat{\boldsymbol{W}}_{\boldsymbol{K}}\widehat{\boldsymbol{q}}^\top$. For a $\boldsymbol{K}$=1 component model, PLS coefficients and weights are proportional to the univariable OLS coefficients; this is not the case for a PLS model with $\boldsymbol{K}$>1, in which coefficients are weighted across components.

Sparse partial least squares regression

Sparseness, in this context, means that a solution is obtained with a subset of the initial

input variables. Noisy or uninformative variables are eliminated. To achieve sparsity, penalisation (also called shrinkage) is introduced, in which regression coefficients are shrunk (down-weighted) via a penalty function towards zero or set to zero, depending on the penalty.

In sparse partial least squares (sPLS) regression, penalisation is applied during the dimension reduction step. We applied the sPLS algorithm of Chun and Keleş,[21] as implemented in the R *spls* package.[22-24] In brief, a penalty (η) is applied to a surrogate of the direction vector ($\boldsymbol{w}$, which is close to the original direction vector, as elaborated in Chun and Keleş[21]). The sPLS sparsity penalty (η) approximates the L_1 penalty of LASSO[25]: $\min_{\beta} \| \boldsymbol{y} - \boldsymbol{X}\beta\|^2 + \lambda\|\boldsymbol{\beta}\|_1$ where $\|\boldsymbol{\beta}\|_1 = \sum_{j=1}^{p} |\boldsymbol{\beta}_j|$.
[N.B.: L_1 corresponds, in the Bayesian setting, to a Laplace or double-exponential prior distribution.[26]] The univariate sPLS penalisation can be simplified to[21,27]:

$$\widehat{\boldsymbol{w}} = \max\left(0, |\widetilde{w}| - \eta \max_{1\le i\le p}|\widetilde{\boldsymbol{w}}_i|\right) \cdot sign(\widetilde{\boldsymbol{w}})$$

where $\widetilde{\boldsymbol{w}} = \left(\widetilde{w}_1 \ldots . \widetilde{w}_p\right)^{\top}$ are the estimated PLS direction vectors with $\widetilde{\boldsymbol{w}}_1 = \boldsymbol{X}^{\top}\boldsymbol{y}/\| \boldsymbol{X}^{\top}\boldsymbol{y} \|_2$ and $0 \le \eta \le 1$ (sparsity increases as η approaches 1, and if $\eta=0$ then the model is equivalent to PLS). A fraction of each direction vector is retained. Thus, sPLS is a two-stage procedure; once sparsity has been applied on the direction vectors (and implicitly, a subset of $\boldsymbol{X}$-variables selected), coefficients are derived from ordinary PLS regression.

Imputation: exposure data

For the exposure data, we imputed values < LOD (0–18%) and, for sPLS-regression analyses only, values missing-at-random (12–16% for metals, 4% for PCB-153 and *p,p'*-DDE, and 2% for other compounds). Data was considered missing-at-random because some serum (n = 13) and whole blood (n = 71) samples were untraceable or depleted in the time since the baseline study. Further, regarding measurement of metals in whole blood, for some samples (n = 26) there was insufficient volume to measure all three metals and a choice was made to analyze Hg and not Cd and Pb.

We used a maximum likelihood method to impute values < LOD based on the distribution estimated from detected values and conditional on the structure of the $\boldsymbol{X}$-matrix, and under the assumption that measurements follow a parametric (log-normal) underlying distribution. Specifically, we performed iterative imputation in which the mean of the imputation distribution for each missing exposure value was dependent on the study population (Greenland/Warsaw/Kharkiv) and levels of the other exposures, while the (residual) standard deviation was allowed to vary by study population. Each value < LOD was substituted with one imputed value (single imputation), which yields approximately unbiased estimates when measurements < LOD are less than 30%.[28]

Imputation: covariate data

We applied a minimal set of *a priori* selected confounders (specified in the main text, Table

1 and Table S3). As a substantial portion of data was missing for abstinence period (n = 45) and time of blood sampling (n = 98; all Greenlandic), we imputed missing data for these two covariates for the primary analyses. We performed single, fill-in imputation: for abstinence period, we assumed missing data followed the same distribution as the available data did; for time of blood sampling, we assumed the same proportion of Greenlandic participants were sampled prior to 12:00 hr as for the available data for Greenland (~ 20%), and randomly imputed a dichotomous (morning yes/no) value resulting in this proportion. In addition, missing values for age (n = 5) and body mass index (BMI) (n = 7) were replaced with the population-specific median values. Missing values for cotinine (n = 13) were replaced with the respective median cotinine value for smokers and non-smokers, based on self-reported smoking status; and values <LOD (0.7 ng/mL, 35%) were imputed based on a log-normal distribution, as described above.

Table S1. Analytical reproducibility of exposure and outcome biomarkers, and variability across study populations.

	Reproducibility		Inter population-variation: ICC[c]
	Coefficient of variation[a] (%)	Concentration[b] (ng/mL)	
Exposure			
Phthalate metabolites			
MEHHP	8	2.4	0.86
MEOHP	9	3.0	0.98
MECPP	18	1.3	0.83
MHiNP	8	2.2	0.91
MOiNP	7	2.0	0.85
MOiCP	19	3.5	1.00
Metals			
Hg	6	2.0	0.30
Cd	4	24	0.84
Pb	6	1.0	0.89
Perfluoroalkyl acids[3]			
PFHxS	8	1.5	0.19
PFOA	6	3.9	0.30
PFOS	5	26	0.15
PFNA	9	1.6	0.72
PFDA	9	0.6	0.33
PFUnDA	10	0.7	0.24
PFDoDA	22	0.08	0.32
Organochlorines[2]			
HCB	37	0.1	0.24
PCB-153	10	0.5	0.26
p,p'-DDE	8	3	0.80
Outcome			
Reproductive hormones in serum[7]			
FSH (IU/L)	3.5 4.1[d]	5.5 IU/L 23.6 IU/L	0.96
LH (IU/L)	5.2 2.3	4.0 IU/L 19.3 IU/L	1.00
Inhibin B (ng/L)	< 7	–	0.93
SHBG (nmol/L)	3.7 6.7	29 nmol/L 85 nmol/L	0.92
Total testosterone (nmol/L)	2.8 302	2.9 nmol/L 8.1 nmol/L	0.78

Table S1. *Contintued.*

	Reproducibility		Inter population-variation: ICC[c]
	Coefficient of variation[a] (%)	Concentration[b] (ng/mL)	
Free testosterone (nmol/L)	N/A	–	0.77
Estradiol (pmol/L)	17.4 6.7	44 pmol/L 303 pmol/L	0.84
Conventional semen characteristics[10]			
Semen volume (mL)	N/A	–	1.00
Sperm concentration (million/mL)	8.1	–	1.00
Total sperm count (million/ejaculate)	N/A	–	1.00
Morphologically normal sperm (%)	N/A	–	1.00
Progressive sperm motility (%)	11	–	0.98
Sperm chromatin integrity[13]			
SCSA DFI (%)	6.0	–	0.92
High DNA stainability (%)	4.8	–	0.95
TUNEL DFI (%)	<5	–	0.60
Apoptotic markers[14]			
Fas positivity (%)	6	–	0.83
Bcl-xL positivity (%)	9	–	0.66
Epididymal and accessory sex gland function[16]			
NAG (mU/ejaculate)	N/A	–	0.93
PSA (µg/ejaculate)	N/A	–	0.93
Zinc (µmmol/ejaculate)	N/A	–	0.94
Fructose (µmmol/ejaculate)	N/A	–	0.99
Y:X chromosome sperm cells[17]			
Y chromosome (%)	2.3, 3.3[e]	–	0.93

Abbreviations: DFI, DNA fragmentation index; FSH, follicle-stimulating hormone; HCB, hexachlorobenzene; LH, luteinizing hormone; MECPP, mono-(2-ethyl-5-carboxypentyl) phthalate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxo-hexyl) phthalate; MOiCP, mono-(4-methyl-7-carboxyheptyl) phthalate; MHiNP, mono-(4-methyl-7-hydroxyloctyl) phthalate; MOiNP, mono-(4-methyl-7-oxooctyl) phthalate; N/A, not available or not applicable; NAG, neutral α-glucosidase; PCB-153, polychlorinated biphenyl 153; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOS, perfluorooctane sulfonic acid; PFOA, perfluorooctanoic acid; *p,p'*-DDE, 1,1-dichloro-2,2-bis(p-chlorophenyl)-ethylene; PSA, prostate-specific antigen; SCSA, sperm chromatin structure assay; SHBG, sex hormone-binding globulin; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end-labeling.

[a] The coefficient of variation for the exposures was calculated as the standard deviation/mean (|σ/µ|*100) of duplicate quality control samples worked-up and analysed on different days.[2,3]

[b] The concentration(s) in quality control samples from which the reproducibility was determined.

[c] The intraclass correlation coefficient (ICC) was calculated from the within-population and the between-population variances from a one-way ANOVA. The higher the ICC value, the more similar the biomarker distributions across study populations.

[d] For most reproductive hormones, a coefficient of variation was determined for two different quality control concentrations.

[e] Interobserver and intraobserver coefficients of variation, respectively.

Table S2. Blood levels[a] of measured contaminants in male partners of pregnant women.

Exposure	LOD (ng/mL)	% >LOD	n[b]	All 3 populations (n=602) GM (5, 95 P)	Greenland (n=199) GM (5, 95 P)	Warsaw, Poland (n=197) GM (5, 95 P)	Kharkiv, Ukraine (n=206) GM (5, 95 P)	*p*-value[c]
Phthalate metabolites[d] (ng/mL)								
MEHHP	0.2	98	580	0.73 (0.26, 2.45)	1.00 (0.44-2.55)	0.62 (0.26, 1.54)	0.63 (0.21, 3.26)	<0.001
MEOHP	0.2	49	287	—[e] (<LOD, 0.52)	— (<LOD, 0.55)	— (<LOD, 0.40)	— (<LOD, 0.63)	—
MECPP	0.1	100	589	1.61 (0.58, 5.63)	1.17 (0.45, 3.72)	1.62 (0.75, 4.56)	2.16 (0.71, 8.93)	<0.001
ΣDEHPom	—	—	—	2.74 (1.22, 7.87)	2.54 (1.12, 5.85)	2.55 (1.39, 6.25)	3.17 (1.17, 10.55)	<0.001
ΣDEHPom (nmol/mL)	—	—	—	0.009 (0.004, 0.026)	0.008 (0.004, 0.019)	0.008 (0.005, 0.021)	0.010 (0.004, 0.035)	<0.001
MHiNP	0.1	93	549	0.24 (<LOD, 0.83)	0.30 (0.12, 0.86)	0.23 (0.10, 0.58)	0.20 (<LOD, 1.04)	<0.001
MOiNP	0.03	39	231	— (<LOD, 0.13)	— (<LOD, 0.11)	— (<LOD, 0.08)	— (<LOD, 0.34)	—
MOiCP	0.1	99	586	0.60 (0.19, 3.43)	0.57 (0.21, 1.61)	0.61 (0.29, 1.60)	0.61 (0.16, 5.72)	0.62
ΣDiNPom	—	—	—	0.91 (0.36, 4.11)	0.96 (0.40, 2.33)	0.90 (0.48, 2.25)	0.88 (0.27, 7.51)	0.48
ΣDiNPom (nmol/mL)	—	—	—	0.003 (0.001, 0.013)	0.003 (0.001, 0.007)	0.003 (0.002, 0.007)	0.003 (0.001, 0.024)	0.48
Metals (ng/mL)								
Hg	0.1	100	531	2.10 (0.38, 33.02)	8.66 (0.85, 49.12)	1.01 (0.39, 2.60)	0.84 (0.31, 2.24)	<0.001
Cd	0.02	100	505	0.50 (0.12, 2.59)	0.72 (0.13, 2.95)	0.33 (0.13, 2.16)	0.53 (0.10, 2.74)	<0.001
Pb	0.08	100	505	27.60 (14.47, 66.06)	29.95 (14.12, 84.90)	22.93 (14.00, 38.63)	31.15 (16.34, 69.21)	<0.001
Perfluoroalkyl acids (ng/mL)								
PFHxS	0.06	100	588	0.97 (0.21, 3.71)	2.39 (1.18, 6.15)	1.16 (0.68, 2.02)	0.35 (0.16, 0.72)	<0.001
PFOA	0.6	97	573	3.05 (0.78, 8.30)	4.60 (2.76, 7.36)	4.86 (2.54, 9.27)	1.33 (0.44, 3.74)	<0.001
PFOS	0.2	100	589	18.11 (4.52, 73.20)	47.39 (25.66, 103.02)	17.69 (9.61, 29.14)	7.32 (3.65, 14.13)	<0.001
PFNA	0.2	100	589	1.31 (0.59, 3.54)	1.85 (0.74, 4.65)	1.20 (0.66, 2.20)	1.02 (0.53, 2.13)	<0.001
PFDA	0.2	82	481	0.41 (<LOD, 1.66)	0.88 (0.33, 2.24)	0.39 (0.21, 0.73)	0.20 (<LOD, 0.47)	<0.001
PFUnDA	0.3	39	232	— (<LOD, 2.92)	— (<LOD, 4.08)	— (<LOD, 0.35)	— (<LOD, 0.37)	—
PFDoDA	0.07	29	180	— (<LOD, 0.31)	— (<LOD, 0.43)	— (<LOD, 0.08)	— (<LOD, <LOD)	—
Organochlorines (ng/g lipid)								
HCB	0.05	93	539	46.53 (6.48, 294.96)	58.83 (17.77, 211.83)	12.25 (4.57, 31.71)	135.22 (55.36, 469.21)	<0.001
PCB-153	0.05	95	551	55.68 (8.52, 579.82)	223.20 (50.00, 1092.55)	16.80 (6.36, 37.68)	44.69 (15.18, 138.61)	<0.001

Table S2. *Continued.*

Exposure	LOD (ng/mL)	% >LOD	n[b]	All 3 populations (n=602) GM (5, 95 P)	Greenland (n=199) GM (5, 95 P)	Warsaw, Poland (n=197) GM (5, 95 P)	Kharkiv, Ukraine (n=206) GM (5, 95 P)	*p*-value[c]
p,p'-DDE	0.1	100	577	677.83 (196.18, 2223.44)	567.93 (108.66, 2188.36)	516.79 (224.18, 1093.09)	1051.35 (415.09, 2906.75)	<0.001

Cd, cadmium; GM, geometric mean; HCB, hexachlorobenzene; Hg, mercury; LOD, limit of detection; MECPP, mono-(2-ethyl-5-carboxypentyl) phthalate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxo-hexyl) phthalate; MOiCP, mono-(4-methyl-7-carboxyheptyl) phthalate; MHiNP, mono-(4-methyl-7-hydroxyloctyl) phthalate; MOiNP, mono-(4-methyl-7-oxooctyl) phthalate; P, percentile; Pb, lead; PCB-153, polychlorinated biphenyl 153; PFDoDA, perfluorododecanoic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOS, perfluorooctane sulfonic acid; PFOA, perfluorooctanoic acid; PFUnDA, perfluoroundecanoic acid; *p,p'*-DDE, 1,1-dichloro-2,2-bis(p-chlorophenyl)-ethylene.

[a] Values <LOD were imputed.

[b] Available for analysis (589 for phthalates, PFAAs and HCB; 531 for Hg; 505 for Cd and Pb; 578 for PCB-153 and p,p'-DDE) and measured value >LOD.

[c] Test for difference in levels between the three populations (ANOVA).

[d] The molar sums (nmol/mL) of the three oxidative DEHP and DiNP metabolites were calculated (ΣDEHPom and ΣDiNPom), and are also presented corrected for molecular weight, based on the weighted average molecular weight (ng/mL).

[e] GM and ANOVA p-value not calculated if >30% of data was below the LOD.

Table S3. Exposure–outcome associations identified from sPLS population-adjusted[a] and further adjusted[b] models: (1) sPLS and (2) OLS regression coefficients per ln-unit change in exposure, and corresponding percent changes in outcome per interquartile range increase in exposure.

			(1) Multi-pollutant sPLS models					(2) Single-pollutant OLS models		
			Pop.-centered inputs[a]		Pop.-centred and 'pre-standardised'[b]			Adj. pop.[a]	Further adjusted[b]	
Outcome (mean)	Exposure[c] (IQR)	n	K, η (Q^2 %)[d]	β_{sPLS}	K, η (R^2, Q^2 %)[d]	β_{sPLS}	% Δ / IQR[e]	β_{OLS}[d]	β_{OLS}[d] (95% CI)	% Δ /IQR[e] (95% CI)
LH[c] (IU/L)	*p,p'*-DDE (416.87–1143.00 ng/g)	456	—	—	1, 0.99 (2.13,1.27)	0.083	8.70	0.080[f]	0.083[f,g,h] (0.031, 0.135)	8.73 (3.18, 14.59)
Inhibin B (182.3 ng/L)	Hg (0.698–4.852 ng/mL)	456	1, 0.99 (0.95)	10.580	1, 0.99 (2.05,1.12)	10.788	11.48	10.588[f]	10.816[f] (3.899, 17.733)	11.51 (4.15, 18.86)
SHBG[c] (nmol/L)	MEHHP (0.440–1.237 ng/mL)	455	1, 0.53 (1.83)	-0.015	—	—	—	-0.046	-0.024[g] (-0.068, 0.020)	-2.45 (-6.79, 2.09)
	MHiNP (0.146–0.353ng/mL)			-0.014		—	—	-0.043	-0.033[g] (-0.076, 0.010)	-2.87 (-6.48, 0.88)
	MOiCP (0.354–0.868 ng/mL)			-0.017		—	—	-0.041	-0.035[g] (-0.069, -0.001)	-3.09 (-6.00, -0.09)
	Cd (0.205–1.114 ng/mL)			0.015		—	—	0.033	0.005[g] (-0.033, 0.043)	0.85 (-5.43, 7.55)
	Pb (20.55–35.41 ng/mL)			0.021		—	—	0.105	0.064[g] (-0.011, 0.139)	3.54 (-0.60, 7.86)
	HCB (17.31–107.02 ng/g)			0.026		—	—	0.083[f]	0.057[g,h] (0.012, 0.103)	10.94 (2.21, 20.64)
	PCB-153 (19.59–131.02 ng/g)			0.027		—	—	0.074[f]	0.045[g,h] (0.005, 0.085)	8.93 (0.95, 17.53)
	p,p'-DDE			0.021		—	—	0.062	0.035[g,h] (-0.007, 0.078)	3.59 (-0.70, 8.19)
Total testosterone (15.81 nmol/L)	MECPP (1.020–2.265 ng/mL)	456	1, 0.58 (2.80)	-0.329	1, 0.90 (3.13,1.05)	—	—	-0.811	-0.727[g] (-1.357, -0.097)	-3.67 (-6.85, -0.49)
	MHiNP			-0.535		-1.141	-6.36	-1.166[f]	-1.153[f,g] (-1.741, -0.565)	-6.43 (-9.70, -3.15)
	MOiCP			-0.526		—	—	-0.746	-0.684[g] (-1.162, -0.206)	-3.88 (-6.59, -1.17)
	Cd			0.653		—	—	0.759[f]	0.515 (-0.015, 1.045)	5.51 (-0.16, 11.19)
	ΣDiNPom (0.0018–0.0039 nmol/mL)			*NT*		*NT*	*NT*	-0.976[f]	-0.929[f] (-1.459, -0.399)	-4.70 (-7.39, -2.02)
Free testosterone (0.339 nmol/L)	MECPP	455	1, 0.61 (0.95)	-0.0055	1, 0.64 (2.87,0.13)	—	—	-0.013	-0.013[g] (-0.025, 0.000)	-3.06 (-5.88, 0.00)
	MHiNP			-0.0084		-0.0113	-2.93	-0.019[f]	-0.019[f,g] (-0.032, -0.007)	-4.93 (-8.31, -1.82)
	MOiCP			-0.0059		-0.0091	-2.41	-0.011	-0.010[g] (-0.020, 0.000)	-2.64 (-5.28, 0.00)
	Cd			0.0083		0.0091	4.52	0.014[f]	0.012 (0.001, 0.023)	5.98 (0.50, 11.47)

Table S3. *Continued.*

			(1) Multi-pollutant sPLS models					(2) Single-pollutant OLS models		
			Pop.-centered inputs[a]		Pop.-centred and 'pre-standardised'[b]			Adj. pop.[a]	Further adjusted[b]	
Outcome (mean)	Exposure[c] (IQR)	n	K, η (Q^2 %)[d]	β_{sPLS}	K, η (R^2, Q^2 %)[d]	β_{sPLS}	% Δ / IQR[e]	β_{OLS}[d]	β_{OLS}[d] (95% CI)	% Δ /IQR[e] (95% CI)
Semen volume[c] (mL)	MEHHP	535	1, 0.99 (0.82)	-0.110	1, 0.99 (2.12,1.21)	-0.106	-10.35	-0.110[f]	-0.106[f] (-0.167, -0.045)	-10.38 (-15.86, -4.55)
Progressive sperm (57%)	PCB-153	565	1, 0.99 (1.18)	-3.488	1, 0.99 (1.70,1.00)	-3.365	-11.22	-3.488[f]	-3.365[f,h] (-5.484, -1.246)	-11.22 (-18.29, -4.16)
TUNEL DFI[c] (%)	MEHHP	462	1, 0.63 (2.72)	-0.102	1, 0.99 (3.00,2.25)	—	—	-0.177[f]	-0.185[f,g] (-0.303, -0.068)	-17.41 (-26.90, -6.79)
	MHiNP			-0.133		-0.218	-17.48	-0.217[f]	-0.218[f,g] (-0.332, -0.104)	-17.47 (-25.36, -8.76)
	Cd			-0.161		—	—	-0.130[f]	-0.090 (-0.189, 0.008)	-14.13 (-27.37, 1.36)
NAG[c] (mU/ejaculate)	MEHHP	448	2, 0.99 (3.76)	-0.170	1, 0.77 (2.73,0.62)	-0.163	-15.52	-0.178[f]	-0.164[f] (-0.255, -0.073)	-15.60 (-23.18, -7.27)
	Cd			-0.118		—	—	-0.123[f]	-0.109[g] (-0.188, -0.030)	-16.85 (-27.25, -4.95)

Adj., adjusted; Pop., study population; *NT*, not tested; —, indicates association was not selected in sPLS model.

[a] The 'unadjusted' models were only adjusted for study population: exposure and outcome variables were mean-centered by study population prior to sPLS modelling, and study population was included as a covariate in OLS models.

[b] 'Adjusted' models included additional potential confounders. sPLS models were constructed with outcome and exposure variables 'pre-standardised' by confounders, inputting the residuals of linear regression models of each outcome versus confounders, and each exposure versus confounders. Confounders were included as covariates in OLS models. All models were adjusted for study population and cotinine, and additionally for age, BMI and time of blood sampling (morning, yes/no) for the reproductive hormones; for ln-abstinence period for all conventional semen characteristics except proportion normal sperm; and for age and ln-abstinence period for markers of sperm chromatin integrity, apoptotic markers, and markers of epididymal and accessory sex gland function.

[c] All exposures and some outcomes, as indicated, were ln-transformed in statistical analyses.

[d] *K* and η represent the tuning parameters for the sPLS model; *K*, the number of components used to construct the model, and η , the degree of sparsity (with η approaching 1 yielding a sparser model). R^2 is the explained variance of ***y*** by ***X***. It represents the partial variance explained by the exposure(s) only, as input ***X***-exposure and ***y***-outcome data were pre-standardised for covariates. Q^2 represents the cross-validated fraction of predicted ***y***-variation (or predictive ability of the model); $Q^2 = 1 - PRESS / SS$, where $\mathrm{PRESS} = \sum(\hat{y}_i - y_i)^2$ is the predictive residual error sum of squares, and $\mathrm{SS} = \sum(y_i - \bar{y}_i)^2$ is the sum of squares of ***y*** corrected for the mean.

[e] sPLS and OLS regression coefficients derived per ln-unit exposure were transformed to represent the percent change in outcome associated with the interquartile range in exposure (IQR; the 75th compared to the 25th percentile in ln-exposure). For ln-transformed outcomes, this is the proportional change: $\left(e^{\beta_{ij} * IQR_{\ln(x_i)}} - 1\right) * 100$.
For untransformed outcomes, this is the absolute change in the outcome relative to the arithmetic mean outcome level: $\left(e^{\beta_{ij} * IQR_{\ln(x_i)}} - 1\right) * 100$. Mean outcome values for the untransformed outcomes are presented. We used IQRs for the full population (n=602), and present untransformed values.

[f] Significant after adjustment for multiple comparisons (FDR <10%): 330 tests in the primary analysis; 374 tests in the additional analysis with ΣDEHPom and ΣDiNPom.

[g] Interaction *p*-value <0.10 for the cross-product term between exposure and study population (see supplemental figure S3 for population-stratified regression plots).

[h] Sensitivity analysis: adjusted β_{OLS} (95% CI) for models with organochlorines unadjusted for lipids (ng/mL), and with total lipids (g/L) included as an additional covariate: LH and *p,p'*-DDE, 0.070 (0.017, 0.123); SHBG and HCB, 0.036 (-0.010, 0.082); PCB-153, 0.043 (0.002, 0.083); *p,p'*-DDE, 0.018 (-0.024, 0.061); progressive sperm and PCB-153: -3.375 (-5.543, -1.207).

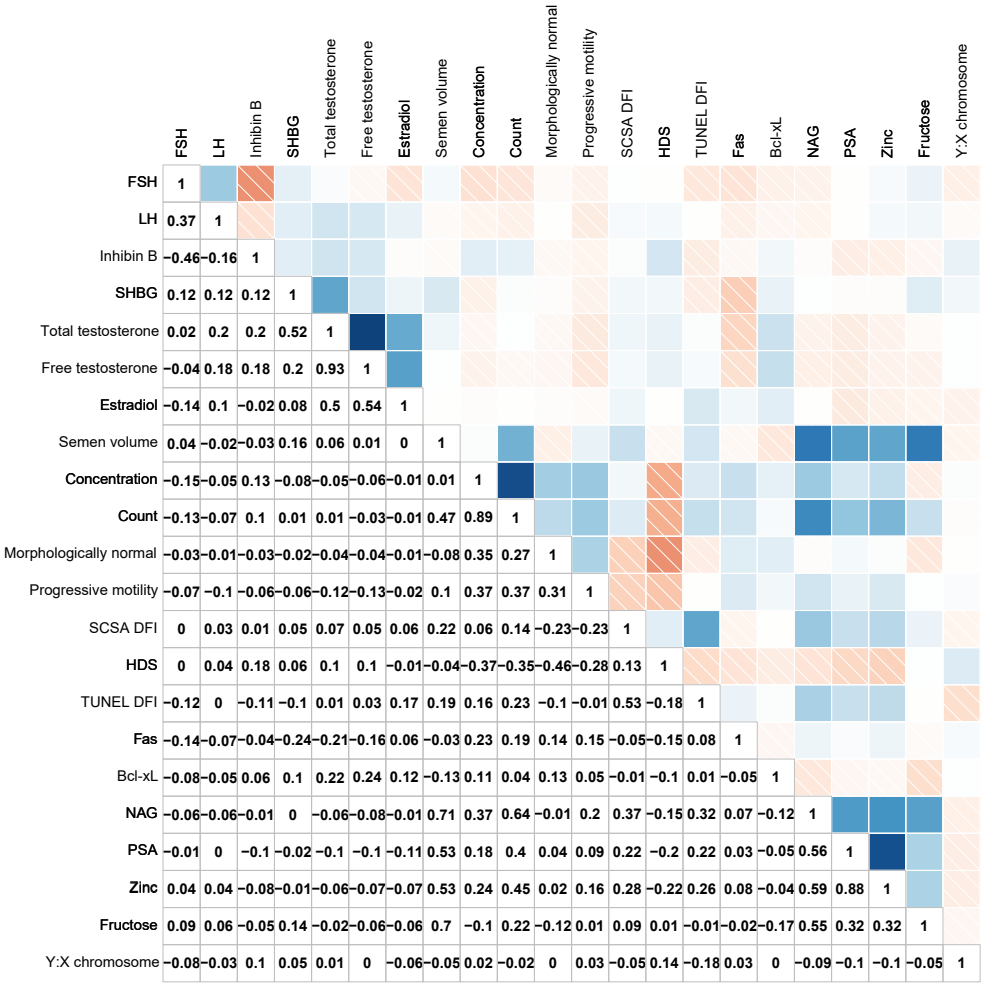

Figure S1. Pearson correlation coefficients, also represented as a heat map, between the biomarkers of reproductive function.

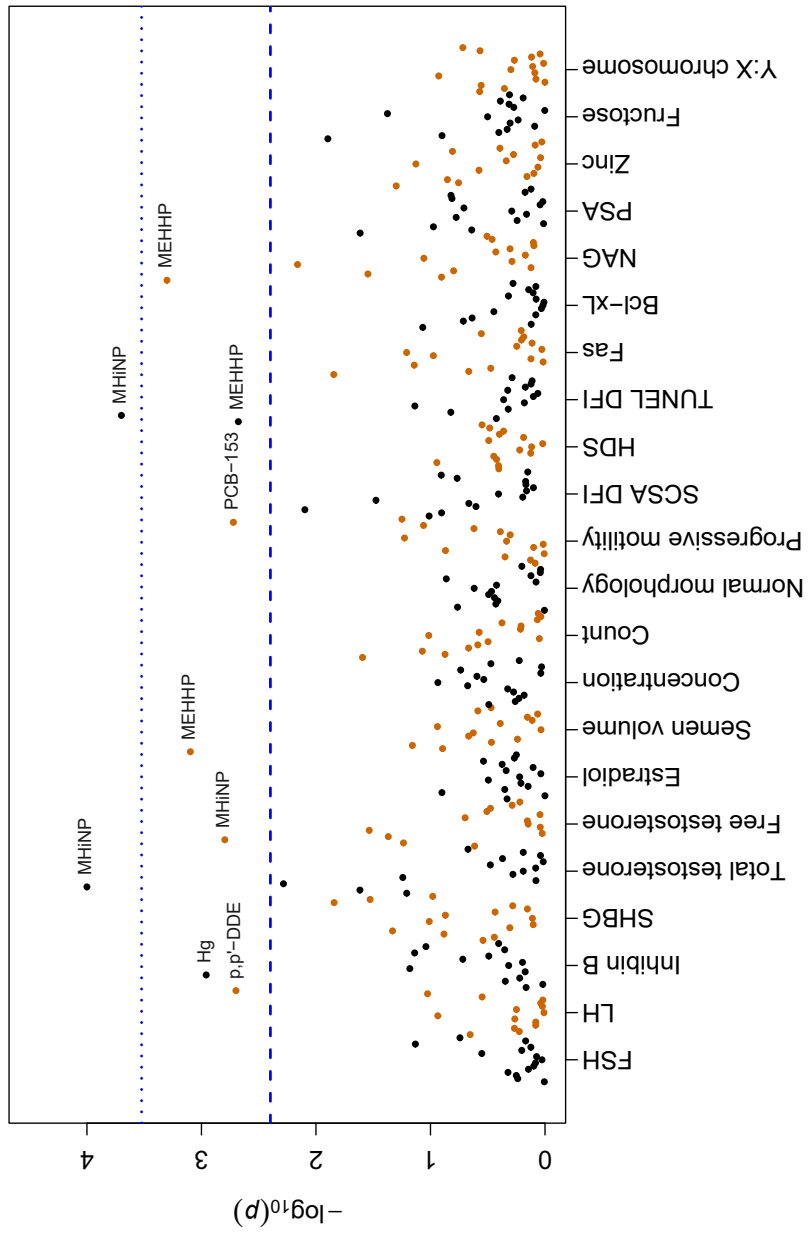

Figure S2. The *p*-values ($-\log_{10}$ scale) from single exposure–outcome OLS regression analyses, plotted per outcome. Analyses are adjusted for study population and cotinine, and variably adjusted for age, BMI, abstinence period and time of blood sampling as indicated in Tables 1 and S3. The dotted and dashed lines demarcate a false discovery rate of <5% and <10%, respectively. Each dot corresponds to the *p*-value from a single exposure–outcome association, and alternating black and orange colours delineate outcomes.

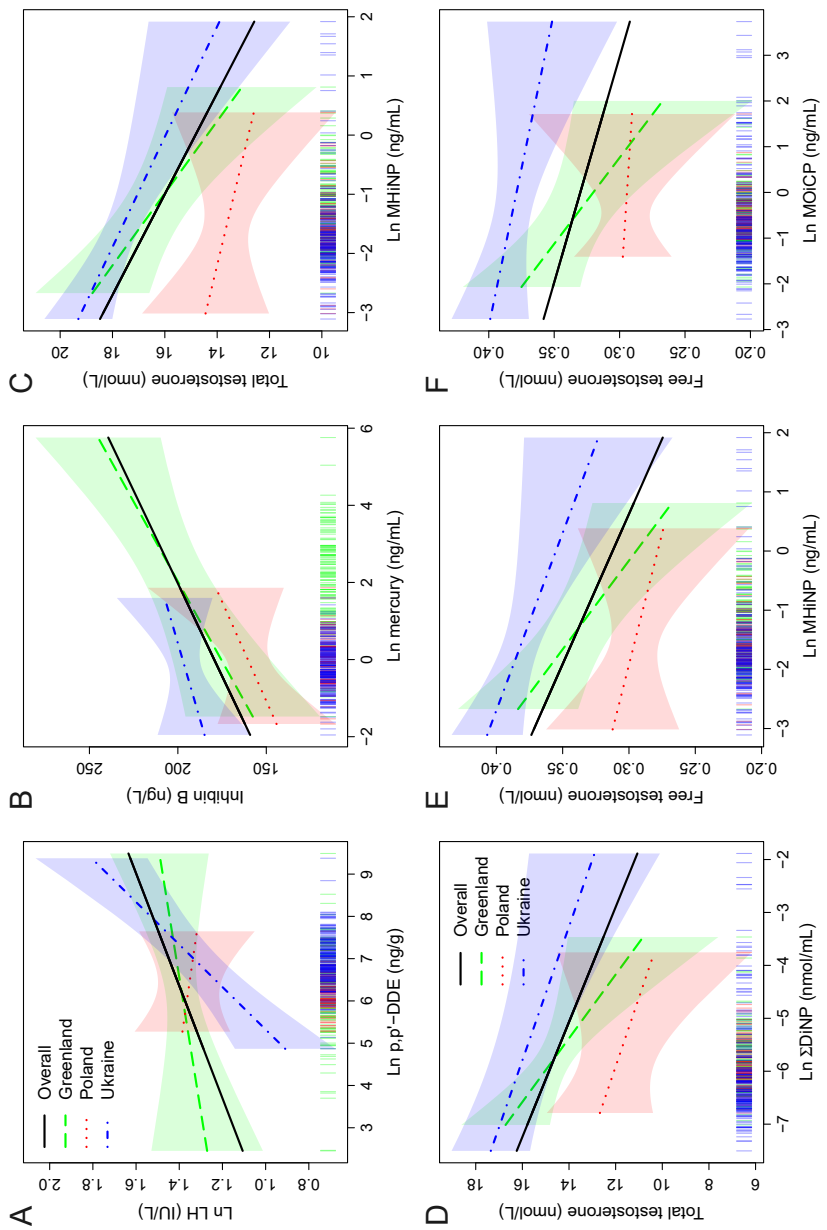

Figure S3. Exposure–outcome associations, plotted as linear regressions across all and stratified by study population **(A-L)**.
Models are adjusted for study population and cotinine, and variably adjusted for age, BMI, abstinence period, and time of blood sampling as indicated in the footnotes of Table S3. Predicted functions, with confounders set at the mean of continuous confounders and morning time of blood sampling are presented: population-specific exposure-outcome relationships (dashed lines) and 95% confidence intervals (shaded), and an overall exposure–outcome relationship for the pooled analysis, plotted at the Greenland-specific intercept (solid black line). Rug plots display the density of the exposure data.

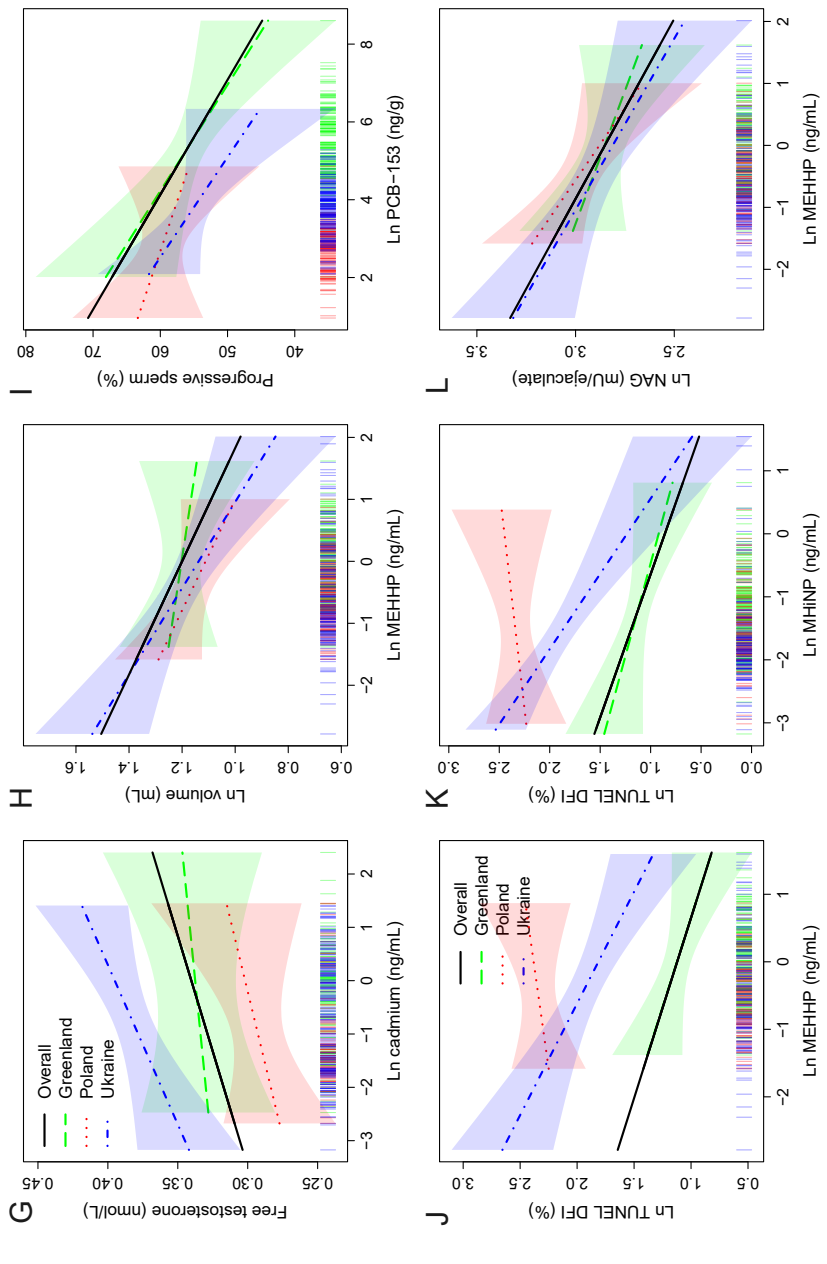

Figure S3. *Continued.*

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Chapter 4

Prenatal phthalate, perfluoroalkyl acid, and organochlorine exposures and term birth weight in three birth cohorts: multi-pollutant models based on elastic net regression

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Abstract

Background: Some legacy and emerging environmental contaminants are suspected risk factors for intrauterine growth restriction. However, the evidence is equivocal, in part due to difficulties in disentangling the effects of mixtures.

Objectives: We assessed associations between multiple correlated biomarkers of environmental exposure and birth weight.

Methods: We evaluated a cohort of 1250 term (≥ 37 weeks gestation) singleton infants, born to 513 mothers from Greenland, 180 from Poland, and 557 from Ukraine, who were recruited during antenatal care visits in 2002–2004. Secondary metabolites of diethylhexyl and diisononyl phthalates (DEHP, DiNP), eight perfluoroalkyl acids, and organochlorines (PCB-153 and *p,p′*-DDE) were quantifiable in 72–100% of maternal serum samples. We assessed associations between exposures and term birth weight, adjusting for co-exposures and covariates, including prepregnancy body mass index. To identify independent associations, we applied the elastic net penalty to linear regression models.

Results: Two phthalate metabolites (MEHHP, MOiNP), perfluorooctanoic acid (PFOA), and *p,p´*-DDE were most consistently predictive of term birth weight based on elastic net penalty regression. In an adjusted, unpenalised regression model of the four exposures, 2-SD increases in natural log–transformed MEHHP, PFOA, and *p,p´*-DDE were associated with lower birth weight: –87 g (95% CI: –137, –340 per 1.70 ng/mL), –43 g (95% CI: –108, 23 per 1.18 ng/mL), and –135 g (95% CI: –192, –78 per 1.82 ng/g lipid), respectively; and MOiNP was associated with higher birth weight (46 g; 95% CI: –5, 97 per 2.22 ng/mL).

Conclusions: This study suggests that several of the environmental contaminants, belonging to three chemical classes, may be independently associated with impaired fetal growth. These results warrant follow-up in other cohorts.

Introduction

Reduced birth weight is associated with increased short- and long-term morbidities and mortality.[1,2] This is in line with the developmental origins of health and disease hypothesis: that *in utero* and early-life stressors can impact certain chronic disease risks throughout the life course.[3] There is a growing although inconsistent body of evidence that some environmental exposures, including background levels of human-made chemicals, are risk factors for impaired fetal growth.[4,5]

Numerous studies have investigated the relationship between organochlorine compounds, including polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT), or its main metabolite dichlorodiphenyldichloroethylene (*p*,*p*´-DDE), and markers of fetal growth. A recent pooled analysis of 14 European study populations showed that prenatal exposure to PCB-153 was associated with a significant decrease in birth weight, and *p*,*p*´-DDE with a nonsignificant decrease.[6] A smaller though substantial number of studies have investigated perfluoroalkyl and polyfluoroalkyl substances [PFASs; also known as perfluorinated compounds (PFCs)] in relation to birth weight. Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) have been studied most extensively (e.g.,[7-9]; perfluorohexane sulfonate (PFHxS), perfluorononanoic acid (PFNA), and perfluoroundecanoic acid (PFUA) have also been assessed.[10,11] Associations have generally been null or in the direction of a negative effect on size metrics at birth, although inconsistent across PFASs. The few studies on phenols and phthalates have yielded inconsistent results (e.g.,[12,13]).

Measurements in maternal biospecimens, cord blood, and amniotic fluid indicate widespread fetal exposure to these environmental contaminants.[6,14-16] Diet is the primary source of adult exposure, via lipid-rich food or food packaging. Although PCBs and DDT have been banned or restricted, exposure will continue due to their environmental persistence. PFASs are surfactants with diverse industrial and commercial applications. Some are being phased out (e.g., PFOS, PFOA), but biomonitoring suggests they are being replaced by other PFASs.[15] Diethylhexyl phthalate (DEHP) and diisononyl phthalate (DiNP) are high-molecular-weight phthalates used as plasticisers in various products including polyvinyl chloride, which, despite their relatively rapid elimination, have high detection rates.

Previous studies have rarely evaluated more than one chemical class simultaneously. In the present study of mother–infant pairs from Greenland, Poland, and Ukraine, metabolites of two phthalates (DEHP, DiNP), eight PFASs, PCB-153, and *p*,*p*´-DDE were measured in serum of pregnant women. Our objective was to characterise associations between the multiple, correlated biomarkers of prenatal exposure and birth weight. We used a variable selection method, elastic net regression, in tandem with unpenalised regression models, to estimate the independent effects of exposures.

Methods

Study populations. Pregnant women were enrolled between June 2002 and May 2004 during

routine antenatal care visits at *a*) local hospitals or clinics in 19 municipalities and settlements throughout Greenland, *b*) three hospitals and eight antenatal clinics in Kharkiv, Ukraine, and *c*) a large central hospital in Warsaw, Poland. Eligible participants were at least 18 years old and born in the country of study. At enrolment, women participated in an interview on time to pregnancy and were invited to donate a nonfasting venous blood sample. The study was approved by the local ethics committees, and written informed consent was obtained from all participating women. Details on the INUENDO cohort study design and data collection have been published.[17-19] Briefly, 1710 couples were interviewed, with a participation rate of 90% in Greenland, 68% in Poland, and 26% in Ukraine; of those, a blood sample was collected and available for analysis for 96%, 55%, and 96% of women, respectively. Blood samples were collected on average later during pregnancy for participants in Poland than in Greenland and Ukraine (median of 33, 25, and 23 gestational weeks, respectively).

There were 1321 mother–singleton infant pairs with complete exposure and birth weight data. Twenty-three pairs (1.7%) with missing data on maternal age, body mass index (BMI), parity, gestational duration, or infant sex were further excluded.

Outcome and covariate ascertainment. Birth outcome data were extracted from hospital maternity records by medical personnel. Gestational age was assigned based on self-reported date of last menstrual period. The outcome of interest, birth weight, was analysed for only infants delivered at term (≥ 37 completed weeks of gestation, $n = 1250$).[20] We did not analyse preterm birth because of the limited power ($n = 48$).

Information on sociodemographic and lifestyle factors and reproductive history was ascertained during the baseline interview. Serum cotinine levels were used as an indicator of exposure to tobacco smoke during pregnancy. Prepregnancy BMI was calculated using self-reported weight and height.

Exposure assessment. PFASs and phthalates, along with cotinine and vitamin D, were simultaneously determined in 100-µL aliquots of serum by liquid chromatography–tandem mass spectrometry, following an optimised protocol based on Lindh et al.[21] and Specht et al.[22] Secondary oxidative metabolites of DEHP [mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP, alternatively 5OH-MEHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP, alternatively 5oxo-MEHP), mono(2-ethyl-5-carboxypentyl) phthalate (MECPP, alternatively 5cx-MEPP)] and DiNP [mono(4-methyl-7-hydroxyloctyl) phthalate (MHiNP, alternatively 7OH-MMeOP), mono(4-methyl-7-oxo-octyl) phthalate (MOiNP, alternatively 7oxo-MMeOP), and mono(4-methyl-7-carboxyheptyl) phthalate (MCiOP, alternatively 7cx-MMeHP)] were analysed. Analysed perfluoroalkyl acids (PFAAs), a subset of PFASs, included perfluoroheptanoic acid (PFHpA), PFHxS, PFOS, PFOA, PFNA, perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), and perfluorododecanoic acid (PFDoDA).

PCB-153 and *p,p´*-DDE were analysed by gas chromatography–mass spectrometry,

as previously described.[17] Lipids were determined by enzymatic methods, and lipid adjustment of organochlorines was based on total lipids, calculated as 1.13 + 1.31 × (triglycerides + cholesterol).[23] Limits of detection (LODs) ranged from 0.005 to 0.2 ng/mL, and the interassay coefficients of variation in quality control samples were between 6% and 21% (median, 13.5%) (see Supplemental Table S1).

Statistical analysis. In the case of multicollinearity, multiple linear regression models may yield unreliable parameter estimates. Therefore, to assess which exposures are associated with the outcome while simultaneously adjusting for other exposures, we used elastic net regression modelling (Equation 1): generalised linear models fit with a hybrid of the lasso and ridge penalty functions.[24,25] Ridge penalises the square of the regression coefficients for the predictors, shrinking coefficients from correlated predictors proportionally toward zero. Lasso imposes a penalty on the absolute value of the coefficients, shrinking coefficients by a constant factor, and can select a subset of predictors by shrinking coefficients for the least predictive predictors exactly to zero. Whereas ridge retains all predictors, and lasso tends to select only one predictor from a group of correlated predictors, elastic net can perform selection while enabling the inclusion of collinear predictors in the final model.

A set of elastic net coefficients (β_{EN}) are estimated by minimising the residual sum of squares, subject to a weighted sum of the lasso and ridge penalties:

$$\min_{\beta_0,\beta_j,\beta_k}\left[\frac{1}{2n}\sum_{i=1}^{n}\left(y_i-\beta_0-\sum_{j=1}^{e}\beta_j X_{ij}-\sum_{k=1}^{c}\beta_k Z_{ik}\right)^2+\lambda\sum_{j=1}^{e}\left(\frac{1-\alpha}{2}\beta_j^2+\alpha\left|\beta_j\right|\right)\right] \quad [1]$$

where $0 \leq \alpha \leq 1$ and $\lambda \geq 0$; and $\boldsymbol{y}_i$ is the $n \times 1$ vector of outcome values for $i = 1,\ldots,n$ participants, β_0 is the intercept, β_j and β_k are vectors of regression coefficients that correspond to the main effects of respectively $\boldsymbol{X}_{ij}$, the $n \times e$ matrix of $j = 1,\ldots,e$ standardised exposures, and $\boldsymbol{Z}_{ik}$, the $n \times c$ matrix of $k = 1,\ldots,c$ standardised potentially confounding covariates. To fully adjust for potential confounders, we selected confounders *a priori*, without subjecting them to variable selection, and included them in the elastic net regression models as unpenalised variables. Thus, α controls the balance between the lasso ($\alpha = 1$) and ridge ($\alpha = 0$) penalties, and λ represents the penalty (or regularisation) parameter, with the degree of shrinkage increasing as λ increases for a given α value.

We used cross-validation (CV) to determine the optimal degree of penalisation. We tested models over a grid of α and λ sequences, and selected the combination which yielded the minimum mean-squared error (MSE) of prediction from repeated 10-fold CV. CV was repeated 100 times, each time with different data partitions, to achieve more stable selection than a single CV. CV has been shown in simulations to have good sensitivity (true positive rate) of selection, yet can suffer from inflated false discovery rates.[26] Therefore, to complement CV and to aid in statistical inference of the results, we also estimated the more stringent, at a loss of power, covariance test *p*-values of Lockhart et al.[27]; this assesses the significance of each

exposure upon its inclusion in the model as the degree of penalisation decreases, conditional on other important predictors having been included in the model first.

For the subset of exposures selected via elastic net, and inferred to be important—giving consideration to selection consistency across adjustment models, the covariance test, and the magnitude of the coefficients—we refit multiple-exposure ordinary least squares (OLS) regression models to obtain unpenalised, mutually adjusted coefficient estimates.

Before modelling, exposure variables were natural log (ln)–transformed to reduce the influence of outliers. Data below the LOD (0–27%) were singly imputed from a log-normal probability distribution, dependent on the study population and observed values for other contaminants, and allowing residual variances to vary by population.[28] For regression models, we mean-centred all predictor variables and scaled continuous variables by two times their respective standard deviations (SD)[29] to impart variables with the same prior importance in penalised regressions, and to improve the comparability of coefficients for continuous and binary predictors.

We identified the minimal sufficient adjustment set using a directed acyclic graph (see Supplemental Figure S1): study population (an indicator variable for participant location), maternal age (27–31, 32–45, vs. 18–26 years; categories based on the lowest Akaike Information Criterion of birth weight on age), prepregnancy BMI (kilograms per meter squared), and parity (≥ 1 vs. 0). Gestational age at birth (weeks) was added in a separate model because it may represent an intermediate variable (or collider)—though it presumably has no direct effect on measured maternal serum levels, it affects the unmeasured cumulative exposure of the fetus, and may also be causally affected by exposures—and there is no consensus on how to adjust for this variable in the literature. In further adjusted models, additional potential confounders were added, possibly leading to over- or unnecessary adjustment: infant sex (female vs. male), maternal height (centimetres), alcohol consumption around the period of conception (≥ 7 vs. < 7 drinks/week), maternal serum cotinine (nanograms per millilitre), and maternal serum vitamin D (nanograms per millilitre). Because of the large proportion of missing data for maternal education, seafood consumption, and season of blood sampling, these variables were included only in a sensitivity analysis.

For comparison, we assessed associations between single exposures and term birth weight with OLS models, controlling the false discovery rate (FDR) at 5%.[30] In sensitivity analyses, we assessed the linearity of adjusted single-exposure–outcome relationships using generalised additive models (GAMs), smoothing the exposure term with a penalised regression spline. We also tested for effect measure modification by study population, infant sex, prepregnancy BMI (< 25, ≥ 25 kg/m^2), and smoking (< 5, ≥ 5 ng/mL cotinine[31]) in stratified analyses and by introducing product interaction terms between potential modifiers and selected exposures in OLS models. Recognizing that using lipid-standardised organochlorine levels may lead to overadjustment if lipids represent an intermediate, coefficients for wet weight PCB-153 and *p,p´*-DDE were additionally estimated, with total lipids included as a co-

variate.[32] Lipids were not included in the primary multiple-exposure models to avoid overadjustment of the phthalate and PFAS coefficients. Additional models were tested including the molar sum of both DEHP and DiNP metabolites instead of their individual metabolites. The default statistical significance level was set at $\alpha = 0.05$. We analysed data using R version 3.0.3,[33] and fit elastic net models using the *glmnet* package.[24]

Results

Participant characteristics varied somewhat across study populations (Table 1). Women from Poland were older than women from Greenland and Ukraine, and more women from Greenland were multiparous, overweight, or obese, and had higher cotinine levels, indicating exposure to tobacco smoke during pregnancy. The mean birth weight was 3651, 3530, and 3302 g for term newborns from Greenland, Poland, and Ukraine, respectively, and 19 infants had a low birth weight (< 2500 g).

All 16 exposure biomarkers were quantifiable in at least 72% of serum samples, and 11 were quantifiable in at least 98% (see Supplemental Table S1). Exposure distributions differed across study populations for nearly all contaminants (Figure 1; see also Supplemental Table S1). For nine contaminants, median concentrations were highest for women from Greenland. Pooled across countries, wet weight concentrations were highest for PFOS, followed by *p,p´*-DDE (median, 8.43 and 3.39 ng/mL). Spearman correlation coefficients between exposures ranged from –0.34 to 0.78 (Figure 2), and were generally similar across the three study populations (see Supplemental Table S2).

We observed several associations between birth weight, selected exposures, and characteristics of the total study population consistent with the literature (see Supplemental Table S3). For instance, maternal age exhibited an inverted U-shaped association and parity a positive association with term birth weight, whereas maternal age was positively associated and parity negatively associated (for some, nonsignificantly) with persistent contaminant concentrations.

In the primary variable selection analysis, with minimal sufficient adjustment, eight exposures were selected ($\beta \neq 0$; seven exposures with $\beta_{EN} > |1.0|$) in the multiple-exposure elastic net regression of term birth weight (Table 2). MEHHP, MOiNP, PFOA, and *p,p´*-DDE were consistently selected in elastic net modelling, and exhibited the largest magnitude beta coefficients, across the three adjusted models. In the covariance test, *p,p´*-DDE was consistently significant, and MEHHP was significant in two models (Table 2). Upon inputting the four exposures into multiple-exposure unpenalised models, β_{OLS} estimates for MEHHP and *p,p´*-DDE were negative and statistically significant across models, whereas estimates for PFOA were negative but significant only in the further adjusted model, and the estimates for MOiNP were positive but did not reach statistical significance (Table 3). These four exposures were not highly correlated ($r_s = –0.19$ to 0.14) (Figure 2). PFHxS, PFNA, and PFDoDA were also variably selected with nontrivial coefficients ($\beta_{EN} > |1.0|$) across adjusted models (Table

Table 1. Characteristics of the study populations (2002–2004, n=1250) [n (%) or mean ± SD].

Characteristic	Greenland (n=513)	Warsaw, Poland (n=180)	Kharkiv, Ukraine (n=557)
Maternal age at delivery (years)			
18–24	235 (45.8)	12 (6.7)	299 (53.7)
25–29	119 (23.2)	110 (61.1)	164 (29.4)
30–34	82 (16.0)	55 (27.8)	76 (13.6)
35–45	77 (15.0)	8 (4.4)	18 (3.2)
Prepregnancy BMI (kg/m^2)			
<18.5	14 (2.7)	11 (6.1)	79 (14.2)
18.5–24.9	306 (59.6)	154 (85.6)	410 (73.6)
25.0–29.9	136 (26.5)	13 (7.2)	56 (10.1)
≥30	57 (11.1)	2 (1.1)	12 (2.2)
Maternal height (cm)	162.0 ± 6.8	166.3 ± 5.2	165.28 ± 6.0
Parity			
0	161 (31.4)	153 (85.0)	440 (79.0)
1	153 (29.8)	24 (13.3)	95 (17.1)
2	104 (20.3)	2 (1.1)	12 (2.2)
≥3	95 (18.5)	1 (0.6)	10 (1.8)
Serum cotinine (ng/mL)			
<5.0	208 (40.5)	178 (98.9)	465 (83.5)
5.0–49.9	87 (17.0)	2 (1.1)	54 (9.7)
≥50.0	218 (42.5)	0 (0.0)	38 (6.8)
Maternal education			
No post-secondary	244 (50.9)	8 (4.4)	221 (39.7)
Some post-secondary	235 (49.1)	172 (95.6)	335 (60.3)
Missing	34	0	1
Alcohol intake (drinks/week)[a]			
<7	450 (87.7)	169 (93.9)	553 (99.3)
≥7	63 (12.3)	11 (6.1)	4 (0.7)
Fish or seafood (days/week)	1.87 ± 1.53	1.28 ± 1.06	1.08 ± 1.53
Missing	12	10	60
Serum vitamin D (ng/mL)	17.56 ± 9.51	28.34 ± 11.73	22.46 ± 10.73
Timing of blood sampling (gest. weeks)			
1–13	66 (13.3)	1 (0.6)	157 (28.9)
14–26	229 (46.1)	11 (6.2)	156 (28.7)
≥27	202 (40.6)	166 (93.3)	231 (42.5)
Missing	16	2	13
Season of blood sampling			
October–March	274 (53.5)	135 (75.8)	353 (64.5)
April–September	238 (46.5)	43 (24.2)	194 (35.5)
Missing	1	2	10
Infant sex			
Female	237 (46.2)	92 (51.1)	264 (47.4)
Male	276 (53.8)	88 (48.9)	293 (52.6)
Term birth weight (g)			
<2500	11 (2.1)	1 (0.6)	7 (1.3)
2500–2999	44 (8.6)	18 (10.0)	98 (17.6)
3000–3499	142 (27.7)	71 (39.4)	269 (48.3)
3500–3999	175 (34.1)	66 (36.7)	158 (28.4)
≥4000	141 (27.5)	24 (13.3)	25 (4.5)
Gestational age (weeks)	39.85 ± 1.34	39.53 ± 1.25	39.21 ± 1.00

[a] During the period around conception.

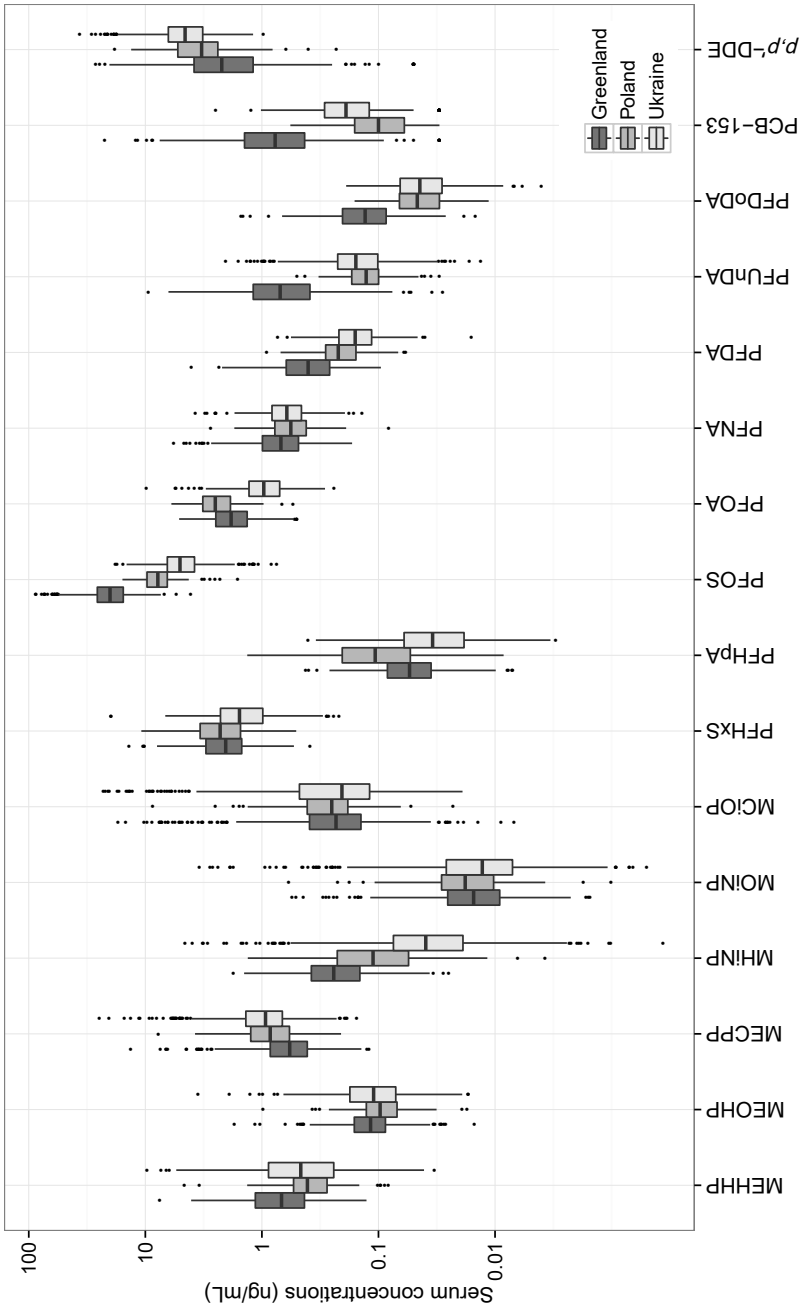

Figure 1. Box plots of distributions of exposure biomarker concentrations per study population. Horizontal lines correspond to medians, and boxes to the 25th–75th percentiles; whiskers extend to data within the interquartile range times 1.5, and data beyond this are plotted as dots. Wet weight concentrations are presented for PCB-153 and *p,p′*-DDE.

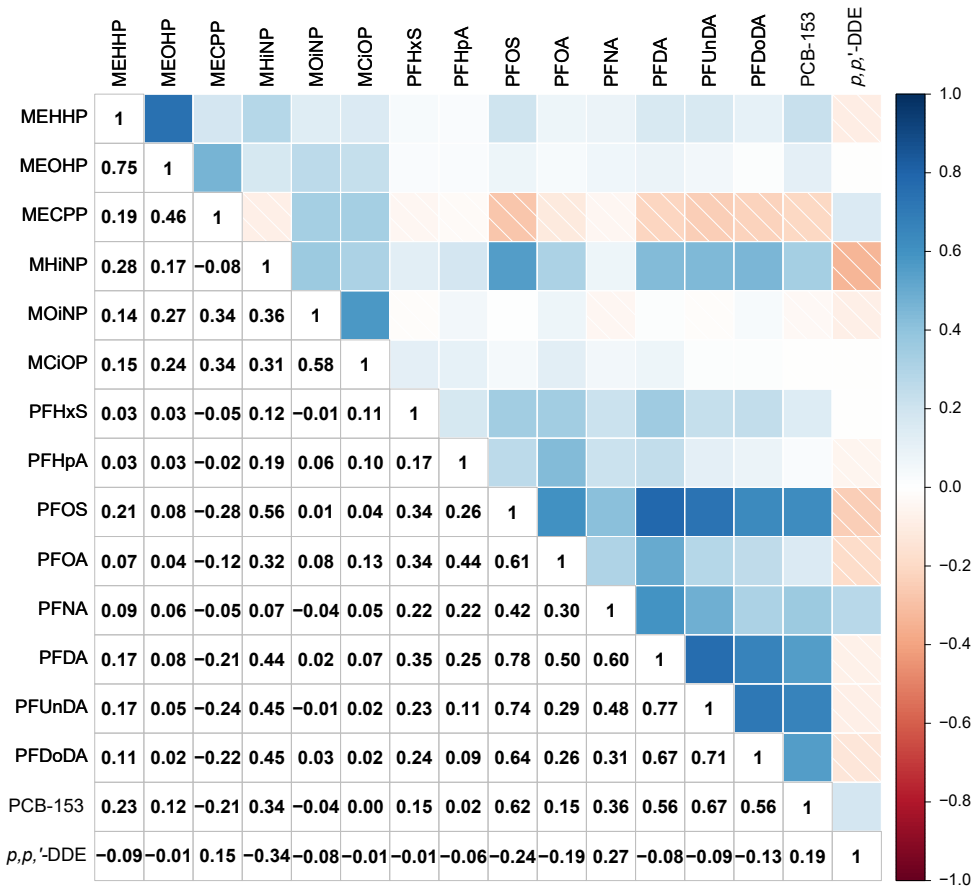

Figure 2. Spearman correlation coefficients between exposure biomarkers. The colour intensity of shaded boxes indicates the magnitude of the correlation. Blue indicates a positive correlation, and red with white diagonal lines indicates a negative correlation.

2). These exposures and the four primary-selected exposures showed some moderate correlations ($r_s \leq |0.34|$; variance inflation factors 1.04–2.17 for exposure terms), yielding potentially less reliable multiple-exposure β_{OLS} estimates for differences in term birth weight (grams) when added to the four-exposure model in turn: per 2-SD increase in ln-transformed PFHxS, −19.68 [95% confidence interval (CI): −74.20, 34.83]; PFNA, −17.34 (95% CI: −77.47, 42.78); PFDoDA, −56.23 (95% CI: −125.05, 12.59).

The magnitude of associations (β_{EN} and β_{OLS}) generally decreased slightly upon adjustment for gestational age, and more so upon adjustment for further covariates, with the notable exception of PFOA, which exhibited a more pronounced reduction in further adjusted models (Table 3). The estimated associations for contaminants (−135 to around 40 g per 2-SD increase in levels) were of a similar magnitude as some other predictors of term

Table 2. Multiple-exposure elastic net penalised regression models[a] (β_{EN}) for term birth weight.

Potential predictor (increment)	Adjusted	Plus gestational age	Further adjusted
Intercept	3532.69	3475.94	3594.68
ln–MEHHP (1.70 ng/mL)	–64.67	–59.43[b]	–48.61
ln–MEOHP (1.29 ng/mL)	–0.15	0	0
ln–MECPP (1.42 ng/mL)	0	0	0
ln–MHiNP (2.74 ng/ml)	0	0	0
ln–MOiNP (2.22 ng/mL)	23.81	22.26	16.31
ln–MCiOP (2.32 ng/mL)	0	0	0
ln–PFHxS (1.24 ng/mL)	–3.49	0	0
ln–PFHpA (1.84 ng/mL)	0	0	0
ln–PFOS (1.60 ng/mL)	0	0	0
ln–PFOA (1.18 ng/mL)	–11.51	–10.11	–38.82
ln–PFNA (1.03 ng/mL)	–7.05	–7.69	0
ln–PFDA (1.40 ng/mL)	0	0	0
ln–PFUnDA (2.10 ng/mL)	0	0	0
ln–PFDoDA (1.67 ng/mL)	–22.56	0	0
ln–PCB-153 (2.43 ng/g)	0	0	0
ln–*p,p´*-DDE (1.82 ng/g)	–106.39[b]	–76.63[b]	–47.02[b]
Population: Poland	–55.03	5.78	–90.89
Ukraine	–226.64[b]	–131.66[b]	–247.33[b]
Maternal age: 27–31	85.17	76.84	62.01
32–45	25.67	31.63	30.55
BMI (8.62 kg/m^2)	205.50[b]	180.03[b]	192.93[b]
Parity: multiparous	67.21[b]	84.01[b]	92.22
Gestational age (2.45 weeks)		346.02[b]	332.29[b]
Infant sex: female			–115.49
Maternal height (12.93 cm)			137.22[b]
Alcohol: ≥7 drinks/week			33.01
Cotinine (113.51 ng/mL)			–143.59[b]
Vitamin D (22.05 ng/mL)			17.23

Regression coefficients (β_{EN}) represent the change in birth weight (g) for term infants per increment: 2-SD increase in ln–transformed exposure biomarker or untransformed continuous covariate levels, or per category for categorical covariates.
[a] The cross-validated optimum penalisation was α=1.00, λ=3.32 (MSE=205 061) for the adjusted model (minimal sufficient set: study population, maternal age, prepregnancy BMI, and parity); α=1.00, λ=3.32 (MSE=177 179) for the model additionally adjusted for gestational age; and α=0.98, λ=2.46 (MSE=166 329) for the further adjusted model (infant sex, maternal height, alcohol, cotinine, and vitamin D). All models, n=1250.
[b] Covariance test[27] p<0.05.

Table 3. Multiple-exposure unpenalised linear regression models for the exposures and covariates selected via elastic net regression and term birth weight.

	Adjusted		Plus gestational age		Further adjusted	
Predictor	**β_{OLS} (95% CI)**	***p*-value**	**β_{OLS} (95% CI)**	***p*-value**	**β_{OLS} (95% CI)**	***p*-value**
Intercept	3528.90		3482.27		3120.16	
MEHHP	−86.75 (−139.18, −34.32)	0.001	−83.94 (−132.68, −35.19)	0.001	−70.22 (−117.59, −22.85)	0.004
MOiNP	45.85 (−4.84, 96.54)	0.076	45.62 (−1.51, 92.74)	0.058	37.64 (−7.99, 83.27)	0.106
PFOA	−42.77 (−108.19, 22.65)	0.200	−41.02 (−101.83, 19.80)	0.186	−63.77 (−122.83, −4.71)	0.035
p,p´-DDE[a]	−134.73 (−191.93, −77.53)	<0.001	−100.75 (−154.13, −47.36)	<0.001	−66.70 (−119.38, −14.02)	0.013
Population: Poland	−40.24 (−133.58, 53.11)	0.398	4.84 (−82.16, 91.85)	0.913	−93.16 (−183.72, −2.6)	0.044
Ukraine	−218.89 (−300.66, −137.13)	<0.001	−142.98 (−219.73, −66.22)	<0.001	−256.90 (−338.77, −175.02)	<0.001
Maternal age: 27–31	88.14 (23.05, 153.24)	0.008	80.01 (19.48, 140.53)	0.010	65.44 (6.74, 124.14)	0.029
32–45	30.53 (−42.52, 103.57)	0.413	38.08 (−29.83, 105.99)	0.272	37.19 (−28.83, 103.21)	0.270
BMI	209.00 (155.53, 262.46)	<0.001	181.37 (131.51, 231.22)	<0.001	194.00 (145.42, 242.58)	<0.001
Parity: multiparous	58.72 (−3.63, 121.06)	0.065	76.33 (18.32, 134.34)	0.010	85.92 (29.39, 142.44)	0.003
Gestational age			343.78 (295.61, 391.94)	<0.001	330.43 (283.7, 377.15)	<0.001
Infant sex: female					−115.40 (−160.38, −70.43)	<0.001
Maternal height					135.83 (88.38, 183.28)	<0.001
Alcohol: ≥7 drinks/week					34.43 (−61.76, 130.62)	0.483
Cotinine					−140.41 (−191.92, −88.89)	<0.001
Vitamin D					18.77 (−29.39, 66.93)	0.445

Regression coefficients (β_{OLS}) represent the change in birth weight (g) for term infants per 2-SD increase in ln–transformed exposure biomarker or untransformed continuous covariate levels (refer to Table 2 for increments), or per category for categorical covariates. Variance inflation factors for contaminant exposure terms ranged from 1.04–1.74.
[a] β_{OLS} for models including wet weight *p,p´*-DDE (ng/mL), adjusted for total lipids: −134.22, −99.91, −67.16.

birth weight, including parity, infant sex, and maternal smoking (specifically, a 2-SD increase in cotinine). When summed phthalate metabolites instead of individual metabolites were included, ΣDEHPom was selected (β_{EN} = –36.94; multiple-exposure β_{OLS} = –65.43; 95% CI: –116.06, –14.80 per 1.27 mol/mL) and ΣDiNPom was not selected.

In the comparative analysis with single-exposure OLS regression models, nine exposures were significantly associated (FDR $q < 0.05$) with term birth weight in the primary adjusted model, of which four were also selected ($\beta_{EN} > |1.0|$) in the elastic net model. In the models additionally adjusted for gestational age, four were selected in the OLS and five in the elastic net, and three in both models. In the further adjusted OLS and elastic net models, three of the same exposures were selected (MEHHP, PFOA, and *p,p´*-DDE), and an additional exposure (MOiNP) was selected in the elastic net model (see Supplemental Table S4).

There was some evidence, although nonsignificant (interaction $p > 0.05$), of effect measure modification by study population for MOiNP and PFOA, because the direction of effect estimates was heterogeneous. There was no evidence that infant sex modified associations. Greater reductions in birth weight were observed for overweight/obese compared with normal-prepregnancy-weight women for *p,p´*-DDE (interaction $p = 0.03$) and for smokers compared with nonsmokers for MEHHP and PFOA (interaction $p = 0.03$) (see Supplemental Table S5).

There was significant nonlinearity for the overall exposure–outcome relationship for only PFOS and birth weight (see Supplemental Figure S2), which appeared to be an artefact of differing exposure ranges and a slight positive slope for Poland and highly negative slope for Greenland ($p = 0.52$ and $p = 0.001$, respectively, for linear terms). Only minor differences in coefficients were observed when wet weight rather than lipid-standardised organochlorines were modelled (Table 3, footnote; see also Supplemental Table S4) and when unpenalised models were additionally adjusted for covariates with missing data (maternal education, season, and seafood) (data not shown).

Discussion

In this study of mother–newborn pairs from Greenland, Poland, and Ukraine, prenatal exposure to a DEHP metabolite (MEHHP), PFOA, and *p,p´*-DDE were independently associated with lower birth weight in term newborns. This study considers one of the largest numbers of environmental contaminants to date, in relation to birth weight, and has one of the larger sample sizes.

To overcome the challenge of assessing associations for correlated exposures, we used a multi-pollutant penalised regression approach to select a subset of the most predictive environmental exposure variables. Elastic net regression forces coefficients of the least predictive variables to zero. We inputted the exposures selected using elastic net regression (MEHHP, MOiNP, PFOA, and *p,p´*-DDE) into multiple-exposure unpenalised regression models to obtain estimates of the linear associations between exposures and term birth weight

which were not shrunken (or were unpenalised).

Selection based on single-exposure, unpenalised OLS models somewhat coincided with elastic net selection. However, in these models, each exposure was not mutually adjusted for other exposures, and some associations likely reflect false positives that are "tracking" with correlated exposure(s) truly associated with the outcome. For instance, the magnitude of associations for PFOS and PCB-153 decreased markedly when modelled with other exposures (data not shown). We decided to use a penalised regression approach because we expected, based on the correlation matrix, that an OLS regression model including all exposures would suffer from multicollinearity. *A posteriori*, we confirmed this. The variance inflation factor exceeded three for six of the 16 exposures, and the direction of the coefficient flipped from negative to positive for four exposures (data not shown). Using a more conventional backward–forward stepwise regression yielded generally similar selection results as the elastic net models (data not shown). However, selection accuracy would theoretically be expected to suffer given that exposures are not mutually adjusted at each step. Elastic net would be expected to have even more power in a data scenario with a higher number of exposures and might offer a complementary selection strategy to the single-exposure environment-wide association study (EWAS) approach.

There are caveats to applying a prediction method to an etiologic research question. With two highly correlated variables, elastic net will select a slightly more predictive variable, and shrink the coefficient of the other variable, which may be etiologically relevant. Nevertheless, with conventional (unpenalised) regression modelling, the strength of an association is usually also interpreted as having etiologic implications. Selection based on minimising prediction error in CV, as performed in the present study, has been shown to overselect predictors. Thus we also applied the recently proposed covariance test for post-selection inference,[27] which has been shown to have lower power than CV-based selection but also a lower FDR.[26] This resulted in consistent, although more conservative (sparser), selection than CV, and indicated strongest support for associations between MEHHP and *p,p´*-DDE and birth weight (Table 2). Other selection strategies have been proposed, such as assessing the stability of selection via subsampling of data,[34] which also indicated that MEHHP and *p,p´*-DDE were most robustly selected (data not shown). Improving the statistical inference of elastic net models, including explicitly addressing multiple testing, warrants further research and validation.

We restricted our analysis to term births because exposures may represent causally independent risk factors for preterm birth and growth restriction. Results did not differ appreciably upon inclusion of preterm births (data not shown). We did not have data on gestational weight gain, a proxy of lipid gain, which has been shown in simulations to confound associations between organochlorines and birth weight[35] but has also been suggested to partially mediate associations.[6] Inclusion of gestational weight gain or estimated fat mass in a pooled analysis of a subset of the European cohorts with available data ($n = 4266$) reduced the effect estimates for cord blood PCB-153 and birth weight by 48% and 34%, respectively.[6] For

PFASs, a lower rate of pregnancy-related plasma volume expansion or glomerular filtration increase might confound PFAS–birth weight associations.[36]

Further, we did not have data on interpregnancy interval, gestational diabetes, preeclampsia, or detailed information on fish intake, possibly contributing to residual confounding. We adjusted for study population to limit bias due to unmeasured confounding, but a corollary is reduced contrast in exposure. We also cannot exclude the possibility that unmeasured (contaminant) exposures confound the observed associations. Furthermore, differential gestational timing of sampling across study populations may have led to less precise and biased coefficients.

The phthalate metabolites have shorter half-lives than the other contaminants. Braun et al.[14] demonstrated high intraindividual variability of urinary ΣDEHP oxidative metabolites (intraclass correlation coefficient of 0.09) in a study with serial sampling of women throughout pregnancy. Thus, repeated measurements would have been preferable. Given that exposure assessment of the phthalates was relatively imprecise due to their short half-lives, leading to possibly attenuated estimates and a loss of statistical power, it is perhaps surprising that we identified a consistent association with MEHHP. Although most studies assessed exposure to phthalates using urine samples, several studies support that secondary oxidative metabolites can be reliably assessed in serum samples because they, unlike the primary monoesters, are not susceptible to lipase activity and external contamination by phthalate diesters in sampling devices and *ex vivo* conversion during analysis and storage.[37,38]

Most previous assessments of contaminant exposures and birth weight have used single-exposure, sometimes two-exposure, modelling. Two studies (n = 287 and 404) which tested 11 and 10 urinary phthalate (including DEHP) metabolites, respectively, found no associations for phthalates and some associations for phenols.[12,13]

Of the larger studies ($n \geq 400$–849) of environmental PFAS exposure and birth weight, one reported significant inverse associations for all PFASs evaluated (PFOS, PFOA, and PFHxS)[11]; another reported nonsignificant inverse associations for PFOS and PFOA and birth weight z-scores[39]; and two studies reported inverse associations for PFOS, but not for PFOA[9] or PFOA, PFNA, and PFUA.[10] A large study within the Danish National Birth Cohort (n = 1388), observed a mean PFOS plasma level of 35.3 ng/mL and PFOA level of 5.6 ng/mL, higher than levels in the present study; 12.9 and 1.7 ng/mL serum, respectively. A significant association was reported for PFOA (–10.63 g; 95% CI: –20.79, –0.47 per 1 ng/mL for all births, and –8.73 g; 95% CI: –19.53, 2.06 for term births) but not PFOS—in line with our findings—and the association for PFOA increased slightly in magnitude upon adjustment for PFOS.[8] Comparisons of concentrations in paired maternal–cord blood samples, suggest that PFOA crosses the placental barrier more readily than PFOS, which may partly account for the observed dominance of PFOA estimates over PFOS.[8] Inconsistent with this finding, in a community exposed to high levels of PFOA in Ohio, USA (n = 1470), PFOA (geometric mean, 16.2 ng/mL) was not associated with term birth weight, whereas PFOS (geometric mean, 13.2

ng/mL) was inversely associated, albeit not consistently statistically significant across analyses, and coefficients remained stable in a two-pollutant model.[7]

Our findings for the organochlorines somewhat contradict those of a pooled analysis of European cohorts ($n \sim 9000$), including the study populations of this study, although risk estimates displayed heterogeneity across cohorts. Cord serum PCB-153 (measured or estimated from maternal serum, whole blood, or breast milk levels) was significantly associated with reduced birth weight, but *p,p´*-DDE was not significantly associated: –194 g (95% CI: –314, –74) per 1 µg/L PCB-153 and –9 g (95% CI: –19, 7) per 1 µg/L *p,p´*-DDE. Associations were reported not to change in a two-pollutant model.[6]

Postulated mechanisms include *a*) increased oxidative stress[40] and *b*) modulation of sex hormone or thyroid hormone homeostasis, which both play a role in development and growth velocities. Cord blood organochlorine compounds and maternal serum PFASs have been found to be inversely associated with thyroid hormone levels.[41,42] There is also experimental evidence that PFASs interfere with lipid metabolism via activation of the peroxisome proliferator-activated receptor alpha (PPARα).[43] Mid-pregnancy PFAS levels have been associated with high-density lipoprotein (HDL) and total cholesterol.[44] In addition, a study of pregnant women found that bisphenol A and a few phthalates, including metabolites of DEHP, were associated with perturbations in biomarkers of angiogenesis linked to placental development and function, with potential adverse consequences for fetal growth.[45]

Our results suggest that smoking during pregnancy and prepregnancy overweight/obesity may confer greater susceptibility to the effects of some contaminants on birth weight, although mechanisms underlying these potential interactions remain unclear. Casas et al.[6] assessed effect measure modification for PCB-153 and birth weight, and reported stronger inverse associations for maternal prepregnancy overweight/obese, and smoking during pregnancy (although only the latter interaction was significant at $p < 0.05$); however, the evidence for potential effect modifiers is generally limited or inconsistent for contaminants assessed in the present analysis.

Conclusions

We found indications that several environmental contaminant exposures, representing three chemical classes (phthalates, PFASs, and organochlorines), are independently associated with reduced birth weight, with possible implications for health trajectories. Cautious interpretation is warranted in light of possible confounding due to pregnancy-related pharmacokinetic issues and unmeasured contaminant exposures. We used penalised elastic net regression to assess a mixture of environmental contaminants; this modelling approach may prove useful for similar environmental epidemiology analyses of multiple (correlated) exposures.

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Supplemental Material

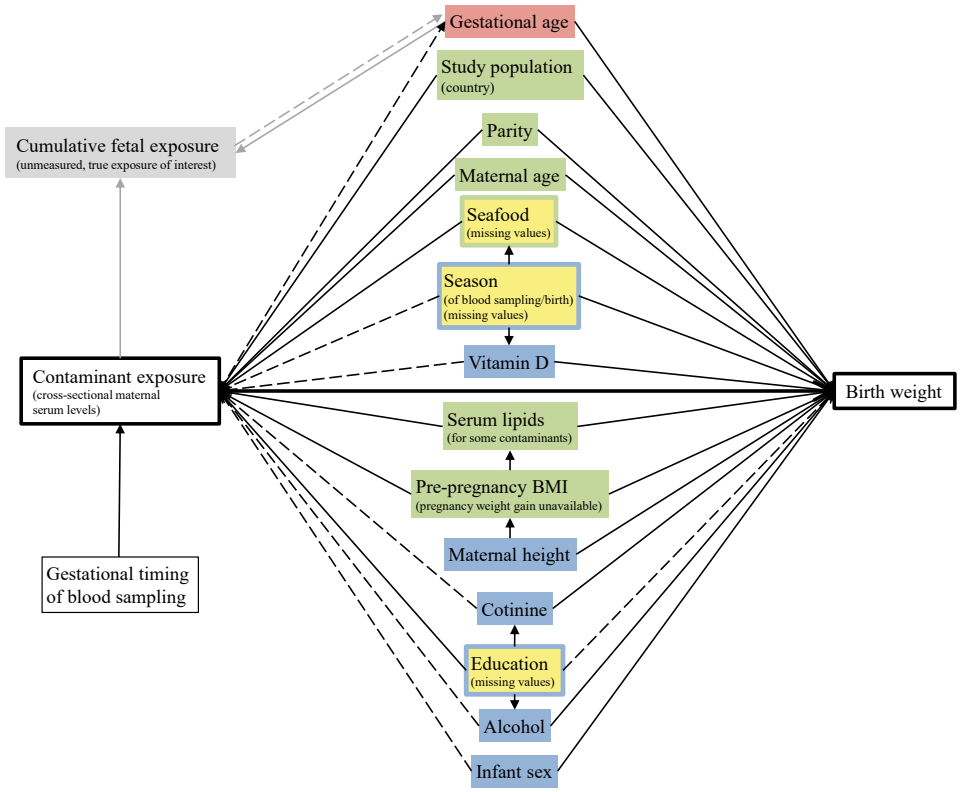

Figure S1. Directed acyclic graph to describe the authors' view of the relationships between the contaminant exposures, covariates, and birth weight.
The minimal sufficient adjustment set for estimating the total effect of contaminants on birth weight: study population, maternal age, pre-pregnancy body mass index (BMI), and parity (green boxes). For birth weight, serum lipids likely represent a confounder for the organochlorines (PCB-153 and *p,p'*-DDE), but not the PFASs or phthalate metabolites. Data on gestational weight gain—a proxy for gain in fat mass during pregnancy, which is an important confounder—was not available. Exposure of the fetus to environmental contaminants (grey box) was assessed by a proxy measure, cross-sectional maternal serum concentrations. While gestational age has an effect on cumulative fetal exposures, it is has no direct causal effect on cross-sectional maternal concentrations. However, fetal/maternal concentrations may have a causal effect on gestational age. Thus gestational age was considered a potential intermediate, and was added separately to the models. Seafood consumption represents a potentially important confounder for some contaminants (e.g., PCB-153); however, this variable represents consumption of different types of seafood/fish in the different study populations (countries). Furthermore, seafood consumption, along with season and maternal education, had a large number of missing values, so these variables were only included in sensitivity analyses (yellow boxes). The relationships between some covariates and contaminants and birth weight are less certain (denoted by a dashed line), and these covariates (blue boxes) were added in a secondary analysis, 'further adjusted models'; this adjustment set may represent overadjustment or unnecessary adjustment. This is a simplified representation of inter-relationships. Other permutations of the model could be argued. For example, maternal BMI affects measured contaminant levels, but may also represent an intermediate, causally affected by contaminant levels.

Table S1. Serum concentrations[a] of exposure biomarkers in pregnant women from Greenland (n=513), Warsaw, Poland (n=180), and Kharkiv, Ukraine (n=557), 2002–2004.

Analyte (ng/mL)	CV (%)[b]	LOD (ng/mL)[c]	% >LOD (n=1250)	GR 5 P	GR 50 P	GR 95 P	PL 5 P	PL 50 P	PL 95 P	UA 5 P	UA 50 P	UA 95 P	GR vs. PL *p*-val.[d]	GR vs. UA *p*-val.[d]	PL vs. UA *p*-val.[d]
Phthalate metabolites															
MEHHP	15, 17	0.01	100.0	0.24	0.68	2.36	0.17	0.41	1.07	0.11	0.46	2.94	<0.001	<0.001	0.011
MEOHP	12, 19	0.02	99.3	0.05	0.12	0.28	0.04	0.10	0.23	0.04	0.11	0.38	<0.001	0.062	0.011
MECPP	15, 18	0.007	100.0	0.25	0.58	2.00	0.34	0.85	2.22	0.35	0.93	4.00	<0.001	<0.001	0.034
ΣDEHPom[e] (nmol/L)	–	–	–	2.37	4.83	13.64	2.06	4.56	9.67	1.99	5.53	21.17	0.091	0.003	<0.001
ΣDEHPom[e]	–	–	–	0.71	1.46	4.11	0.62	1.38	2.92	0.60	1.67	6.38	0.091	0.003	<0.001
MHiNP	14, 16	0.01	95.5	0.07	0.24	0.79	0.02	0.11	0.54	<LOD	0.04	0.48	<0.001	<0.001	<0.001
MOiNP	16, 20	0.005	90.2	<LOD	0.02	0.07	0.005	0.02	0.06	<LOD	0.01	0.24	0.047	0.078	0.003
MCiOP	13, 14	0.03	100.0	0.07	0.23	3.25	0.11	0.25	0.88	0.05	0.21	4.57	0.062	0.221	0.011
ΣDiNPom[e] (nmol/L)	–	–	–	0.68	1.65	12.18	0.67	1.37	3.88	0.28	0.84	18.48	<0.001	<0.001	<0.001
ΣDiNPom[e]	–	–	–	0.22	0.52	3.86	0.21	0.44	1.23	0.09	0.27	5.86	<0.001	<0.001	<0.001
Perfluoroalkyl acids															
PFHxS	14, 18	0.02	100.0	0.99	2.05	5.07	0.97	2.28	5.95	0.45	1.56	4.09	0.058	<0.001	<0.001
PFHpA	11, 15	0.02	82.4	<LOD	0.05	0.15	0.02	0.11	0.60	<LOD	0.03	0.13	<0.001	<0.001	<0.001
PFOS	9, 11	0.2	100.0	10.23	20.09	49.47	4.38	7.81	12.40	2.27	5.04	9.48	<0.001	<0.001	<0.001
PFOA	6, 10	0.04	100.0	0.78	1.84	3.55	1.34	2.51	4.36	0.45	0.96	2.10	<0.001	<0.001	<0.001
PFNA	13, 13	0.03	100.0	0.33	0.69	1.99	0.30	0.56	1.34	0.30	0.61	1.37	<0.001	<0.001	0.033
PFDA	12, 13	0.03	99.9	0.16	0.40	1.18	0.10	0.22	0.45	0.07	0.16	0.34	<0.001	<0.001	<0.001
PFUnDA	19, 21	0.04	98.3	0.17	0.70	2.54	0.06	0.13	0.25	0.06	0.16	0.50	<0.001	<0.001	<0.001
PFDoDA	11, 15	0.04	72.4	0.04	0.13	0.40	<LOD	0.05	0.11	<LOD	0.04	0.11	<0.001	<0.001	0.194
Organochlorines															
PCB-153	10, 18	0.05	95.0	0.15	0.77	3.62	<LOD	0.10	0.29	0.06	0.19	0.53	<0.001	<0.001	<0.001
PCB-153 (ng/g)	–	–	95.0	21.03	106.82	526.47	2.59	10.75	27.02	8.50	26.98	68.06	<0.001	<0.001	<0.001
p,p´-DDE	7, 11	0.1	99.3	0.39	2.21	9.53	1.06	3.29	9.48	2.02	4.56	11.27	<0.001	<0.001	<0.001
p,p´-DDE (ng/g)	–	–	99.3	47.78	302.22	1289.32	137.29	347.82	876.12	276.01	653.44	1657.00	0.017	<0.001	<0.001
Total lipids (g/L)	–	–	100.0	4.10	7.37	13.53	7.51	9.63	11.98	4.44	7.18	10.91	<0.001	0.174	<0.001

Table S1. *Continued.*

CV, coefficient of variation; DEHP, diethylhexyl phthalate; DiNP, diisononyl phthalate; GM, geometric mean; GR, Greenland; LOD, limit of detection; MCiOP, mono-(4-methyl-7-carboxyheptyl) phthalate; MECPP, mono-(2-ethyl-5-carboxypentyl); MEHHP, mono-(2-ethyl-5-hydroxylhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MHiNP, mono-(4-methyl-7-hydroxyloctyl)phthalate; MOiNP, mono-(4-methyl-7-oxo octyl)phthalate; P, percentile; PCB-153, 2,2´,4,4´,5,5´-hexachlorobiphenyl; PFDA, perfluorodecanoic acid; PFHxS, perfluorohexane sulfonic acid; PFHpA, perfluoroheptanoic acid; PFOS, perfluorooctane sulfonic acid; PFOA, perfluorooctanoic acid; PFNA, perfluorononanoic acid; PFUnDA, perfluoroundecanoic acid; PFDoDA, perfluorododecanoic acid; PL, Poland; *p,p'*-DDE, 1,1-dichloro-2,2-bis(p-chlorophenyl)-ethylene; UA, Ukraine.

[a] Values below the LOD were imputed (see main text for details).

[b] CV for the between-day precision in 2 different quality control samples (n=76).

[c] LOD determined as the concentration corresponding to 3 times the standard deviation of the chemical blank signal.

[d] Pairwise comparisons of concentrations between study populations: Mann-Whitney U-test.

[e] Sum of DEHP or DiNP secondary oxidative metabolites, calculated as (1) the molar sum (nmol/L) and (2) corrected for molecular weight, based on the weighted average molecular weight (ng/mL).

Table S2. Spearman correlation coefficients between exposure biomarkers[a] in pooled samples, and per study population.

		MEHHP	MEOHP	MECPP	MHiNP	MOiNP	MCiOP	PFHxS	PFHpA	PFOS	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PCB-153	*p,p'*-DDE
MEHHP	**All**	1.00	0.75*	0.19*	0.28*	0.14*	0.15*	0.03*	0.03*	0.21*	0.07*	0.09*	0.17*	0.17*	0.11*	0.23*	-0.09*
	GR	1.00	0.65*	0.24*	0.32*	0.10*	0.05	-0.09*	0.02	-0.09*	0.01	-0.02	-0.06	-0.11*	-0.13*	-0.15*	-0.19*
	PL	1.00	0.62*	0.30*	0.24*	0.13	0.07	0.00	0.12	0.03	-0.01	0.07	0.00	-0.05	-0.04	0.11	0.01
	UA	1.00	0.85*	0.38*	0.11*	0.18*	0.26*	0.08	0.01	0.10*	0.09*	0.08	0.11*	-0.01	-0.10*	0.13*	0.10*
MEOHP	**All**		1.00	0.46*	0.17*	0.27*	0.24*	0.03*	0.03*	0.08*	0.04*	0.06*	0.08*	0.05*	0.02	0.12*	-0.01
	GR		1.00	0.53*	0.27*	0.26*	0.16*	-0.01	0.03	0.03	0.10*	0.02	0.03	-0.04	-0.02	-0.05	-0.08
	PL		1.00	0.50*	-0.06	0.25*	0.30*	0.15*	0.06	0.01	0.11	0.15*	0.11	0.00	-0.06	0.03	-0.03
	UA		1.00	0.54*	0.18*	0.31*	0.31*	0.03	0.04	0.07	0.04	0.04	0.06	-0.03	-0.09*	0.14*	0.11*
MECPP	**All**			1.00	-0.08*	0.34*	0.34*	-0.05*	-0.02*	-0.28*	-0.12*	-0.05*	-0.21*	-0.24*	-0.22*	-0.21*	0.15*
	GR			1.00	0.12*	0.33*	0.30*	0.05	0.06	0.04	0.07	-0.02	0.02	-0.02	0.00	0.01	-0.02
	PL			1.00	-0.03	0.34*	0.47*	0.08	0.07	0.02	0.07	0.12	0.05	0.12	0.04	-0.11	-0.09
	UA			1.00	0.31*	0.44*	0.4*1	-0.01	0.00	0.04	0.03	-0.03	0.00	-0.03	0.00	0.09*	0.08
MHiNP	**All**				1.00	0.36*	0.31*	0.12*	0.19*	0.56*	0.32*	0.07*	0.44*	0.45*	0.45*	0.34*	-0.34*
	GR				1.00	0.16*	0.17*	-0.11*	0.07	0.03	-0.02	0.03	0.05	0.01	0.05	-0.15*	-0.16*
	PL				1.00	0.16*	0.02	0.03	-0.06	0.10	-0.21*	-0.18*	-0.05	0.05	0.18*	0.09	0.15*
	UA				1.00	0.63*	0.53*	-0.08	0.04	0.02	0.02	0.03	0.04	0.09*	0.08*	0.03	-0.08
MOiNP	**All**					1.00	0.58*	-0.01	0.06*	0.01	0.08*	-0.04*	0.02	-0.01	0.03*	-0.04*	-0.08*
	GR					1.00	0.41*	-0.04	0.00	-0.12*	0.05	-0.14*	-0.11*	-0.15*	-0.04	-0.08	-0.08
	PL					1.00	0.58*	-0.03	0.04	0.02	0.06	0.06	0.06	0.15*	0.07	-0.03	-0.01
	UA					1.00	0.68*	-0.05	0.04	-0.02	0.04	0.01	0.05	0.01	0.07	-0.02	-0.05
MCiOP	**All**						1.00	0.11*	0.10*	0.04*	0.13*	0.05*	0.07*	0.02	0.02	0.00	-0.01
	GR						1.00*	0.09	0.08	0.07*	0.09	0.01	0.06	0.02	0.06	0.03	0.02
	PL						1.00	0.26*	0.17*	0.03	0.13	0.12	0.13	0.08	-0.01	-0.18*	-0.05
	UA						1.00	0.08	0.05	0.03	0.16*	0.09*	0.08*	0.02	-0.03	0.03	0.04
PFHxS	**All**							1.00	0.17*	0.34*	0.34*	0.22*	0.35*	0.23*	0.24*	0.15*	-0.01
	GR							1.00	0.03	0.37*	0.19*	0.31*	0.41*	0.30*	0.34*	0.28*	0.27*
	PL							1.00	0.10	0.27*	0.17*	0.18*	0.19*	0.07	0.06	0.09	0.09
	UA							1.00	0.10*	0.24*	0.26*	0.17*	0.19*	0.14*	0.06	0.03	0.04

Table S2. *Continued.*

		MEHHP	MEOHP	MECPP	MHiNP	MOiNP	MCiOP	PFHxS	PFHpA	PFOS	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PCB-153	*p,p'*-DDE
PFHpA	**All**								1.00	0.26*	0.44*	0.22*	0.25*	0.11*	0.09*	0.02*	-0.06*
	GR								1.00	0.21*	0.20*	0.27*	0.19*	0.17*	0.10*	0.20*	0.15*
	PL								1.00	0.22*	0.44*	0.53*	0.38*	0.25*	0.07	0.14	0.08
	UA								1.00	0.17*	0.33*	0.16*	0.11*	0.06	-0.05	0.01	0.09*
PFOS	**All**									1.00	0.61*	0.42*	0.78*	0.74*	0.64*	0.62*	-0.24*
	GR									1.00	0.50*	0.74*	0.72*	0.64*	0.48*	0.52*	0.50*
	PL									1.00	0.55*	0.58*	0.47*	0.37*	0.08	0.20*	0.24*
	UA									1.00	0.53*	0.54*	0.52*	0.41*	0.11*	0.23*	0.14*
PFOA	**All**										1.00	0.30*	0.50*	0.29*	0.26*	0.15*	-0.19*
	GR										1.00	0.24*	0.15*	-0.03	-0.02	0.08	0.12*
	PL										1.00	0.70*	0.55*	0.24*	0.06	0.19*	0.17*
	UA										1.00	0.46*	0.47*	0.30*	0.06	0.14*	0.14*
PFNA	**All**											1.00	0.60*	0.48*	0.31*	0.36*	0.27*
	GR											1.00	0.85*	0.83*	0.63*	0.65*	0.61*
	PL											1.00	0.79*	0.47*	0.16*	0.15*	0.10
	UA											1.00	0.53*	0.44*	0.08	0.20*	0.14*
PFDA	**All**												1.00	0.77*	0.67*	0.56*	-0.08*
	GR												1.00	0.86*	0.74*	0.62*	0.60*
	PL												1.00	0.54*	0.30*	0.11	0.12
	UA												1.00	0.57*	0.26*	0.16*	0.09*
PFUnDA	**All**													1.00	0.71*	0.67*	-0.09*
	GR													1.00	0.75*	0.65*	0.60*
	PL													1.00	0.48*	0.18*	0.11
	UA													1.00	0.39*	0.15*	0.06
PFDoDA	**All**														1.00	0.56*	-0.13*
	GR														1.00	0.50*	0.46*
	PL														1.00	-0.05	0.01
	UA														1.00	0.08*	-0.01

Table S2. *Continued.*

		MEHHP	MEOHP	MECPP	MHiNP	MOiNP	MCiOP	PFHxS	PFHpA	PFOS	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PCB-153	*p,p'*-DDE
PCB-153	**All**															1.00	0.19*
	GR															1.00	0.92*
	PL															1.00	0.54*
	UA															1.00	0.50*
***p,p'*-DDE**	**All**																1.00
	GR																1.00
	PL																1.00
	UA																1.00

All represents pooled study populations; GR, Greenland; PL, Poland; UA, Ukraine.
[a] Phthalates and PFASs in ng/mL serum, and organochlorines in ng/g lipids.
* $p<0.05$; considered statistically significant.

Table S3. Adjusted associations (β [95% CI]) between term birth weight and selected exposures and demographic, reproductive, and lifestyle factors.

Covariate (increment)	Term birth weight (g)	ΣDEHPom (nmol/L)	ΣDiNPom (nmol/L)	PFOS (ng/mL)	PFOA (ng/mL)	PCB-153 (ng/g)	*p,p´*-DDE (ng/g)
Model 1 *(n=1250)*							
Intercept	3707.7 (3620.8, 3794.7)*	1.72 (1.59, 1.85)*	0.64 (0.43, 0.86)*	3.05 (2.96, 3.14)*	0.73 (0.63, 0.83)*	4.47 (4.30, 4.64)*	5.48 (5.31, 5.64)*
Study population (ref.: Greenland) Poland	-97.0 (-187.2, 6.9)*	-0.22 (-0.36, 0.08)*	-0.29 (-0.51, 0.06)*	-1.03 (-1.12, 0.93)*	0.19 (0.09, 0.29)*	-2.41 (-2.58, 2.24)*	0.43 (0.26, 0.60)*
Ukraine	-235.8 (-301.1, 170.6)*	0.06 (-0.04, 0.16)	-0.45 (-0.61, 0.29)*	-1.45 (-1.52, 1.38)*	-0.74 (-0.81, 0.67)*	-1.35 (-1.47, 1.22)*	0.96 (0.84, 1.08)*
Maternal total lipids (5.11 g/L)	33.0 (-14.9, 80.9)	0.04 (-0.03, 0.11)	0.11 (-0.01, 0.23)	-0.06 (-0.12, 0.01)*	-0.12 (-0.17, 0.07)*	-0.27 (-0.37, 0.18)*	-0.40 (-0.49, 0.31)*
Gestational age (2.45 weeks)	336.2 (289.2, 383.2)*	-0.03 (-0.11, 0.04)	-0.02 (-0.14, 0.09)	-0.06 (-0.11, 0.01)*	-0.02 (-0.07, 0.03)	-0.05 (-0.14, 0.04)	-0.12 (-0.21, 0.03)*
Infant sex (ref.: male): female	-116.2 (-161.5, 70.9)*	-0.02 (-0.09, 0.05)	-0.04 (-0.15, 0.07)	-0.03 (-0.07, 0.02)	-0.01 (-0.06, 0.04)	0.02 (-0.06, 0.11)	-0.01 (-0.10, 0.08)
Maternal age (years)[a] (ref: 18–26) 27–31	53.2 (-5.7, 112.1)	0.09 (0.00, 0.18)*	0.13 (-0.02, 0.27)	0.07 (0.00, 0.13)*	0.05 (-0.02, 0.12)	0.24 (0.13, 0.36)*	0.17 (0.06, 0.28)*
32–45	14.5 (-51.4, 80.3)	0.09 (-0.01, 0.19)	0.03 (-0.14, 0.19)	0.15 (0.08, 0.22)*	0.11 (0.04, 0.19)*	0.53 (0.40, 0.65)*	0.34 (0.21, 0.46)*
Pre-pregnancy BMI (8.62 kg/m^2)	192.8 (143.9, 241.6)*	-0.09 (-0.16, 0.01)*	-0.11 (-0.23, 0.01)	0.05 (-0.00, 0.10)	0.04 (-0.01, 0.10)	-0.12 (-0.22, 0.03)*	0.02 (-0.07, 0.11)
Maternal height (12.93 cm)	141.5 (94.0, 188.9)*	-0.04 (-0.12, 0.03)	0.04 (-0.08, 0.15)	-0.00 (-0.05, 0.05)	0.06 (0.00, 0.11)*	-0.16 (-0.25, 0.07)*	-0.14 (-0.23, 0.05)*
Parity (ref.: nulliparous): multiparous	102.6 (46.8, 158.3)*	-0.05 (-0.14, 0.04)	0.02 (-0.12, 0.15)	-0.08 (-0.13, 0.02)*	-0.23 (-0.29, 0.17)*	-0.11 (-0.21, 0.00)	-0.07 (-0.18, 0.03)
Serum cotinine (113.51 ng/mL)	-147.6 (-199.0, -96.1)*	-0.03 (-0.11, 0.05)	0.29 (0.16, 0.42)*	0.04 (-0.01, 0.10)	-0.02 (-0.08, 0.04)	0.21 (0.11, 0.31)*	0.22 (0.12, 0.31)*
Vitamin D (22.05 ng/mL)	13.2 (-35.3, 61.6)	0.00 (-0.07, 0.08)	0.01 (-0.11, 0.12)	0.13 (0.08, 0.19)*	0.05 (-0.00, 0.10)	0.22 (0.13, 0.31)*	0.12 (0.03, 0.21)*
Alcohol intake (ref.: <7): ≥7 drinks/weeks[b]	34.5 (-61.9, 130.8)	-0.17 (-0.32, 0.02)*	0.04 (-0.20, 0.27)	0.11 (0.01, 0.22)*	0.00 (-0.11, 0.11)	0.25 (0.07, 0.44)*	0.28 (0.10, 0.47)*
Adjusted R^2	*0.32*	*0.02*	*0.07*	*0.71*	*0.41*	*0.58*	*0.29*
Model 2: *Model 1 + the following covariates (n=1122)*							
Maternal education (ref.: none): some post-secondary	-14.2 (-68.0, 39.6)	0.09 (0.01, 0.17)*	-0.05 (-0.18, 0.08)	0.02 (-0.03, 0.08)	0.09 (0.04, 0.15)*	-0.08 (-0.19, 0.02)	-0.01 (-0.11, 0.09)
Season of blood sampling (ref.: Oct.–March): April–Sept.	6.0 (-45.6, 57.7)	-0.18 (-0.26, 0.10)*	-0.25 (-0.37, 0.13)*	0.07 (0.02, 0.12)*	-0.01 (-0.07, 0.04)	0.09 (-0.01, 0.18)	0.09 (-0.00, 0.19)
Fish/seafood (3.03 days/week)[b]	-20.5 (-37.1, 3.9)*	-0.01 (-0.03, 0.02)	0.05 (0.01, 0.09)*	0.05 (0.03, 0.07)*	0.02 (0.01, 0.04)*	0.07 (0.04, 0.10)*	0.06 (0.03, 0.09)*
Adjusted R^2	*0.32*	*0.04*	*0.09*	*0.72*	*0.44*	*0.60*	*0.30*

ref, Reference category.
Regression coefficients (β) are estimated from multivariable OLS linear regression models of term birth weight or ln-exposures on (Model 1) covariates with no missing data, and (Model 2) with the addition of covariates with missing data. Continuous covariates were mean-centred and rescaled to 2 times their standard deviations (increment). Variance inflation factors (VIF, a measure of multicollinearity) were all <1.50, except for study population (VIF=2.48) in term birth weight models.
* Considered statistically significant: The 95% confidence interval for β did not include unity.
[a] These cutoffs for age yield a low AIC for a model of birth weight and age, which most closely matches the AIC from a GAM model with age fitted with a smoothing spline (best fit was an inverted U-shape).
[b] With reference to period attempting to conceive.

Table S4. Single-exposure unpenalised OLS-regression models for term birth weight (n=1250).

	Geometric	Ln,	Adjusted		Plus gestational age		Further adjusted	
Exposure	mean	2-SD[a]	β_{OLS} (95% CI)	*p*-value	β_{OLS} (95% CI)	*p*-value	β_{OLS} (95% CI)	*p*-value
MEHHP (ng/mL)	0.559	1.700	-73.89 (-125.96, -21.81)	0.006*	-72.66 (-120.92, -24.39)	0.003*	-63.63 (-110.44, -16.83)	0.008*
MEOHP (ng/mL)	0.113	1.293	-58.72 (-109.66, -7.79)	0.024*	-48.39 (-95.65, -1.12)	0.045	-44.75 (-90.45, 0.96)	0.055
MECPP (ng/mL)	0.813	1.421	-12.15 (-65.57, 41.26)	0.656	-2.82 (-52.36, 46.73)	0.911	-12.52 (-60.44, 35.39)	0.609
ΣDEHPom (mol/mL)	5.430	1.265	-60.48 (-111.50, -9.46)	0.020*	-52.50 (-99.83, -5.17)	0.030	-53.23 (-98.98, -7.47)	0.023
MHiNP (ng/mL)	0.096	2.735	28.60 (-34.90, 92.10)	0.378	28.78 (-30.09, 87.65)	0.338	33.55 (-23.48, 90.58)	0.249
MOiNP (ng/mL)	0.016	2.219	34.93 (-15.60, 85.47)	0.176	34.04 (-12.82, 80.89)	0.155	26.11 (-19.17, 71.39)	0.259
MCiOP (ng/mL)	0.275	2.321	-10.52 (-61.09, 40.06)	0.684	-3.85 (-50.75, 43.05)	0.872	11.79 (-33.97, 57.54)	0.614
ΣDINPom (mol/mL)	1.471	2.074	-0.69 (-52.90, 51.52)	0.979	6.70 (-41.72, 55.12)	0.786	20.75 (-26.28, 67.78)	0.387
PFHxS (ng/mL)	1.842	1.241	-47.74 (-101.07, 5.59)	0.080	-11.39 (-61.15, 38.37)	0.654	-6.28 (-55.18, 42.61)	0.801
PFHpA (ng/mL)	0.047	1.837	-10.17 (-66.19, 45.84)	0.722	-11.12 (-63.05, 40.82)	0.675	-7.05 (-57.18, 43.08)	0.783
PFOS (ng/mL)	9.357	1.600	-114.36 (-206.81, -21.91)	0.015*	-69.53 (-155.59, 16.53)	0.114	-68.84 (-152.90, 15.22)	0.109
PFOA (ng/mL)	1.421	1.175	-68.94 (-134.25, -3.63)	0.039	-61.06 (-121.63, -0.49)	0.048	-78.52 (-137.01, -20.03)	0.009*
PFNA (ng/mL)	0.652	1.028	-78.62 (-130.57, -26.66)	0.003*	-60.92 (-109.20, -12.63)	0.014*	-44.67 (-92.04, 2.69)	0.065
PFDA (ng/mL)	0.245	1.397	-103.28 (-169.84, -36.72)	0.002*	-64.80 (-126.87, -2.73)	0.041	-43.93 (-104.83, 16.97)	0.158
PFUnDA (ng/mL)	0.275	2.099	-102.04 (-174.80, -29.28)	0.006*	-65.99 (-133.74, 1.77)	0.057	-37.15 (-103.86, 29.56)	0.275
PFDoDA (ng/mL)	0.068	1.672	-83.97 (-150.78, -17.16)	0.014*	-35.64 (-98.07, 26.79)	0.263	-24.40 (-86.33, 37.54)	0.440
PCB-153 (ng/g)	39.620	2.432	-143.74 (-218.92, -68.57)	0.000*	-121.09 (-190.93, -51.24)	0.001*	-78.01 (-147.15, -8.88)	0.027
p,p´-DDE (ng/g)	419.856	1.823	-137.24 (-193.92, -80.56)	0.000*	-103.11 (-156.04, -50.17)	0.000*	-73.02 (-125.21, -20.83)	0.006*
PCB-153 (ng/mL)[b]	0.299	2.331	-141.82 (-216.21, -67.43)	0.000*	-103.75 (-171.60, -35.89)	0.003*	-65.25 (-132.29, 1.80)	0.057
p,p´-DDE (ng/mL)[b]	3.144	1.774	-134.91 (-191.88, -77.95)	0.000*	-93.14 (-145.99, -40.29)	0.001*	-64.83 (-116.78, -12.89)	0.015

Regression coefficients (β_{OLS}) represent the change in birth weight (g) for term infants per 2-standard deviation (SD) increase in natural-log (ln)–transformed exposure concentration. To convert the β_{EN} or β_{OLS} presented per 2-SD increase in ln-transformed exposure to a β coefficient per 1 unit increase in ln-exposure, apply the formula: (1 / 2-SD) * β. Adjusted models were adjusted for study population (Poland, Ukraine vs. Greenland); maternal age (27-31 and 32-45 vs. 18-26); pre-pregnancy BMI (kg/m^2); and parity (multiparous vs. nulliparous). Further adjusted models were additionally adjusted for gestational age (weeks); infant sex (female vs. male); maternal height (cm); alcohol (≥ drinks/week); cotinine (ng/mL); and vitamin D (ng/mL).

[a] 2 times the SD of ln–transformed concentrations (used for scaling in analyses).

[b] Wet weight PCB-153 and *p,p´*-DDE models were additionally adjusted for total lipids (g/L).

* *p*-value (two-sided) considered statistically significant at a false discovery rate (FDR)<5% (*q*-value <0.05) for the 16 exposures tested in the primary analysis; and significant at FDR<5% for all 20 exposures tested for the additional analysis, including summed phthalate metabolites [ΣDEHPom (mol/mL) and ΣDiNPom (mol/mL)] and wet weight organochlorines [PCB-153 (ng/mL) and *p,p´*-DDE (ng/mL)].

Table S5. Assessment of potential effect modification of the associations between contaminant exposures and term birth weight.

Potential modifier	n	MEHHP (1.70 ng/mL) β_{OLS} (95% CI)	MOiNP (2.22 ng/mL) β_{OLS} (95% CI)	PFOA (1.18 ng/mL) β_{OLS} (95% CI)	*p,p´*-DDE (1.82 ng/g) β_{OLS} (95% CI)
Base model	1250	-86.75 (-139.18, -34.32)*	45.85 (-4.84, 96.54)	-42.77 (-108.19, 22.65)	-134.73 (-191.93, -77.53)*
Study population					
Greenland	513	-118.80 (-234.15, -3.45)*	-10.76 (-121.04, 99.51)	-94.50 (-225.36, 36.37)	-153.00 (-234.70, -71.30)*
Poland	180	-134.24 (-315.62, 47.14)	148.65 (-10.48, 307.78)	60.21 (-118.31, 238.73)	-290.86 (-472.79, -108.92)*
Ukraine	557	-79.13 (-136.09, -22.16)*	57.07 (1.72, 112.42)*	-45.56 (-123.43, 32.32)	-42.42 (-148.4, 63.55)
p-interaction[a]		0.728	0.211	0.463	0.104
Infant sex					
Female	593	-97.53 (-170.53, -24.53)*	58.12 (-13.17, 129.40)	-32.76 (-123.77, 58.26)	-171.24 (-251.97, -90.52)*
Male	657	-70.73 (-145.35, 3.88)	42.10 (-29.10, 113.31)	-38.05 (-131.45, 55.35)	-111.20 (-191.02, -31.38)*
p-interaction[a]		0.946	0.910	0.835	0.263
Pre-pregnancy BMI					
<25 kg/m^2	972	-102.97 (-160.13, -45.81)*	61.39 (6.23, 116.55)*	-33.39 (-105.51, 38.73)	-82.32 (-150.81, -13.84)*
≥25 kg/m^2	278	-45.07 (-176.79, 86.66)	-53.12 (-181.46, 75.22)	-99.23 (-255.31, 56.85)	-246.81 (-352.90, -140.72)*
p-interaction[a]		0.169	0.158	0.759	0.029**
Smoking: serum cotinine					
Non-smoker: <5 ng/mL	851	-68.83 (-127.20, -10.46)*	34.91 (-19.51, 89.33)	-36.93 (-109.75, 35.88)	-47.58 (-118.22, 23.06)
Smoker: ≥5 ng/mL	399	-155.43 (-261.12, -49.74)*	64.90 (-48.19, 177.98)	-80.35 (-211.94, 51.23)	-186.32 (-284.07, -88.57)*
p-interaction[a]		0.025**	0.446	0.033**	0.198

The primary-selected exposures from elastic net modelling (see main text) were modelled in multiple-exposure unpenalised OLS regression models.
Regression coefficients (β_{OLS}) represent the change in birth weight (g) for term infants per 2-SD increase in ln-transformed exposure concentration for the pooled study populations (increment indicated in the table heading). Models were adjusted for the minimal adjustment set (maternal age, pre-pregnancy BMI, parity, and study population), plus the potential modifier. Variance inflation factors (VIF) were <2.00 for all terms, across stratified models, except for study population (VIF=2.21—3.74 across models).

[a] *p*-values for interaction were calculated from a likelihood ratio test comparing multiple-exposure OLS models with and without the cross-product interaction term (exposure x potential modifier).

* Considered significant: The 95% confidence interval (CI) for β_{OLS} did not include unity.

** *p*-interaction < 0.05.

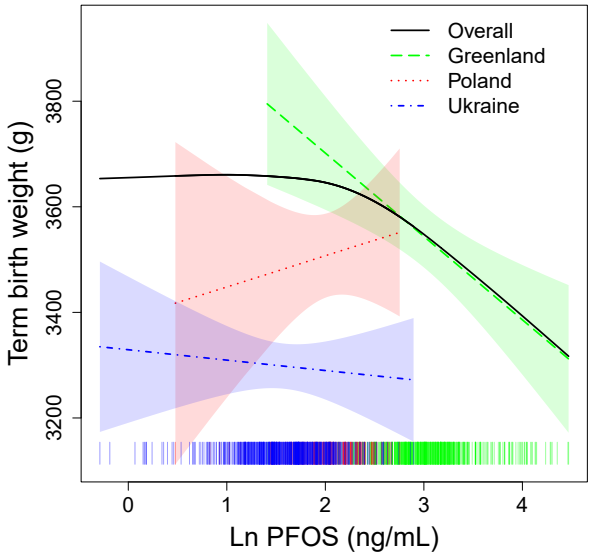

Figure S2. Generalised additive models for the single-pollutant exposure–outcome relationship for PFOS and term birth weight, fitted with a smoothing spline term for PFOS (with restricted maximum likelihood estimation).
The other exposure and term birth weight models did not show evidence of significant non-linearity. The models were adjusted for study population (overall model), maternal age (categorical), pre-pregnancy BMI, and parity (as in the primary analyses). Predicted functions, with BMI set at the mean (22.89 kg/m^2) and fixed at 18–26 years of age, and nulliparous, are presented: three population-specific exposure–outcome relationships (dashed lines) and 95% confidence intervals (shaded), and an overall exposure–outcome relationship for the pooled analysis, plotted at the Greenland-specific intercept (solid black line). Rug plots display the density of the PFOS exposure biomarker data.

Chapter 5

Early-life exposure to persistent organic pollutants, gut microbiota diversity and metabolites, and respiratory health in Norwegian children

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Submitted

Abstract

Background and objectives: There is suggestive evidence that prenatal and early-life exposure to certain environmental contaminants may increase the risk of developing asthma and allergic diseases. We assessed associations between multiple persistent organic pollutants (POPs) and risk of asthma and lower respiratory tract infection (LRTI) in childhood. We additionally explored the potential independent and mediating effects of early-life gut microbiota diversity and metabolites, which have been shown to be immunomodulatory and possibly protect against allergic disease development.

Methods: We studied Norwegian singleton children, born 2002–2009, and their mothers (HUMIS-NoMIC cohort). Concentrations of 26 POPs [polychlorinated biphenyls (PCBs), brominated flame retardants, perfluoroalkyl substances, and organochlorine pesticides] were quantified in breast milk samples, reflecting pre- and postnatal exposures (n = 993). Gut microbiota diversity and short-chain fatty acids (SCFAs), products of microbial metabolism of dietary fibres, were assessed in fecal samples collected at multiple time points before 2 years of age in a subset of children (n = 438). Asthma cases (n = 44) were identified based on diagnosis in a national registry by 2014, when children were a median of 10.4 years of age. Maternal-reported asthma (n = 60) and LRTI (n = 156) by 2 years of age were also evaluated. We estimated associations between POP exposures, Shannon diversity and SCFAs, and respiratory outcomes.

Results: In adjusted models, Σ_{14}PCBs was associated with a decreased risk and Σ_{4}OCPs with an increased risk of asthma; associations between β-HCH [odds ratio (OR) = 2.99 per 2-SD increase; 95% CI: 1.66, 5.43] and PCB-138 (OR = 0.43; 95% CI: 0.20, 0.91) and asthma by around 10 years of age were most robust. No chemical exposures were associated with LRTI. There were both inverse and positive associations between Shannon diversity and respiratory outcomes, and generally imprecise associations for SCFAs. There was limited evidence that early-life POPs perturbed microbial diversity or production of SCFAs, except for an association between Σ_{14}PCBs and reduced Shannon diversity at 2 years, and there was no clear evidence of mediation effects.

Conclusions: Our findings provide weak support for associations between some POPs and risk of childhood asthma, and provide some indications of a potential independent role of gut microbiota on childhood asthma.

Introduction

Asthma is one of the most common chronic conditions in childhood, and together with lower respiratory tract infections (LRTIs), is a leading cause of hospitalisation in young children.[1,2] Worldwide variability and temporal trends in the prevalence of asthma symptoms—decades of increasing prevalence which may have peaked at the turn of the century in higher-income countries—provide strong support for environmental causal risk factors.[3,4]

Epidemiological evidence suggests that prenatal and early-life exposure to ubiquitous environmental chemicals may disrupt the developing immune system and increase the risk of developing allergic diseases (reviewed by Gascon et al.[5]). Several studies have reported both positive and inverse associations between exposure to persistent organic pollutants (POPs) and asthma, wheeze, and related symptoms; including associations with polychlorinated biphenyls (PCBs), organochlorines pesticides (OCPs), brominated flame retardants including polybrominated diphenyl ethers (PBDEs), and poly-and perfluoroalkyl substances (PFASs), used as surfactants and repellent coatings.[6-9]

While many of these compounds have been prohibited, restricted or phased out, human exposure continues due to their persistence in the environment, and the long product life cycle of some chemically treated household products. The primary source of exposure to most POPs is via ingestion of contaminated food, notably breast milk for infants, although inhalation and ingestion of house dust represent an important exposure route for PBDEs and PFASs.[10-13] *In utero* exposure also occurs via placental transfer.[14,15]

More hygienic conditions and a reduction in infections have been postulated to partly explain rising allergic disease prevalence (the 'hygiene hypothesis').[16,17] This hypothesis has expanded to involve factors linked to urbanisation and Westernisation (reviewed by Douwes and Pearce[18]), and more recently, decreased exposure to organisms humans co-evolved with, including microbes (the biodiversity or 'old friends' hypothesis).[19] Prospective studies provide evidence that early-life LRTIs predict the development of asthma,[20,21] although it remains to be elucidated if this is a causal association or rather reflects underlying susceptibility or common causes.

Gut microbiota play a crucial role in maturation of the immune system,[22,23] and perturbations of microbiota composition (dysbiosis) and bacterial 'imprinting' during sensitive developmental windows may have long-lasting consequences for health. Short-chain fatty acids (SCFAs), which are primarily produced by gut microbiota during fermentation of non-digestible fibres, have also been shown to be immunomodulatory; for example, SCFAs induce regulatory T cells and activate G-protein-coupled receptors (GPR41 and GPR43).[24,25] In mice, a fibre-rich diet and the concomitant increase in circulating SCFAs were protective against allergic airway inflammation.[26] Emerging evidence from prospective epidemiological studies indicate that early-life gut microbiota composition, diversity, and SCFA metabolites may play a role in allergic disease development; greater microbial diversity and higher levels of anti-inflammatory SCFAs have been postulated to be protective.[27-30]

Furthermore, environmental chemicals interact with gut microbiota. Experimental studies in mice have found that metals and a dioxin (2,3,7,8-tetrachlorodibenzofuran) perturb the microbiota composition and metabolic activity.[31,32] Microbiota can also biotransform xenobiotics into more bioavailable forms, as has been observed for e.g., arsenic.[31] We evaluated the risk of asthma and LRTIs in association with early-life chemical profiles and microbiota diversity and metabolic products, and explored potential mediation of chemical–outcome associations by these microbiota markers.

Methods

Study population. We used data from the prospective Norwegian HUMIS-NoMIC birth cohort, which has been described in detail elsewhere.[33,34] Briefly, mothers were recruited for the HUMIS subcohort in 2003–2009 by public health nurses during routine postnatal care home visits in seven counties across Norway. Mothers were recruited for the NoMIC subcohort by a pediatrician at the maternity ward in Østfold hospital in Southern Norway in 2002–2005, and for every mother of a preterm infant (< 37 weeks gestation) enrolled, two mothers of term infants were consecutively enrolled. All mothers were asked to collect breast milk on 8 consecutive days before the child reached 2 months of age. In addition to collecting breast milk samples, participants of the NoMIC subcohort were asked to collect a fecal sample 4 days postpartum and fecal samples from their infants when they were 4, 10 and 30 days, and 4, 12 and 24 months old. Minor deviations in this sampling protocol, such as collection by breast pump, were accepted (as elaborated in the Supplemental Methods). The HUMIS-NoMIC cohort comprises 2606 participants (2052 in HUMIS and 554 in NoMIC). The studies were approved by the Regional Committees for Medical and Health Research Ethics and the Norwegian Data Inspectorate, and written informed consent was obtained from all participating women prior to enrolment.

Due to financial constraints, the pooled breast milk samples for only a subset of women have so far been analysed for environmental chemical levels; up to 1028 samples, depending on the chemical. We restricted analyses to mother–child pairs with singletons. For analyses based on chemicals assayed in breast milk samples, the sample size was 993, of which 157 were oversampled for small for gestational age (SGA) (<10th percentile of sex-specific Norwegian standards[35]), rapid growth in the first six months (change in weight z-score > 0.67[36]), or (at enrolment) for preterm birth; for analyses based on fecal samples, the sample size was 438 (see Supplemental Figure S1 for a flow chart of the study population samples).

Respiratory health outcomes. The primary outcome of interest was childhood asthma. We linked to the Norwegian Patient Registry to identify children who had ever been diagnosed with asthma by a health specialist (based on the International Classification of Diseases, 10th revision (ICD-10) codes J45 and J46). The registry collects diagnostic data from all hospitals and outpatient clinics in Norway. Individual records are available from 2008 onwards, when

children (n = 993 sample) were a median of 3.7 years old [interquartile range (IQR), 2.9–4.5; range, 0–5.1], and were available and up until August 2014 at the time of linkage, when children were 10.4 years old (IQR, 9.6–11.2; range, 4.8–11.8). We also evaluated two secondary outcomes which were ascertained based on questionnaires administered to mothers at 6, 12 and 24 months (2 years) postpartum: current asthma at 2 years of age, and the occurrence of one or more LRTIs by 2 years, defined as maternal-reported pneumonia, bronchitis or respiratory syncytial virus infections. Asthma at 2 years of age may represent early-onset transient or persistent wheeze phenotypes,[37] clinically diagnosed or interpreted by the mother as asthma, which may resolve later in childhood.

Chemical exposure assessment. Chemicals were measured at different laboratories in several batches, as previously described,[38-40] and as elaborated in the Supplemental Methods. We evaluated the 26 contaminants which were measured and detected in ≥ 70% of samples: seven dioxin-like (DL) PCBs (mono-*ortho* congeners 105, 114, 118, 156, 157, 167, and 189) and seven non-dioxin-like (NDL) PCBs (congeners 74, 99, 138, 153, 170, 180, and 194); four organochlorine pesticides (OCPs), hexachlorobenzene (HCB), β-hexachlorocyclohexane (β-HCH), oxychlordane, and dichlorodiphenyldichloroethylene (DDE); six PBDEs, congeners 28, 47, 99, 100, 153, and 154; and two PFASs, perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS).

Values below the limit of detection (LOD) (or limit of quantification, if an LOD was not provided by the laboratory), were imputed (0–2.5% for 24 chemicals, and 10–18% for two chemicals). We used single conditional imputation, including the other exposure variables, maternal age, and parity as predictors, using maximum likelihood estimation assuming a log-normal distribution.[41] Molar concentrations of the DL-PCBs, NDL-PCBs, OCPs, PBDEs, and PFASs were summed. Breast milk was collected at a median of 32 days postpartum (IQR, 24–43). Because we did not observe a significant trend in concentration levels with timing of sampling for nearly all chemicals (the exception was a minor decreasing trend for PFOA), we did not calibrate by sampling period.

SCFAs and gut microbiota diversity. Eight SCFAs were measured at the Karolinska Institute (Stockholm, Sweden) and at Lovisenberg Diaconal Hospital (Oslo, Norway) following previously published analytical protocols,[42-44] as described in the Supplemental Methods. We did not assess *n*-caproic or *i*-caproic in the present analysis as the majority (> 72%) of values were below the detection limit. We imputed SCFA data below the LOD using single conditional imputation.[41] Characterisation of microbiota composition, using DNA extraction and purification, PCR amplification of the V4 region of the 16S rRNA gene, and sequencing and processing, was performed at the University of Colorado (Denver, CO, USA), as elaborated in the Supplemental Methods. We calculated the Shannon diversity index, a robust measure of taxonomic diversity[45] that accounts for both richness and evenness of species.

Covariates. Information on potential confounders was obtained from the questionnaires completed by the mothers 6, 12, and 24 months postpartum, and supplemented with information from the Medical Birth Registry of Norway (child's sex, gestational age, birth weight, and maternal pre-pregnancy height and weight, and smoking during pregnancy).

Statistical analysis. First, we estimated associations between POPs and the three respiratory outcomes. To account for the complex correlation pattern of the POP exposures and to mitigate potential estimation problems due to collinearity, we modelled all POPs simultaneously using a multivariable (multi-pollutant) variable selection method, elastic net regression.[46] A generalised linear model (GLM) is fitted with an added penalty that maximises the penalised log-likelihood in logistic regression, shrinking coefficients towards or exactly to zero, thereby achieving selection. The elastic net penalty is a weighted sum of the ridge penalty, proportional to the sum of the squared regression coefficients, and the lasso penalty, proportional to the absolute value of the coefficients, which allows for selection of groups of collinear variables.

We refit the selected subset in a multi-pollutant unpenalised logistic regression (with ordinary maximum likelihood estimation) model to obtain effect estimates which were not biased (shrunken) due to penalisation. For comparison, we also present the single-pollutant effect estimates from logistic regression models, and applied false discovery rate correction.[47] We also assessed associations with summed molar concentrations of PCBs, OCPs, PBDEs, and PFASs, under the assumptions that each molecule has a single potent binding site, and that chemicals within a group act via common biological pathways.

Based on *a priori* literature-based knowledge, we considered the minimal sufficient adjustment set for the models of POP exposures and asthma and LRTI outcomes to include maternal age (years), parity (nulliparous, multiparous), pre-pregnancy body mass index (continuous), maternal education (≤ 12, > 12 years education, as a proxy of socioeconomic status), diet, and maternal stress (see Supplemental Figure S4A). We also tested models further adjusted for covariates for which evidence for an association with the exposure and/or outcome is weaker: child birth year (continuous), child sex, delivery mode (C-section vs. vaginal), duration of any breastfeeding (months), parental asthma (maternal or paternal), maternal fish consumption in the year before delivery (≤ 23, > 23 servings), and the number of playmates at age 2 years, as a proxy of daycare attendance (≤ 4, > 4 playmates). We did not incorporate sampling weights (16% were oversampled for preterm, SGA, or rapid growth) in any of the models because this may reduce precision when covariates related to sampling are included in models of exposure–outcome associations,[48] and only adjusted for sampling design covariates deemed to be confounders.[49]

We used multiple imputation by chained equations[50] to impute missing outcome data (0% for registry-based asthma by 2014, 13% for self-reported asthma at 2 years, and 21% for LRTI), chemical exposure data (because the sample was not analysed for that chemical; 0–4% for 23 chemicals, and 11% for 3 chemicals) and covariate data (0–3% for covariates

in the minimally adjusted models, and 0–23% in the further adjusted models). We imputed 100 datasets using predictive mean matching for continuous variables and logistic regression for binary variables, and estimates were combined using Rubin's rules (see Supplemental Methods for additional details). As a sensitivity analysis, we also ran complete case analyses.

Chemical exposures were natural log (ln)–transformed in all analyses to reduce the influence of extreme values. We input exposures and covariates which were scaled to two standard deviations (2-SD) to impart variables with same prior probability of selection independent of the contrast in levels, and to facilitate comparison of coefficients across variables.[51] In elastic net models, we set covariates to be unpenalised. We determined the optimal level of penalisation using 10-fold cross-validation, repeated 10 times to improve stability, and selecting the model yielding the minimum prediction error. We repeated elastic net modelling using 100 multiply imputed datasets, and averaged regression coefficients for the exposures that were selected in at least half of the models.[52] We also assessed potential effect modification by child sex and maternal education.

Second, we assessed the associations between Shannon diversity and total SCFA levels and the respiratory outcomes using unpenalised logistic regression models, and for the six individual SCFAs using penalised logistic regression models. We assessed the samples from 4, 12 and 24 months; this was because the most samples had been analysed for these sampling time points, increasing statistical power, and because this represents a time period when colonisation of the gut is well underway but highly plastic[53,54] and when the immune system is still rapidly maturing.[55] Furthermore, there were few complete-case (n = 9 and 85 for SCFAs, and 99 and 122 for diversity for all six and the latter three sampling periods, respectively). We scaled SCFAs to 2-SD prior to modelling. As a sensitivity analysis, we repeated the modelling using the relative abundance of each SCFA, normalised to the total SCFAs, and using isometric log-ratio transformation of SCFAs to account for the compositional data, or dependence between SCFA proportions.[56,57] These models were adjusted for maternal education, preterm birth, and maternal smoking during pregnancy and when the child was one year old. As a sensitivity analysis, models were further adjusted for maternal fish consumption during pregnancy, breastfeeding, and delivery by C-section (see Supplemental Figure S4B).

Third, we assessed associations between POP exposures, summed across chemical classes, and diversity and total SCFAs using linear regression models, and also associations for the 26 POPs using penalised regression. These models were adjusted for maternal education, breastfeeding, delivery via C-section, and whether the child had recently received antibiotics (see Supplemental Figure S4C). We also used multiple imputation for analyses based on microbiota data, and presented estimates based on complete cases for comparison (imputing to n = 438 for microbiota marker–outcome analyses and n = 298 for POP–microbiota marker analyses, with complete cases ranging from 111–390 and 79–271, respectively).

If there was a robust association between a chemical and outcome which overlapped with associations with gut microbiota markers, we inputted these in an exploratory causal

mediation analysis to assess if microbiota mediated the direct association[58] (as illustrated in Supplemental Figure S5). We did not pursue a longitudinal approach given the large number of missing blocks across sampling time points.

We used R software (version 3.2.3; R Foundation for Statistical Computing, Vienna, Austria) to perform statistical analyses, including the *glmnet* package for elastic net modelling,[59] and the *mice* imputation[50] and *mediation*[60] packages.

Results

Study population. Characteristics of the study population (n = 993) are presented in Table 1. 4.4% of children were registered as diagnosed with asthma by 2014. 7.0% were reported by the mother to have asthma at 2 years of age, of which 33.3% of these had a later diagnosis in the registry, and 19.9% had had an LRTI by 2 years of age. More than half of the mothers were older than 30 years when they became pregnant, had given birth before, and were within the normal weight range prior to pregnancy, and 11% smoked at the beginning of pregnancy. Nearly a quarter of children had received antibiotics by one year of age. Characteristics for the subset with fecal samples (n = 438) are presented in Supplemental Table S1, and were similar.

Chemical exposures. Within each chemical class, the highest breast milk concentrations were observed for DDE (46.4 ng/g), PCB-153 followed by PCB-138 (32.2 and 19.9 ng/g, respectively), PBDE-47 (1.1 ng/g), and PFOS (110 ng/L) (Supplemental Table S2). 37% of pairwise correlations ($r_{pearson}$) exceeded 0.5, and 13% exceeded 0.8 (Supplemental Figure S2). Correlations within each chemical class were moderate to high (an IQR of r_p = 0.65–0.82 for PCBs, 0.31–0.65 for OCPs, 0.63–0.94 for PBDEs, and 0.56 for the two PFASs). Correlations between PCBs and OCPs were moderate (r_p = 0.44 for Σ_{14}PCBs and Σ_4OCPs), whereas correlations between other chemical classes were generally low or non-significant.

Gut microbiota metabolites and diversity. The three most abundant SCFAs were acetic, propionic, and butyric acid, comprising a median of >95% of the total SCFAs at all sampling points (Supplemental Figure S3). During the six sampling periods in the first 2 years of life, the proportion of acetic acid decreased and the proportion of all other SCFAs increased. Shannon diversity increased over time, with a marked increase from 4 months to 12 months of age. In contrast, total SCFAs were relatively stable. Both diversity and total SCFAs were weakly correlated over 4 months to 2 years (r_s = –0.08–0.23 and 0.13–0.24, respectively); within sampling periods, the most abundant SCFA, acetic acid, was highly correlated with total SCFAs, and *i*-butyric and *i*-valeric acid, which reflect protein fermentation,[61] were also highly correlated (r_s > 0.93–0.95), whereas the other within-period SCFA correlations were moderate.

Associations with covariates. Associations between exposures or outcomes and covariates were generally in the expected direction (Supplemental Table S3). Having a parent with asth-

Table 1. Selected sample characteristics for mothers and children (HUMIS-NoMIC, Norway, n=993).

	n (%) or median (IQR)	Missing
Maternal characteristics		
Age at start of pregnancy, years		0
16–26	249 (25.1)	
27–33	527 (53.1)	
34–44	217 (21.9)	
Education, years completed		14
<12	139 (14.4)	
12	212 (22.0)	
13-16	392 (40.7)	
≥17	220 (22.8)	
Pre-pregnancy BMI (kg/m^2)		28
Underweight (<18.5)	39 (4.0)	
Normal weight (18.5–24.9)	596 (61.8)	
Overweight (25–29.9)	229 (23.7)	
Obese (≥30)	101 (10.5)	
Parity		1
Primiparous	408 (41.1)	
1	360 (36.3)	
≥2	224 (22.6)	
Caesarean-section	166 (16.7)	0
Smoking during pregnancy	108 (11.0)	10
Fatty fish consumption, servings	23.1 (9.1–48.0)	32
Maternal asthma	70 (7.3)	32
Child characteristics		
Year of birth		0
2002–2003	438 (44.1)	
2004–2005	455 (45.8)	
2006–2009	100 (10.1)	
Sex: girl	451 (45.4)	0
Birth weight, grams	3560 (3180–3958)	0
<2500 g	62 (6.2)	
Small-for-gestational age	119 (12.0)	0
Preterm (<37 weeks gestation)	96 (9.7)	0
Rapid growth, 0–6 months	230 (23.1)	3
Breastfeeding, any, months	13 (9–15)	6
Breastfeeding, any, for at least 12 months	575 (58.3)	6
Either parent has asthma	115 (12.0)	32
>4 playmates at age 2 years	437 (57.2)	229
Pets during infancy	345 (40.5)	141
Antibiotics before 1 year	218 (24.0)	83
Asthma, diagnosed 2008–2014	44 (4.4)	0
Asthma, maternal-reported at age 2 years	60 (7.0)	134
≥1 LRTI before age 2 years	156 (19.9)	211
No. of LRTI episodes before age 2 years		211
0	626 (80.1)	
1	101 (12.9)	
2–9	55 (7.0)	

BMI, body mass index; LRTI, lower respiratory tract infection.

ma was associated with an increased risk of asthma diagnosis. Notably, antibiotic treatment before 1 year of af age was associated with increased risk of asthma and LRTI, and a lowered Shannon diversity. POP concentrations generally increased with maternal age, decreased with parity, and increased with the year the child was born.

Associations between POPs and respiratory outcomes. Coefficients for multi-pollutant penalised regression variable selection models, and the unpenalised selected subset-model, are shown in Figure 1, along with the coefficients for the more traditional single-pollutant modelling approach (numerical results in Supplemental Tables S5–S8). Three exposures were consistently detected in penalised models as associated with registry-based asthma (when most children were around 10 years of age): β-HCH was associated with an increased risk, and PCB-118 and 138 were associated with a decreased risk. The associations for β-HCH and the PCB-138 were the most precise in multivariable regression models [Supplemental Table S8; odds ratio (OR) = 2.99 per 2-SD increase in β-HCH; 95% CI: 1.65, 5.43 and OR = 0.43 for PCB-138; 95% CI: 0.20, 0.91], and both were selected in further adjusted models. In multivariable unpenalised models with summed chemical groups, Σ_4OCPs was positively associated (OR = 2.05; 95% CI: 1.06, 3.94) and Σ_{14}PCBs was inversely associated (OR = 0.39; 95% CI: 0.18, 0.82) with registry-based asthma by 10 years of age. Six exposures were associated with self-reported asthma at 2 years of age; PCB-170 and 189, and β-HCH with an increased risk, and PCB-105 and 138, and BDE-154 with a decreased risk, although variable selection results were less stable across penalised models than those for registry-based asthma by 10 years, and no exposures were consistently selected in further adjusted models. The estimates for PCB-138 (OR = 0.42; 95% CI: 0.18, 0.97) and β-HCH (OR = 1.96; 95% CI: 1.03, 3.73) and asthma at 2 years of age were most precise. Associations with summed chemical groups showed the same directions of effect, although estimates were also less precise for self-reported asthma at 2 years than registry-based asthma at 10 years. None of the single-pollutant associations survived correction for multiple testing. No exposures were associated with occurrence of an LRTI by 2 years. Results based on multiple imputation and complete case analyses were generally similar (Tables S5–S8). There was some evidence of effect modification by child sex for PCB-138 and self-reported asthma at 2 years; a positive association for girls and a negative association for boys. There was little evidence that other associations were modified by child sex (Table S8), or by maternal smoking or education (data not shown).

Associations between gut microbiota markers and respiratory health outcomes. Shannon diversity at 4 months of age was positively associated with asthma ascertained both at age 10 years and at 2 years (Figure 2 and Table S9–S10). Diversity at 12 months of age was inversely associated, although imprecisely, with self-reported asthma at 2 years and occurrence of an LRTI by 2 years. Other estimates for diversity were closer to null. Associations for total SCFAs and most individual SCFAs were imprecise, and generally inconsistent in direction across

the three early-life sampling time points. *i*-valeric acid at 12 months of age was positively associated with asthma at around 10 years. Propionic and *i*-butyric acid at 2 years were associated with decreased risk of an LRTI by 2 years. Estimates for *i*-valeric and *i*-butyric acid were inconsistent in direction in some univariable versus multivariable regression analyses, likely due to their very high inter-correlation levels. In a sensitivity analysis with SCFAs analysed compositionally as log-contrasts and compared to reduced models only including confounders, only the inverse association between SCFAs at 2 years and LRTI was significant (likelihood ratio test $p = 0.01$). Regression coefficients based on multiply imputed data were somewhat inconsistent with complete case analyses, particularly for weak and imprecise associations, and for the microbiota markers for which a large fraction was imputed (i.e., SCFAs at 4 months and diversity at 2 years, for which n < 100 for covariate-adjusted associations).

Associations between POPs and gut microbiota markers. Both DL- and NDL-∑PCB exposures were associated with reduced Shannon diversity at 2 years (Figure 3 and Tables S11). This appeared to be driven by PCB-114, based on selection within penalised regression models (Table S12). $\sum_4$OCPs was imprecisely associated with diversity, both inversely at 1 year and positively at 2 years of age; β-HCH was selected at both time points in penalised models. $\sum_{14}$PCBs was positively associated with total SCFAs at 4 months and 1 year, and $\sum_4$OCPs was inversely associated with total SCFAs at 2 years, although associations with total SCFAs were imprecise. Associations were close to null for $\sum_6$PBDEs and $\sum_2$PFASs, except for an inverse association between $\sum_2$PFASs and total SCFAs at 2 years, which was seemingly driven by an inverse association between PFOA and acetic acid (Table S12). Regression coefficients were generally attenuated and less precise for analyses based on multiply imputed compared to complete case data.

Mediation analyses. We tested if the β-HCH and asthma association was mediated by total SCFAs at 4 months, or if the ∑DL-PCBs and asthma association was mediated by Shannon diversity at 4 months. Neither path showed a significant mediation; i.e., the confidence intervals of the natural indirect (mediated) effects included zero (Figure S5).

Discussion

In this prospective cohort study, we observed that β-HCH was positively associated with asthma at 2 years of age and later childhood asthma. Different PCBs were positively and inversely associated with asthma, including a robust inverse association between the non-dioxin-like PCB-138. We did not observe consistent associations between POPs and LRTIs. We observed suggestive evidence that SCFAs at 4 months were protective for asthma. NDL and DL-PCBs were associated with reduced microbial diversity at 2 years of age, and OCPs with lower SCFAs at 2 years of age. Microbial diversity and metabolites did not appear to mediate chemical–asthma associations.

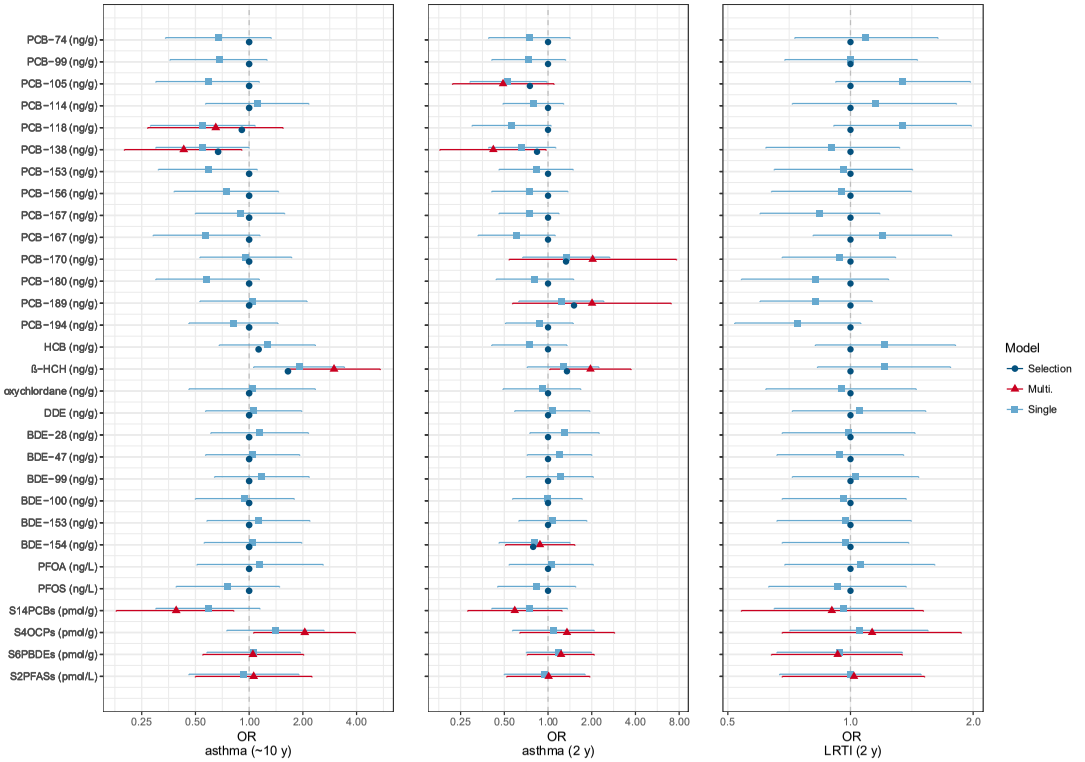

Figure 1. Odds ratios (OR) and 95% confidence intervals for the three respiratory health outcomes per 2-standard deviation increase in natural-log transformed exposure concentrations. Single-pollutant unpenalised logistic regression coefficients (light blue; square); multivariable elastic net penalised logistic regression selection and coefficients (dark blue; circle); and the selected subset of chemicals remodelled in a multivariable unpenalised logistic regression model (red; triangle) are presented. Coefficients from single- and multivariable unpenalised logistic regression models are also presented for the summed chemical groups. Models were adjusted for maternal age, parity, pre-pregnancy BMI, and maternal education; missing data was multiply imputed (n=993; exposure increments and numerical results provided in Supplemental Tables S5–S8). Abbreviations: (P)BDE, (poly)brominated diphenyl ether; DDE, dichlorodiphenyldichloroethylene; HCB, hexachlorobenzene; β-HCH, β-hexachlorocyclohexane; LRTI, lower respiratory tract infection; OCP, organochlorine pesticides; PCB, polychlorinated biphenyls; PFAS, poly-and perfluoroalkyl substances; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.

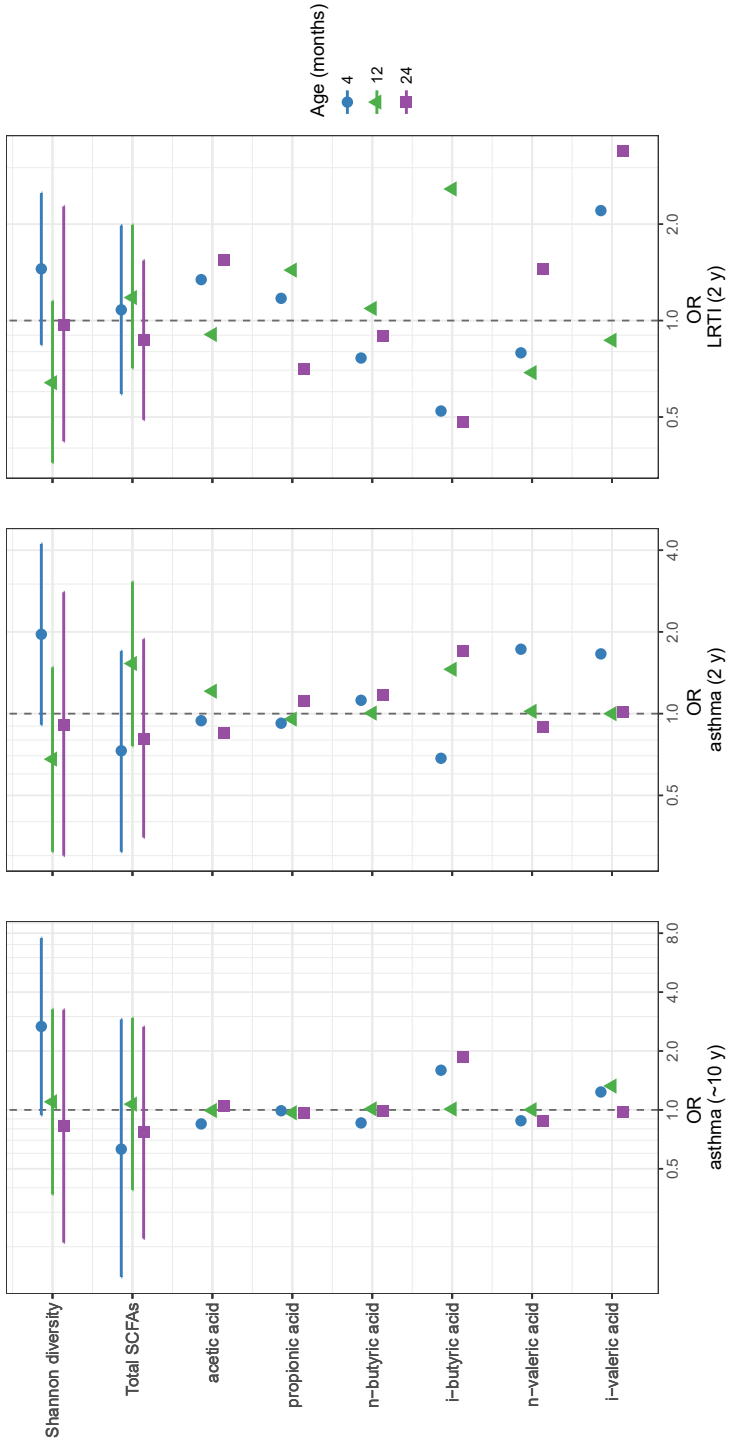

Figure 2. Odds ratios (OR) and 95% confidence intervals for the three respiratory health outcomes per 2-standard deviation increase in Shannon diversity and SCFA levels (mmol/kg) across 3 sampling time periods in early life. Unpenalised logistic regression coefficients are presented for Shannon diversity and total SCFAs, and multivariable penalised (α=0.1) regression coefficients for the individual SCFAs; models were estimated using multiply imputed data (n=438). Models were adjusted for preterm birth, maternal smoking during and after pregnancy, and maternal education (increments and numerical results provided in Supplemental Tables S9–S10). Abbreviations: LRTI, lower respiratory tract infection; SCFAs, short-chain fatty acids.

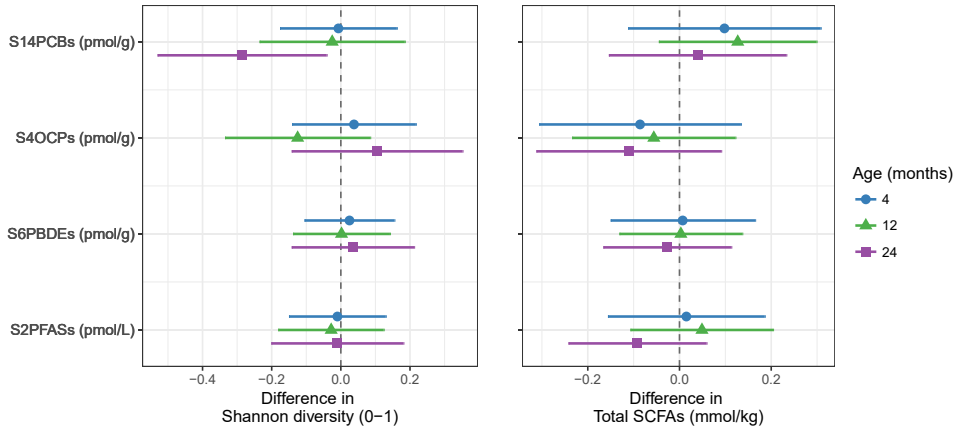

Figure 3. Differences in 2-standard deviations of Shannon diversity and total SCFA concentrations across 3 sampling time periods in early life associated with a 2-standard deviation increase in natural-log transformed exposure concentrations. Multivariable unpenalised logistic regression coefficients are presented, estimated based on multiply imputed data (n=298). Models were adjusted for maternal education, breastfeeding, C-section, and recent antibiotics (numerical results provided in Supplemental Table S11 and for individual POPs in Table S12).

POPs–outcomes. We found associations for β-HCH and PCB-138, highly persistent legacy POPs which have shown declining serum concentrations in the past three decades in Norway.[62] β-HCH is one of the isomers of the technical mixture HCH, which along with isolated γ-HCH (known as Lindane), was widely used as an insecticide until the 1990s.[63] PCB-138 had the highest breast milk levels after PCB-153 in this study population. To our knowledge, this is the first prospective study to assess HCH exposure in relation to risk of asthma. A prospective Danish study (n = 872) that assessed maternal serum levels in association with offspring asthma medication use between 6 and 20 years of age reported an association with the organochlorine fungicide HCB, and a null association with DDE.[7] This study also found positive associations with DL-PCB-118 and weak positive associations with five other PCBs tested. We found both inverse and weak positive associations between PCBs and asthma, but the most robust association was an inverse association between with non-dioxin-like PCB-138. In the same Danish study population, PCBs, HCB, and DDE were also associated with airway obstruction, but not allergic sensitisation.[64]

This relatively large study did not confirm findings of a meta-analysis of 10 European birth cohorts (n = 4608) which reported a robust positive association between prenatal DDE exposure and ever-bronchitis and ever-wheeze by around 18 months, and a weaker and inconsistent association with PCB-153.[65] This meta-analysis included the present study population (HUMIS-NoMIC cohort), although a smaller sample size was then available with measured POP concentrations (n = 386 versus 993 now). Part of the discrepancy may be attributable to the lower levels of POPs in the study population and Norway in general, com-

pared to other European countries.[66] Other, smaller studies have reported both positive[67-70] and inverse associations[67,70] between early-life PCB and OCP exposures and markers of allergic and respiratory diseases, including wheeze, eczema, IgE, and upper and lower RTIs. It has been postulated[68] that early-life exposure to some PCBs, specifically DL-PCBs, may reduce the risk of developing allergic diseases through aryl hydrocarbon receptor (AhR)-mediated immunosuppression.[71]

We did not identify associations between PFOS or PFOA and childhood asthma. A cross-sectional analysis in the United States (NHANES, n = 1877) reported a positive association for PFOA, an inverse association for PFOS, and null results for two other PFASs.[6] Prenatal PFAS exposure has been linked to reduced vaccine antibody titres in childhood, providing suggestive evidence that PFASs exert immunosuppressive effects.[72,73] A few small case-control studies have assessed levels of PBDEs in indoor dust in relation to asthma. A prospective study reported null findings for multiple PBDEs and organophosphate flame retardants.[74] and a cross-sectional study reported a weak inverse association with BDE-99 and null findings for BDE-47 and 209.[75]

Diversity/SCFAs–outcomes. In a seminal study, Arrieta et al.[30] examined early-life gut microbiota composition and SCFAs in relation to wheezing and skin atopy at age 1 year in Canadian infants. Although the sample size for the SCFAs analysis was small (n = 13 cases with atopy and wheeze and n = 13 controls), they observed reduced acetic acid levels at 3 months of age for cases. Furthermore, in a larger sample size (n = 324), they found that compositional dysbiosis (of the genera *Faecalibacterium, Lachnospira, Veillonella*, and *Rothia)*, but not diversity, was associate with risk of asthma and allergic disease. A small study (n = 47) in Sweden previously observed that Shannon diversity at 1 week and 1 month, but not 12 months, was inversely associated with asthma at 7 years of age; diversity was not associated with other atopic disease markers (allergic rhinoconjunctivitis, eczema or skin prick reactivity).[28] A larger study of 411 children in Denmark found that gut microbiota diversity at 1 month and 12 months of age was inversely associated with several markers of allergic sensitisation, but not asthma.[27]

We observed the strongest associations for gut microbiota diversity at 4 months and increased risk of asthma, counter to the *a priori* hypothesis that diversity is protective. The different sample sizes with data available on microbial markers hampered our ability to compare coefficients and identify sensitive windows of exposure. A recent review of mouse-model and human studies on microbial immune system priming and allergic disease development concluded that the first 100 days may the most important early-life critical window.[76] A lack of diversity in early-life may have longer-term consequences for host immunity, whereas SCFAs may have more immediate effects of suppressing immune responses and asthma symptoms. This is supported by a randomised control trial in asthmatics that found a reduction in clinical symptoms and cytokine production following probiotic supplementation with *Lactobacillus gasseri*,[77] and by experimental mouse models, in which increased acetic acid resulting from

a high-fibre diet suppressed airway inflammation via transcription of Foxp3, and promotion of T-regulatory cells.[78] SCFAs have generally been shown to exert anti-inflammatory effects, inhibiting histone deacetylase (HDAC) and inducing T-regulatory cells, and acting as predominantly agonists of G protein-coupled receptors. Maternal vaginal microbiota[79] and early-life nasopharyngeal (upper respiratory tract) microbiota compositional changes[80] have also been found to antedate asthma development. It is unclear if pathways are similar for gut and lung microbiota and immune system signalling.

Maternal and child antibiotic use has been associated with subsequent asthma,[81] and this has been supported by experimental evidence from animal models.[82] This has been attributed to gut microbiota dysbiosis of the child, as antibiotics have been shown to lead to both rapid transient and long-lasting changes to gut and lung microbiota,[83] and maternal microbiota influence offspring composition through vertical transmission. However, antibiotics prescribed for non-respiratory illnesses in early life were not associated with an increased risk of asthma.[84] The causal mechanisms of the antibiotic–asthma association requires further research, and may be partly attributable to common risk factors and underlying susceptibility.[85]

POPs–diversity/SCFAs. As far as we are aware, no human studies have evaluated associations between environmental chemicals and microbiota. We found limited evidence that early-life exposure to PCBs and OCPs perturbed gut microbiota. Σ_{14}PCBs showed a robust association with lower diversity at 2 years of age; however, other association were closer to null or less precise. We speculate that changes in microbiota composition and metabolite production induced by POPs might be relatively small in magnitude, and that there might be greater statistical power for detecting perturbations once gut microbiota are closer to a more stable and resilient state at 2 years compared to the 4 month age, during which microbiota are undergoing succession and exhibit greater intra-individual variability.[86] Recent experimental studies support that the interplay between POPs and microbiota is mediated by the AhR[32]; POPs are potent ligands of the AhR, and AhR activity can modulate microbiota, and conversely, microbial metabolites can activate the AhR, which in turns mediates the toxicity of xenobiotics.[87]

Strengths and limitations. To our knowledge, this study included the largest number of chemicals evaluated simultaneously in relation to respiratory and allergic diseases, and is one of the largest studies to date. We used a multi-pollutant approach to reduce confounding bias due to correlated co-exposures. Another strength of this study is that detailed data on important potential confounders, such as breastfeeding and antibiotic use, was available. Breast milk levels of POPs served as a proxy of early-life offspring exposures, and directly reflect the dose via breastfeeding. We did not attempt to model temporal variability in prenatal and postnatal exposure levels over time, which are highly correlated. Absolute levels of *in utero* exposures to protein-bound PFASs are underestimated by breast milk levels, as transfer of protein-bound PFASs from maternal serum into breast milk is less efficient than for lipophilic

POPs,[88] although the relative ranking would be expected to remain the same.

This study has limitations. Reliance on questionnaire data led to a loss to follow-up in maternal-reported outcomes for the child at age 2 years (13% for asthma and 21% for LRTI), and thereby a loss of statistical power and potential selection bias; this was not a problem for the registry-based asthma outcome. Based on prevalence studies of asthma in Norway, it is likely that the national register captured severe cases, but less severe asthma or chronic wheeze cases, along with children who were younger at the time of linkage, were more likely to have been misclassified as non-asthmatic. Differential misclassification of outcomes with respect to chemical exposures is possible, in that accuracy of maternal-reported outcomes and likelihood of visiting the hospital may differ somewhat with socioeconomic status, and exposures may too. Prevalence of asthma in Norwegian population-based studies is heterogeneous; ever-asthma (lifetime asthma) based on parental-reports and medications in 10 years olds recruited in Oslo was 20.2%[89]; current asthma was 5.7% at 3 years and 5.1% at 7 years of age in a large Norwegian birth cohort, ascertained based on parental-report in combination with asthma medication use.[90]

We cannot exclude that effect estimates suffer from residual confounding due to unmeasured exposures. A small number of prospective studies provide suggestive, although limited, evidence that early-life exposure to other environmental chemicals may be associated with asthma and other allergic diseases, including organophosphate pesticides, phthalates, and bisphenol A[9,91-95]; however, the magnitude of potential confounding bias is expected to be small given that these non-persistent exposures generally exhibit low correlation levels with the POPs considered in the present study.[96]

Furthermore, we cannot exclude that the mediation analyses did not meet the assumption of no residual confounding of the various relationships, including confounding of the mediator–outcome relationship (Figure S5), nor the presumed the temporal ordering of the POP–microbial markers associations, which may exhibit bidirectional effects.[97] With a more complete matrix of repeated measures of gut microbiota markers, we would have pursued longitudinal modelling which has the advantage of increased statistical power. We did not study phylogenetic composition of microbiota in this study; however, it has been postulated that diversity rather than single species are most relevant for optimal immune system development.[98,99]

In conclusion, we found evidence that some organochlorine pesticides were associated with increased risk of developing asthma, and also observed that several PCBs were inversely associated with asthma. The present study provides weak support for the hypothesis that reduced gut microbial diversity and reduced production of microbial metabolites predispose asthma development. Although our study did not identify consistent evidence that gut microbiota diversity and function modulated associations between environmental chemicals and asthma, the interplay between chemical exposures, gut microbiota, and allergic disease warrants further research.

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Supplemental Material

Supplemental Methods

Study population

Recruitment and eligibility criteria: Participants were eligible to participate in the HUMIS and NoMIC cohorts if they spoke fluent Norwegian and were a resident of the county in which they were recruited. The majority of participants were from Finnmark, Oppland, Østfold, Rogaland, Telemark, and Troms, and small number (<20 of the 993 included in the present study) were from Akershus and Nordland. These counties cover northern, southern, inland and coastal areas of Norway. 868 (98.1%) of the parents were both white, and the others were both Asian (0.8%), were both Inuit (0.7%), or other (0.4%; or this data was missing, 10.6%).

Ethics statement: The studies were approved by the Norwegian Data Inspectorate (HUMIS: refs. 2002/1398-2 and 2002/1398-7; NoMIC: ref. 2002/1934-2) and the Norwegian Regional Committees for Medical and Health Research Ethics (HUMIS: ref. S-02122; NoMIC: ref. 2002, S-02216).

Breast milk sample collection and handling: Mothers who consented to participate received pre-washed containers in which to collect their breast milk. They were asked to collect 25 mL of breast milk by hand in the morning on 8 consecutive days before the child reached 2 months of age. Minor deviations in this sampling protocol, such as the total number of sampling days and collection by breast pump, were accepted. 55% of women collected breast milk for fewer than 8 days. The median number of days over which breast milk was collected was 8 [interquartile range (IQR), 5–8 days; data missing for 19%]. 32% reported using a pump instead of hand milking (this information was missing for 7%). Milk samples were transferred to a 250 mL container and were kept frozen after collection; they were allowed to thaw as they were sent by regular mail to the coordinating centre, the Norwegian Institute of Public Health (NIPH), Oslo, Norway. The procedure differed for Østfold County, where samples were collected by study personnel and were kept frozen until transport. Samples were stored at NIPH at −20 °C until analysed.

Fecal sample collection and handling: Mothers who consented to participate received NoMIC questionnaires and containers for fecal sample collection at the maternity ward. Study personnel retrieved maternal and child fecal samples, and kept them frozen during transport. Samples were stored at −20 °C at NIPH until analysed.

Chemical exposure assessment

Analytical protocols and quality control measures have been described in detail elsewhere. We provide the relevant references and briefly describe the protocols here. To date, up to n =

1028 breast milk samples (specifically, aliquots from the samples pooled for each individual) have been assayed for some chemicals. We describe the methods for the set of chemicals measured and detected in ≥ 70% of samples for the study sample size used in the present study (n = 993).

PFASs: We used data for PFOS and PFOA, which were measured in 884 samples of the 993, as previously described.[1] Two batches of breast milk samples (n = 254 and 434) were analysed for PFASs at the Department of Environmental Exposure and Epidemiology (formerly the Department of Analytical Chemistry), NIPH (Oslo, Norway), using high performance liquid chromatography/tandem mass spectrometry (LC-MS/MS) according to a previously described protocol.[2,3] A second batch of samples (n = 196) were analysed at the Institute for Environmental Studies (IVM), Faculty of Earth and Life Sciences, VU University (Amsterdam, the Netherlands), using LC-MS/MS on a triple quadrupole mass spectrometer following a previously described protocol.[4]

PBDEs: We used data for six polybrominated diphenyl ethers (PBDE) congeners (BDE-28, 47, 99, 100, 153 and 154) which were measured in 957 samples of the 993. One batch (n = 393) was measured at NIPH using automated solid-phase extraction (SPE) extraction and a gas chromatography–mass spectrometry (GC–MS) system, as previously described.[5,6] The PBDE standards were obtained from Cambridge Isotope Laboratories (Andover, MA, USA). A second batch (n = 600) was subsequently analysed at NIPH. The same SPE extraction procedure was used,[5] while a GC–high resolution MS (GC-HRMS) with electron impact ionisation was used for quantification.[7,8]

PCBs and OCPs: Fourteen polychlorinated biphenyls [PCBs; non-dioxin-like (74, 99, 138, 153, 170, 180, and 194), and dioxin-like (105, 114, 118, 156, 157, 167, and 189)] and four organochlorine pesticides [(OCPs); hexachlorobenzene (HCB), β-hexachlorocyclohexane (β-HCH), 4,4'-dichlorodiphenyldichloroethylene (*p,p*'-DDE), and oxychlordane] were measured in two batches. The first batch (n = 468 of 993) was measured at the Norwegian University of Life Sciences (formerly the Norwegian School of Veterinary Science; Oslo, Norway), using GC-electron capture detector (GC-ECD) for OC pesticides and non-dioxin like PCBs, and using GC-low resolution MS (GC–LRMS) for the dioxin-like mono-*ortho* PCBs, as previously described.[9-12] A second batch of samples (n = 525 of 993) was measured (at the same time as the second batch analysis of PBDEs) at NIPH using the same SPE extraction procedure,[5] and GC-HRMS as previously described.[8,13]

Lipids: Lipids were determined gravimetrically.

Potential batch effects: The subsets of samples analysed were oversampled for outcomes which

might be related to the outcomes assessed in the present study (n = 157 were oversampled for preterm birth, small for gestational age, or rapid growth). We tested for systematic differences in measured levels between batches ('batch effects') using principal component analysis (PCA) visualisation and linear regression models, adjusted for birth year, maternal age, parity and oversampling-stratum. We found no clear evidence of batch effects, and therefore did not attempt to apply normalisation methods to correct for batch effects.

Analysis of fecal SCFAs

Short-chain fatty acids (SCFAs) are saturated aliphatic organic acids with fewer than 6 carbon atoms. The following fatty acids were analysed: acetic (C_2), propionic (or the IUPAC name, propanoic) (C_3), *iso*-butyric (i-C_4), *n*-butyric (C_4) (or butanoic), *iso*-valeric (i-C_5), *n*-valeric (or pentanoic) (C_5), *iso*-caproic (i-C_6), and *n*-caproic (or hexanoic) (C_6) acids. Caproic acid is often classified as a medium-chain fatty acid (MCFA). Other SCFAs, e.g., succinic acid, lactic acid, and formic acid, can be detected with lower frequencies, and were not measured in the present study. The majority of SCFAs are produced by carbohydrate fermentation (saccharolytic fermentation), although SCFAs are also produced by protein breakdown and amino acid fermentation (proteolytic fermentation). The branched-chain fatty acids (BCFA), i.e. *i*-butyric, *i*-valeric, *i*-caproic and also 2-methylbutyrate, are principally produced by amino acid fermentation (specifically valine, leucine, and isoleucine).[14,15]

SCFAs were measured in three batches. All fecal samples with a sufficient volume for the analysis (0.5 g) were analysed. Two batches (n = 734 and n = 65) were analysed at the Unger-Vetlesen Institute, Lovisenberg Diaconal Hospital (Oslo, Norway), with an LOD of 0.06 mmol/kg for all SCFAs. The fecal material (0.5 g) was homogenised after addition of distilled water containing 3 mmol/L of 2-ethylbutyric acid (as internal standard) and 0.5 mmol/L of H_2SO_4; 2.5 mL of the homogenate was vacuum distilled, according to the method of Zijlstra et al.,[16] as modified by Høverstad et al.[17] The distillate was analysed with gas–liquid chromatography (Agilent 7890 A, Agilent Technologies, Santa Clara, CA, USA), using a capillary column (serial no. USE400345H, Agilent J&W GC columns, CA, USA), and quantified using internal standardisation. Flame ionisation detection was employed. A third batch (n = 336) was analysed at the Department of Microbiology, Tumour and Cell Biology, Karolinska Institute (Stockholm, Sweden) using the same protocol,[18] with an LOD of 0.03 mmol/kg. SCFAs were assessed on a wet weight basis (mmol/kg), and the relative proportion of the total sum of SCFAs at each sample collection point was also calculated.

Analysis of gut microbiota diversity

We also calculated the Shannon diversity index, a measure of within sample (alpha) diversity: $H = -\Sigma p_i \ln(p_i)$, where p_i denotes the relative frequency of operational taxonomic unit (OTU) *i*. Not all samples were analysed, for several reasons, partly due to an industrial production error of the PCR plates used.

DNA extraction: Analysis of OTUs was performed at the Knight Lab at the Department of Chemistry and Biochemistry, University of Colorado (Boulder, Colorado, USA). First fecel samples were prepared for analysis by adding 1 ml Solution 1 (50 mM glucose, 25 mM Tris–HCl pH 8.0, and 10 mM EDTA pH 8.0) per 0.2 g feces. The samples were mixed by vortexing and left for 30–60 min on ice before 400 µL of the supernatant was diluted 1:2 in 4 M guanidinium thiocyanate (GTC). Five hundred microliters of sample were transferred to a sterile FastPrep®-tube (Qbiogene Inc., Carlsbad, CA, USA) containing 250 mg glass beads (106 microns and finer, Sigma-Aldrich, Steinheim, Germany), and samples were homogenised for 40 seconds in FastPrep® Instrument (Qbiogene). Wells in a 96-well Greiner U-plate (Greiner bio-one, Frickenhausen, Germany) were filled with 170 µL sample and 10 µL Silica particles (Merck, Darmstadt, Germany) and transferred to a Biomek® 2000 Workstation (Beckman Coulter, Fullerton, CA, USA). One percent Sarkosyl was added, and the plate was incubated at 65°C for 10 min and at room temperature for 10 min. The supernatant was removed, and the paramagnetic beads were washed twice with 50% ethanol. DNA was eluted from the silica particles by suspension of the particles in 100 µL Buffer C (1 mM EDTA pH 8.0, 10 mM Tris–HCl pH 8·0) at 65 °C for 30 min. The adequacy of the automated DNA extraction procedure was evaluated by repeating the DNA extraction in 20 samples using the modified MoBio 96-well manual extraction method adopted by the Earth Microbiome Project (http://press.igsb.anl.gov/earthmicrobiome/emp-standard-protocols/dna-extraction-protocol/). The samples gave very similar results regardless of DNA extraction method used.

PCR amplification: 1 µL DNA extracted from fecal samples was amplified by PCR reactions by 16S specific primers (515F-806R) (http://www.earthmicrobiome.org/emp-standard-protocols/16s/). All reactions were set up as 25 µL samples in 96 well Thermo-fast 96, low profile, 0.2 mL, non-skirted PCR plates (ABgene Thermo scientific, UK) with Cas1200 Corbett robot (Qiagen). 10 µL HotMastermix enzyme (5PRIME GmbH, Germany), 0.2 µM forward-/reverse primers (ILHS_515fa/ IL_806rcbc) and 13 µL PCR grade water (Qiagen) were used.

Sequence processing: Sequencing of the V4 region of the 16S rRNA gene using the Illumina HiSeq instrument[19] resulted in a total of 2546 samples for all time points after demultiplexing, and quality-filtering (excluding samples of poor quality with OTUs <1000). All data processing was performed using Quantitative Insights Into Microbial Ecology (QIIME) pipeline version 1.7.0.2. OTUs were assigned to bacterial taxa using a closed-reference OTU picking procedure (97% sequence similarity) and mapped against the Greengenes (release 13-8) reference database of 16S rRNA sequences.[20] Data were rarefied to 5906 sequences per sample, the minimum number of reads observed in a sample.

Description of the multiple imputation procedure

- Method: Multiple imputation by chained equations; estimates combined using Ru-

bin's rules
- Software: *mice* package in R[21]
- 3 sets of 100 imputed datasets were created: (1) chemicals – respiratory outcomes (n = 993); (2) microbiota diversity and SCFAs – respiratory outcomes (n = 438); and (3) chemicals – microbiota diversity and SCFAs (n = 298)
- Variables included in the imputation procedure: outcomes, exposures, and covariates (refer to main text for the full set of potential confounders)
- Treatment of variables: predictive mean matching for numeric data, and logistic regression imputation for binary data (factor with 2 levels); toxicant exposure variables were log-transformed
- No statistical interactions were included in the imputation models

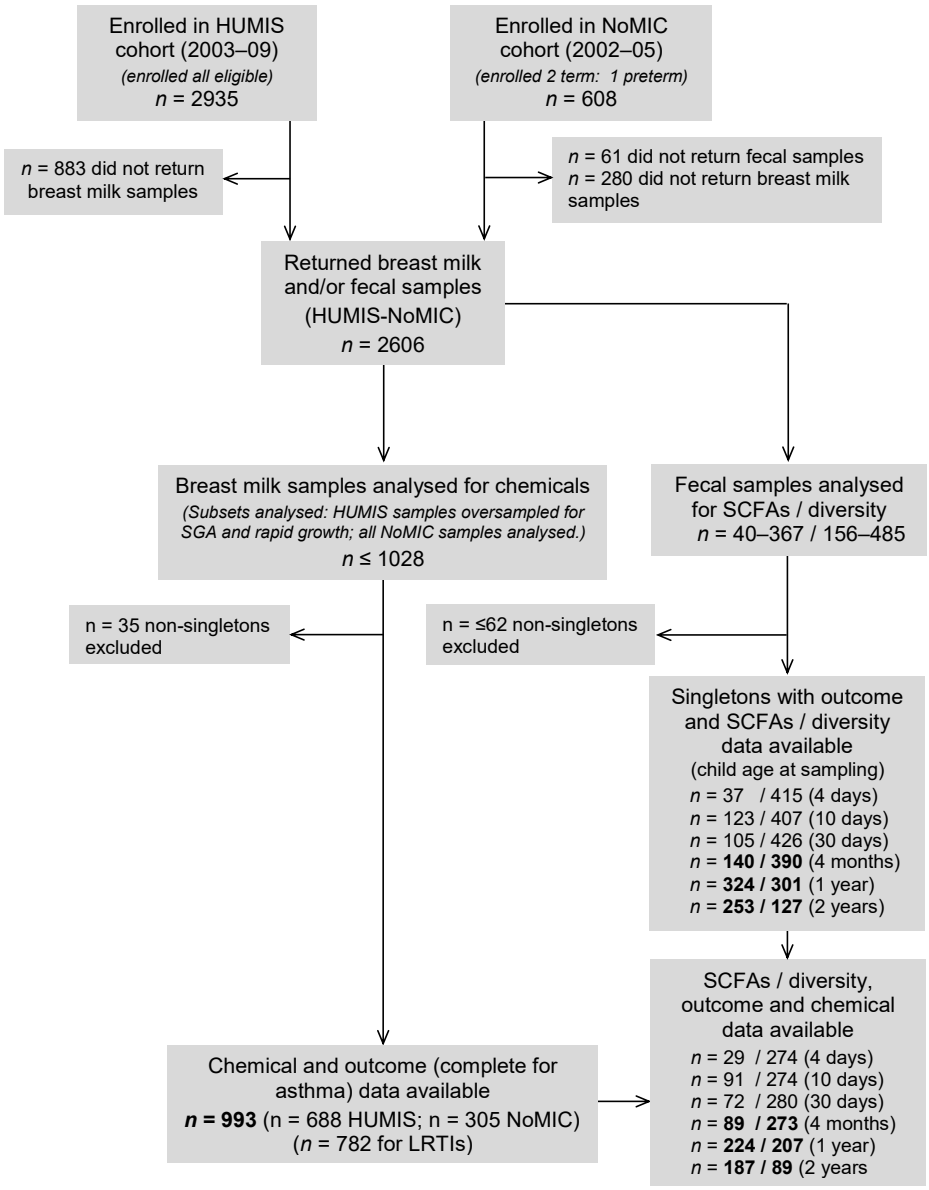


Figure S1. Flow chart of study population selection within the HUMIS-NoMIC cohort, and an overview of available data used in the present analysis (bolded numbers denote the sample sizes for the primary analyses). Abbreviations: LRTI, lower respiratory tract infection; SCFAs, short-chain fatty acids; SGA, small for gestational age.

Table S1. Selected sample characteristics of the cohort with available Shannon diversity or SCFA data (NoMIC, Norway, n=438[a]).

	n (%) or median (IQR)	Missing
Maternal characteristics		
Age at start of pregnancy, years		0
16–26	83 (18.9)	
27–33	250 (57.1)	
34–44	105 (24.0)	
Education, years completed		34
<12	67 (16.6)	
12	114 (28.2)	
13-16	148 (36.6)	
≥17	75 (18.6)	
Caesarean-section	133 (30.4)	0
Smoking during pregnancy	61 (14.1)	5
Smoking 1 year after delivery	56 (14.5)	51
Child characteristics		
Year of birth		0
2002–2003	157 (35.8)	
2004–2005	270 (61.6)	
2007	11 (2.5)	
Sex: girl	209 (47.7)	0
Preterm (<37 weeks gestation)	99 (22.6)	0
Breastfeeding duration, any, months	10.0 (4.3–13.0)	12
Breastfeeding, any, for at least 12 months	181 (42.5)	12
Recent antibiotics, before age 12 months[b]	24 (6.3)	21
Ever antibiotics, before age 24 months	139 (39.2)	83
Asthma, ever hospital admission, 2008–2014[c]	18 (4.1)	0
Asthma, maternal-reported at age 2 years	35 (10.1)	90
LRTI ≥1 by age 2 years	81 (24.6)	109

BMI, body mass index; IQR, interquartile range; LRTIs, lower respiratory tract infections (maternal-reported doctor diagnosis of bronchitis, respiratory syncytial virus, or pneumonia).
[a] This is the sample size for singletons with fecal samples analysed for Shannon diversity and/or SCFAs for the samples collected at 4, 12 or 24 months.
[b] Antibiotics taken in the 2 weeks (or, if unavailable, in 1 month) before to when the day 365 sample was collected.
[c] The median age of children in the subset of 438 at the time of linkage was 10.4 years old (IQR, 9.8–11.1; range, 9.3–11.7).

Table S2. Detection frequencies and concentrations of POP exposure biomarkers[a] in breast milk samples (n=993; HUMIS-NoMIC cohort, Norway, 2002–2009).

Exposure	N[b]	% >LOD	Median LOD[c]	5 P	25 P	50 P	75 P	95 P
PCB-74 (ng/g)	993	100.0	0.005	1.651	2.400	3.250	4.360	7.004
PCB-99 (ng/g)	993	100.0	0.005	2.078	3.210	4.230	5.680	8.382
PCB-105 (ng/g)	993	100.0	0.002	0.638	0.980	1.350	1.852	3.221
PCB-114 (ng/g)	993	98.5	0.001	0.157	0.249	0.335	0.460	0.727
PCB-118 (ng/g)	993	100.0	0.001	3.047	4.540	6.170	8.400	14.202
PCB-138 (ng/g)	993	100.0	0.003	9.408	14.887	19.869	26.140	40.460
PCB-153 (ng/g)	993	100.0	0.005	16.660	24.900	32.180	42.820	68.606
PCB-156 (ng/g)	993	100.0	0.002	1.506	2.328	3.210	4.595	7.611
PCB-157 (ng/g)	993	99.8	0.002	0.288	0.471	0.670	1.020	1.794
PCB-167 (ng/g)	969	100.0	0.001	0.380	0.585	0.786	1.070	1.794
PCB-170 (ng/g)	993	99.7	0.002	1.570	4.560	6.192	8.430	13.741
PCB-180 (ng/g)	993	100.0	0.003	7.843	11.870	15.542	20.990	34.618
PCB-189 (ng/g)	993	98.8	0.001	0.100	0.162	0.230	0.320	0.552
PCB-194 (ng/g)	993	99.8	0.002	0.610	0.950	1.360	1.940	3.172
$\sum_7$DL-PCBs (pmol/g)	—	—	—	19.467	28.491	38.059	51.070	86.540
$\sum_7$NDL-PCBs (pmol/g)	—	—	—	119.789	176.911	228.843	301.537	473.785
$\sum_{14}$PCBs (pmol/g)	—	—	—	142.185	209.678	266.751	350.733	550.665
HCB (ng/g)	993	100.0	0.02	5.998	8.687	10.683	13.090	18.993
β-HCH (ng/g)	993	99.9	0.05	1.361	2.670	3.960	5.810	10.842
oxychlordane (ng/g)	882	100.0	0.05	1.417	2.262	3.018	4.140	7.295
DDE (ng/g)	993	100.0	0.05	18.282	32.048	46.406	71.270	152.787
$\sum_4$OCPs (pmol/g)	—	—	—	95.127	151.185	210.015	296.043	589.453
BDE-28 (ng/g)	955	97.5	0.02[d]	0.031	0.076	0.123	0.214	0.493
BDE-47 (ng/g)	956	100.0	0.02[d]	0.368	0.682	1.051	1.737	4.967
BDE-99 (ng/g)	956	99.9	0.02[d]	0.105	0.182	0.278	0.457	1.359
BDE-100 (ng/g)	956	99.7	0.02[d]	0.104	0.182	0.260	0.407	0.940
BDE-153 (ng/g)	953	99.8	0.02[d]	0.234	0.365	0.501	0.700	1.390
BDE-154 (ng/g)	954	82.3	0.02[d]	<LOQ	0.019	0.030	0.046	0.104
$\sum_6$PBDEs (pmol/g)	—	—	—	1.898	3.172	4.450	6.667	17.347
PFOA (ng/L)	884	89.7	10[d]	9.957	25.000	40.000	63.000	110.000
PFOS (ng/L)	884	100.0	10[d]	41.271	76.000	110.000	150.395	259.538
$\sum_2$PFASs (pmol/L)	—	—	—	132.507	227.043	322.788	454.105	740.038

β-HCH, β-hexachlorocyclohexane; DDE, dichlorodiphenyldichloroethylene; HCB, hexachlorobenzene; LOD, limit of detection; (N)DL, (non)-dioxin-like; OCP, organochlorine pesticide; P, percentile; (P)BDE, (poly)brominated diphenyl ether; PCB, polychlorinated biphenyl; PFAS, poly-and perfluoroalkyl substances; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.

[a] We present data for only those chemicals which were measured and detected in ≥ 70% (n ≥ 696) of the breast milk samples from the 993 mothers included in the present analysis. This excluded, among others, PCB-209 (61%) and DDT (61%), and 3 perfluoroalkyl acids (49%) which were measured in a smaller number of samples. Values <LOD/LOD were imputed prior to calculating percentiles.

[b] The number of mothers whose breast milk samples were analysed.

[c] Sample-specific LOD/LOQs were determined for PCBs and OCPs. The signal/noise (S/N) ratio of the calibration solutions was used to estimate the LOD (S/N = 3) and LOQ (S/N = 10).

[d] A single limit of quantification (LOQ) (and not an LOD) was reported by the laboratory for these analytes.

Table S3. Adjusted associations [β or odds ratio (OR)][a] between covariates and the respiratory health outcomes, selected gut microbiota markers, and selected POP exposures.

	Ever asthma diagnosis	Asthma at 2 years	LRTI by 2 years	ΣSCFA (135 mmol/kg)[b]	Butyric (12.5 % total SCFAs)[b]	Shannon diversity (1.44)[b]	ln-Σ_{14}PCBs (0.83 pmol/g)	ln-Σ_{4}OCPs (1.13 pmol/g)	ln-Σ_{6}PBDEs (1.39 pmol/g)	ln-Σ_{2}PFASs (1.08 pmol/L)
Covariate (2-SD increment)	**OR**	**OR**	**OR**	**β**	**β**	**β**	**β**	**β**	**β**	**β**
Age at start of pregnancy (9.3 years)	1.72	0.80	0.99	0.071	-0.099	-0.053	**0.506**	**0.397**	0.065	**0.100**
Mat. education: >12years	1.21	1.22	1.16	0.003	-0.112	-0.114	0.021	-0.006	-0.072	-0.057
Pre-pregnancy BMI (8.8 kg/m^2)	**0.40**	0.84	1.20	-0.074	-0.084	0.032	**-0.087**	**0.064**	0.066	-0.052
Parity: multiparous	1.37	1.26	1.04	0.103	0.016	0.048	**-0.308**	**-0.312**	**-0.075**	**-0.329**
Caesarean-section	1.20	0.72	1.26	0.141	-0.036	-0.007	0.020	0.076	0.053	0.047
Smoking during pregnancy	1.31	1.29	1.31	-0.094	-0.105	-0.133	0.025	-0.009	0.034	-0.016
Mat. fatty fish (>23 servings/year)	0.52	**0.49**	1.04	-0.047	0.002	-0.002	**0.056**	0.044	0.047	0.048
Child age in 2015 (2.79 years)	1.16	1.22	1.06	**0.270**	0.095	-0.059	**0.182**	**0.174**	**0.163**	**0.268**
Sex: male	1.19	**2.99**	**1.58**	-0.104	-0.031	-0.033	-0.015	0.036	0.024	0.002
Small for gestational age (<10th P)	1.46	1.71	0.57	-0.097	-0.066	-0.191	0.019	-0.013	-0.043	0.055
Preterm (<37 weeks gestation)	2.03	**2.30**	1.69	-0.027	-0.089	0.033	-0.077	-0.053	0.082	-0.004
Breastfeeding duration (10.7 mo.)	0.89	0.94	0.98	-0.070	-0.052	-0.126	**-0.123**	**-0.102**	0.000	0.017
Rapid growth, 0–6 months	1.75	1.74	0.96	0.048	-0.056	0.074	0.006	0.019	-0.074	0.069
Either parent has asthma	**3.91**	1.73	1.07	0.009	-0.041	0.106	-0.019	-0.016	0.031	0.032
No. of playmates at 2 years: >4	0.78	0.84	1.29	-0.016	0.120	0.037	-0.001	-0.004	0.033	**0.087**
Pets during infancy	1.41	1.19	0.97	0.046	**0.211**	0.009	0.007	-0.058	0.000	0.015
Antibiotics <1 year old	**3.14**	**2.54**	**1.94**	-0.016	0.006	**-0.198**	**-0.110**	-0.049	0.031	-0.029
Antibiotics <4 weeks 365 day sample	–	–	–	-0.071	-0.185	-0.275	–	–	–	–

Table S3. *Continued.*

Abbreviations: BMI, body mass index; LRTIs, lower respiratory tract infections; OCPs, organochlorine pesticides; PBDEs, polybrominated diphenyl ethers; PCBs, polychlorinated biphenyls; PFASs, poly-and perfluoroalkyl substances; POP, persistent organic pollutant; SCFA, short-chain fatty acids; SD, standard deviation.

[a] Coefficients from multivariable models are presented (the number of playmates and pets, and subsequently, antibiotics, were added separately to models as they resulted in a smaller sample size). The coefficient is bolded if the 95% confidence interval excluded zero. Continuous exposures and covariates were scaled to 2-standard deviations (these increments are presented in brackets). Sample sizes ranged from n=139–208 for SCFAs and diversity models; and n=592–927 for the asthma and LRTI outcomes and POP exposures. The maximum variance inflation factor ranged from 1.31–1.53 across models.

[b] Butyric acid (relative abundance) and Shannon diversity index for child fecal samples sampled at 1 year of age. We present results for butyric acid as it is one of the most abundant SCFAs and has been more frequently linked to health outcomes relative to the other SCFAs.

Table S4. Detection frequencies and levels[a] of SCFAs and Shannon diversity of microbiota from the fecal samples collected from the children at 4, 12 and 24 months of age.

			Absolute (mmol/kg)			Relative (%)		
	n[b]	% <LOD	25 P	50 P	75 P	25 P	50 P	75 P
4 months								
Acetic acid	140	0.0	56.66	87.47	128.33	74.7	83.1	90.8
Propionic acid	140	1.4	4.42	10.22	19.99	5.0	9.4	16.6
n-butyric acid	140	2.1	0.74	2.68	6.22	0.9	2.4	5.2
i-butyric acid	140	12.9	0.33	0.94	1.66	0.4	0.8	1.4
n-valeric acid	140	52.1	<LOD	<LOD	0.17	0.0	0.0	0.2
i-valeric acid	140	12.9	0.24	1.10	2.10	0.3	0.9	1.8
n-caproic acid	140	79.3	<LOD	<LOD	<LOD	0.0	0.0	0.0
i-caproic acid	140	77.9	<LOD	<LOD	<LOD	0.0	0.0	0.0
Total SCFAs[c]	140	0.0	70.69	110.60	151.37	—	—	—
Shannon diversity[d]	390	0.0	2.69	3.11	3.51	—	—	—
12 months								
Acetic acid	324	0.0	55.71	75.24	106.85	59.6	67.8	77.1
Propionic acid	324	0.0	9.39	15.20	23.75	8.7	14.7	20.1
n-butyric acid	324	0.0	8.06	13.29	20.20	8.4	11.6	16.1
i-butyric acid	324	2.2	0.76	1.51	2.43	0.8	1.4	2.1
n-valeric acid	324	20.7	0.07	0.25	0.67	0.1	0.2	0.4
i-valeric acid	324	1.2	0.88	1.89	3.29	0.9	1.6	2.8
n-caproic acid	324	82.7	<LOD	<LOD	<LOD	0.0	0.0	0.0
i-caproic acid	324	77.5	<LOD	<LOD	<LOD	0.0	0.0	0.0
Total SCFAs	324	0.0	83.93	112.83	157.50	—	—	—
Shannon diversity	301	0.0	3.87	4.37	4.79	—	—	—
24 months								
Acetic acid	253	0.0	54.74	73.23	91.02	56.4	61.3	66.5
Propionic acid	253	0.0	11.65	17.58	25.05	12.0	15.8	19.6
n-butyric acid	253	0.0	11.91	18.07	26.13	12.2	15.7	19.5
i-butyric acid	253	0.8	1.14	1.88	2.84	1.0	1.6	2.5
n-valeric acid	253	3.6	0.50	1.60	2.58	0.4	1.5	2.1
i-valeric acid	253	0.0	1.34	2.43	3.83	1.2	2.2	3.3
n-caproic acid	253	74.3	<LOD	<LOD	<LOD	0.0	0.0	0.0
i-caproic acid	253	84.2	<LOD	<LOD	<LOD	0.0	0.0	0.0
Total SCFAs	253	0.0	88.86	115.96	147.91	—	—	—
Shannon diversity	127	0.0	4.41	4.94	5.29	—	—	—

[a] The absolute concentrations (mmol/kg) and the relative abundance (%) of SCFAs.
[b] The number of samples that were analysed (refer to the Supplemental Methods) at the last 3 collection time points.
[c] Calculated with values <LOD imputed.
[d] Shannon diversity index is unitless, on a scale of 0–1.

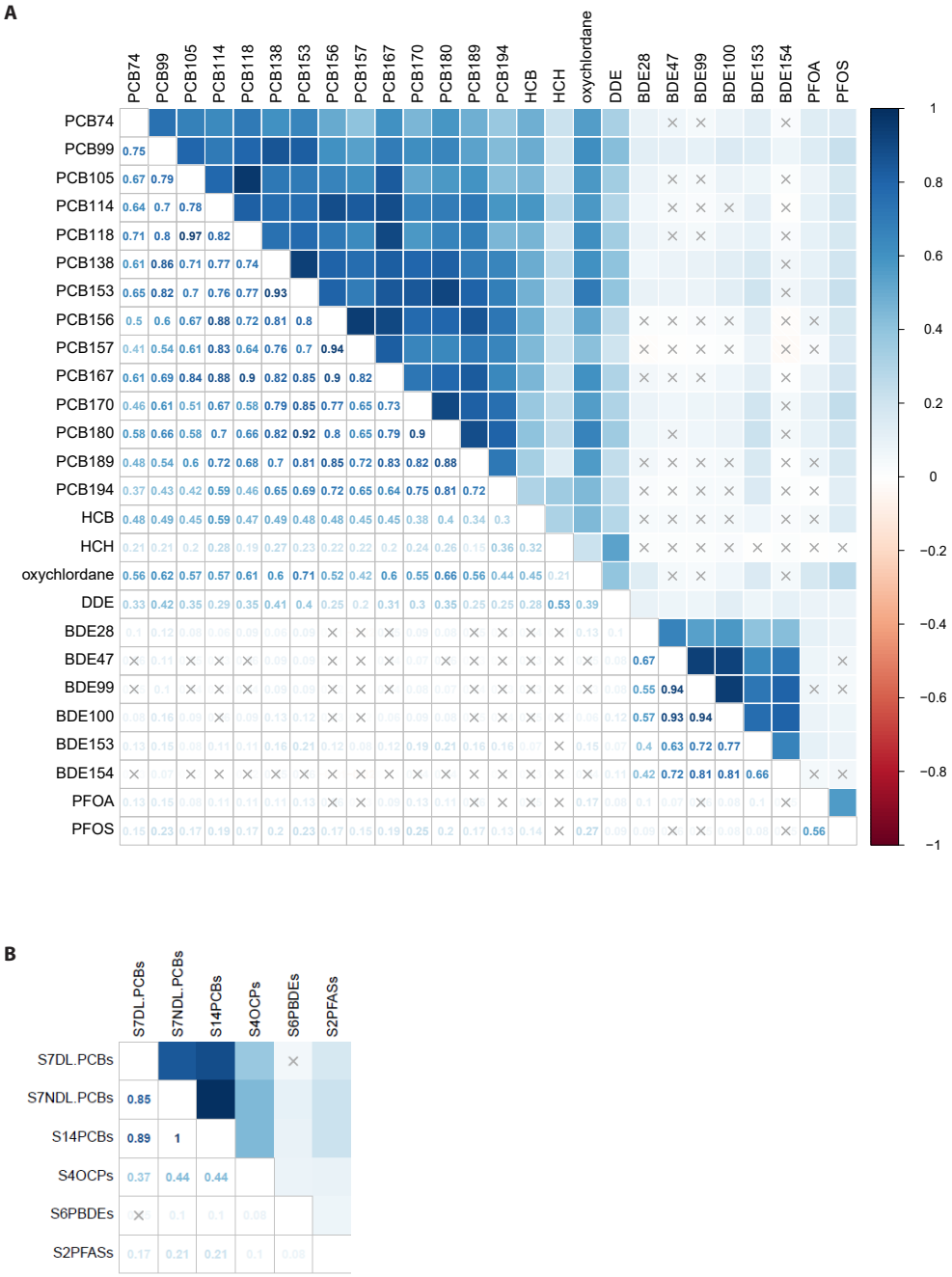


Figure S2. Pearson correlation coefficient matrix for the **(A)** 26 individual POP exposure biomarkers and **(B)** summed chemical groups. The intensity of the shaded squares reflects the magnitude of the correlation coefficient, crosses indicate that the correlation was non-significant ($p > 0.05$).

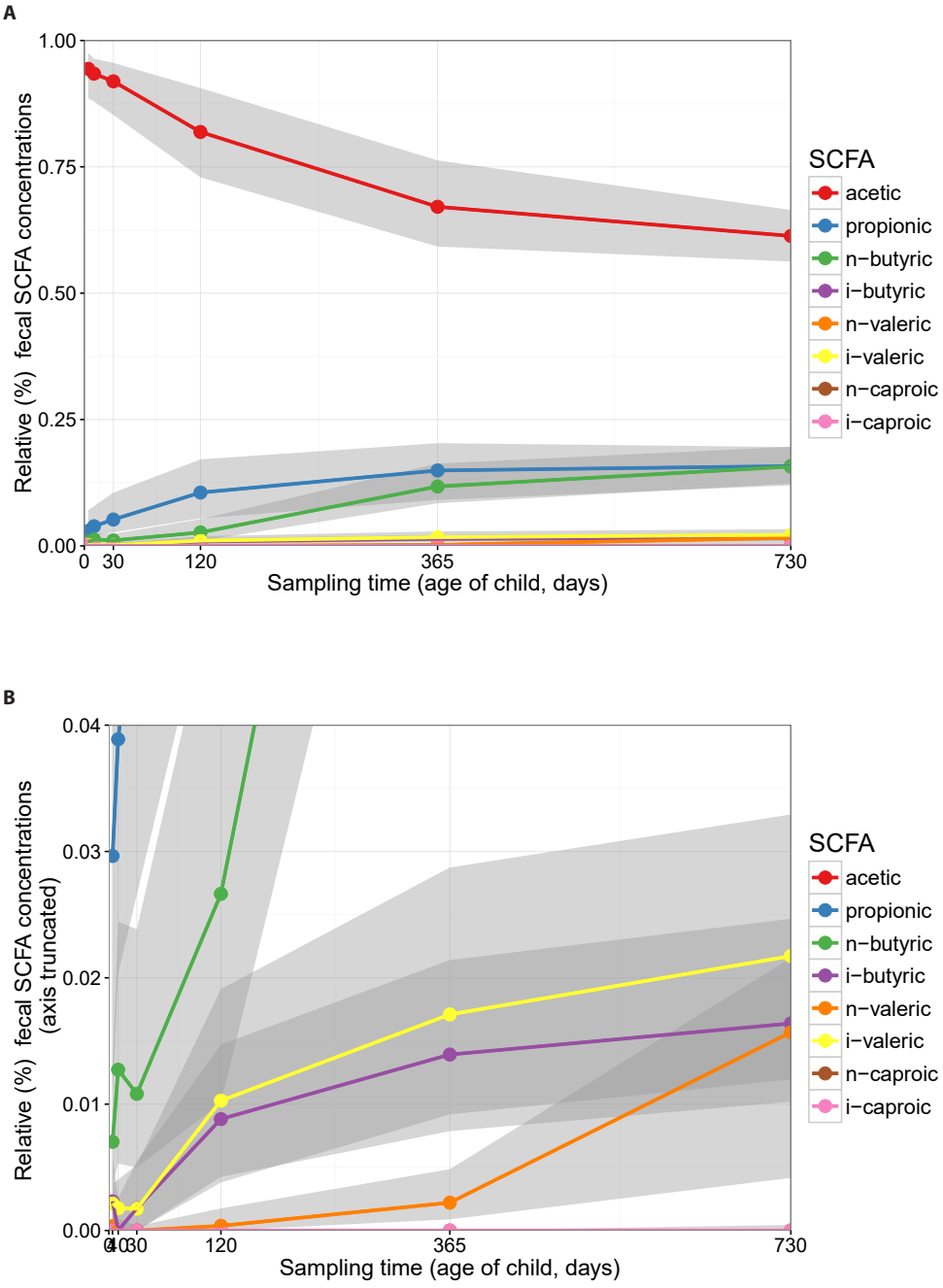
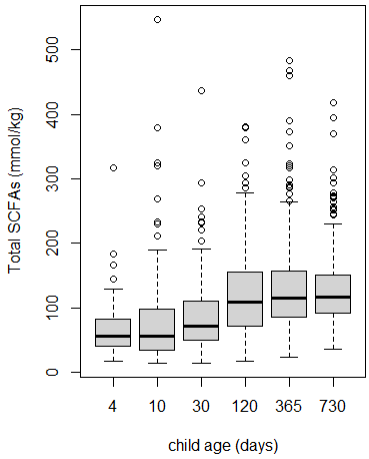
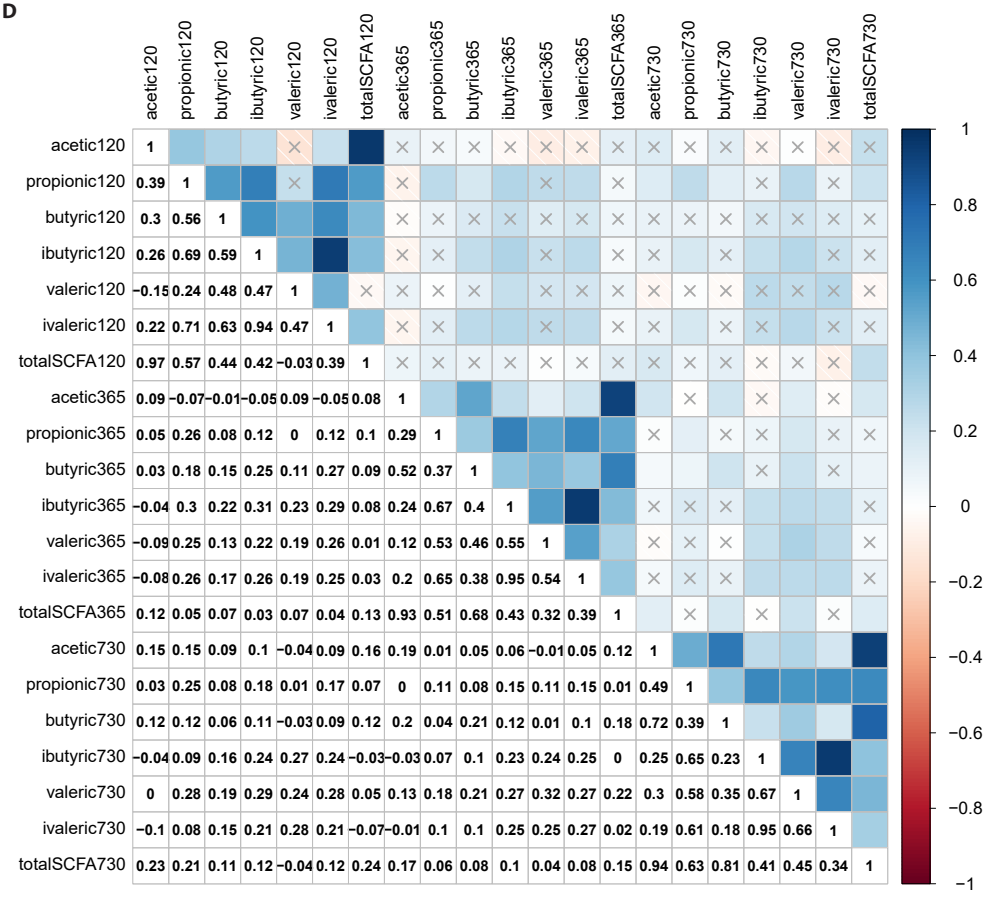


Figure S3. The distribution of the relative abundance of short-chain fatty acids **(A–B)**; and the distribution and Spearman correlations for short-chain fatty acid levels **(C–D)** and Shannon diversity **(E–F)** at the sample collection time points (when the child was 4, 10, and 30 days, and 4, 12, and 24 months old). In plots A and B, dots represent the median values, and shaded bands the interquartile range. In plots D and F, crosses indicate that the correlation was non-significant ($p > 0.05$).

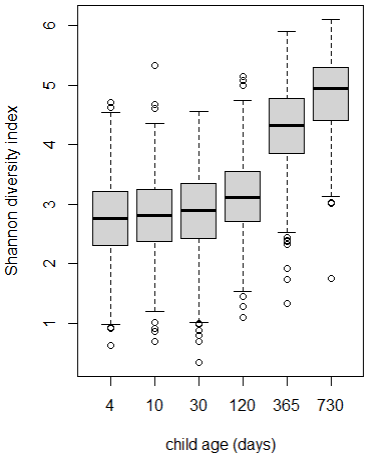
C


Total SCFAs (mmol/kg)
0
100
200
300
400
500
4
10
30
120
365
730
child age (days)

D


acetic120 propionic120 butyric120 ibutyric120 valeric120 ivaleric120 totalSCFA120 acetic365 propionic365 butyric365 ibutyric365 valeric365 ivaleric365 totalSCFA365 acetic730 propionic730 butyric730 ibutyric730 valeric730 ivaleric730 totalSCFA730
1
0.8
0.6
0.4
0.2
0
−0.2
−0.4
−0.6
−0.8
−1

E



F

	diversity.4	diversity.10	diversity.30	diversity.120	diversity.365	diversity.730
diversity.4	1			×	×	×
diversity.10	0.39	1		×	×	×
diversity.30	0.31	0.45	1			×
diversity.120	0.06	0.14	0.36	1		×
diversity.365	0.04	0.03	0.12	0.15	1	
diversity.730	-0.06	-0.01	0.02	-0.08	0.23	1

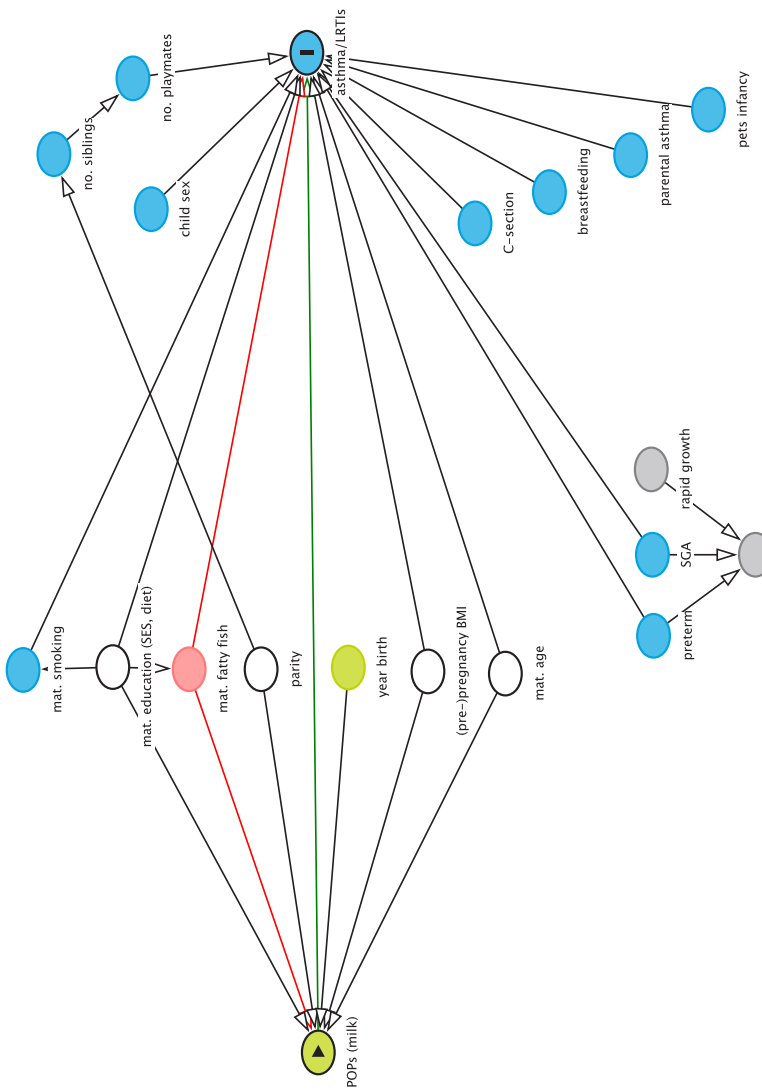


Figure S4. Directed acyclic graph (DAG) of the authors' conceptions of the associations between **(A)** POP exposures, covariates, and the respiratory health outcomes (asthma and LRTIs); between **(B)** SCFAs, covariates and the outcomes, and between **(C)** POPs, covariates and the outcomes. DAGs were created with DAGitty [22]. For POP–asthma/LRTI models **(A)**, we considered the minimal sufficient adjustment set to include maternal age, parity, pre-pregnancy BMI, and maternal education. We also tested models further adjusted for several covariates for which evidence for an association is weaker or which are plausibly associated with the only the outcome (leading to unnecessary adjustment, possibly decreasing precision in a logistic regression model): child birth year, child sex, delivery mode (C-section), duration of any breastfeeding, parental asthma, maternal marine fish consumption in the year before delivery, and the number of playmates at age 2, as a proxy of daycare attendance.[23-25] Covariates are defined in the main text. Abbreviations: BMI, body mass index; LRTIs, lower respiratory tract infections; SCFAs, short-chain fatty acids; SES, socioeconomic status; SGA, small for gestational age.

B

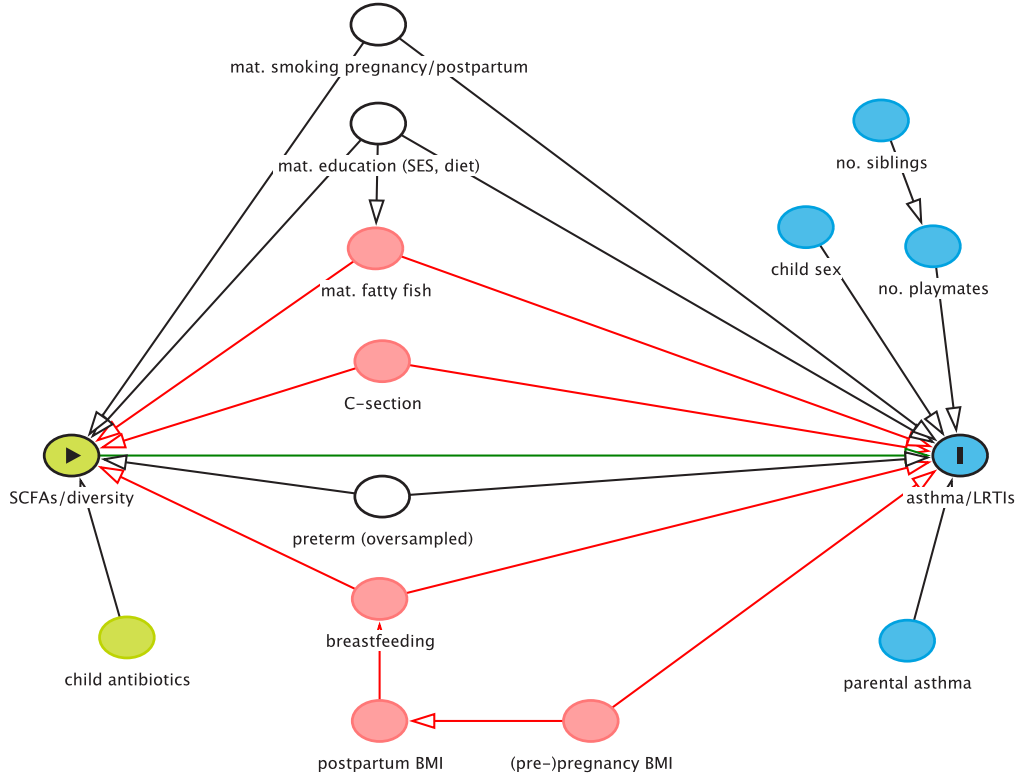


Figure S4. *Continued.*
For SCFA/diversity–asthma/LRTI models **(B)**, we considered the minimal sufficient adjustment set to include preterm birth (the NoMIC cohort was oversampled for preterm birth), maternal education (as a proxy of socioeconomic status, diet, and maternal stress), and maternal smoking during pregnancy, and when the child was one year old. In further adjusted models, we also adjusted for covariates for which associations are less established, including maternal fatty fish consumption during pregnancy, breastfeeding, and delivery by C-section.

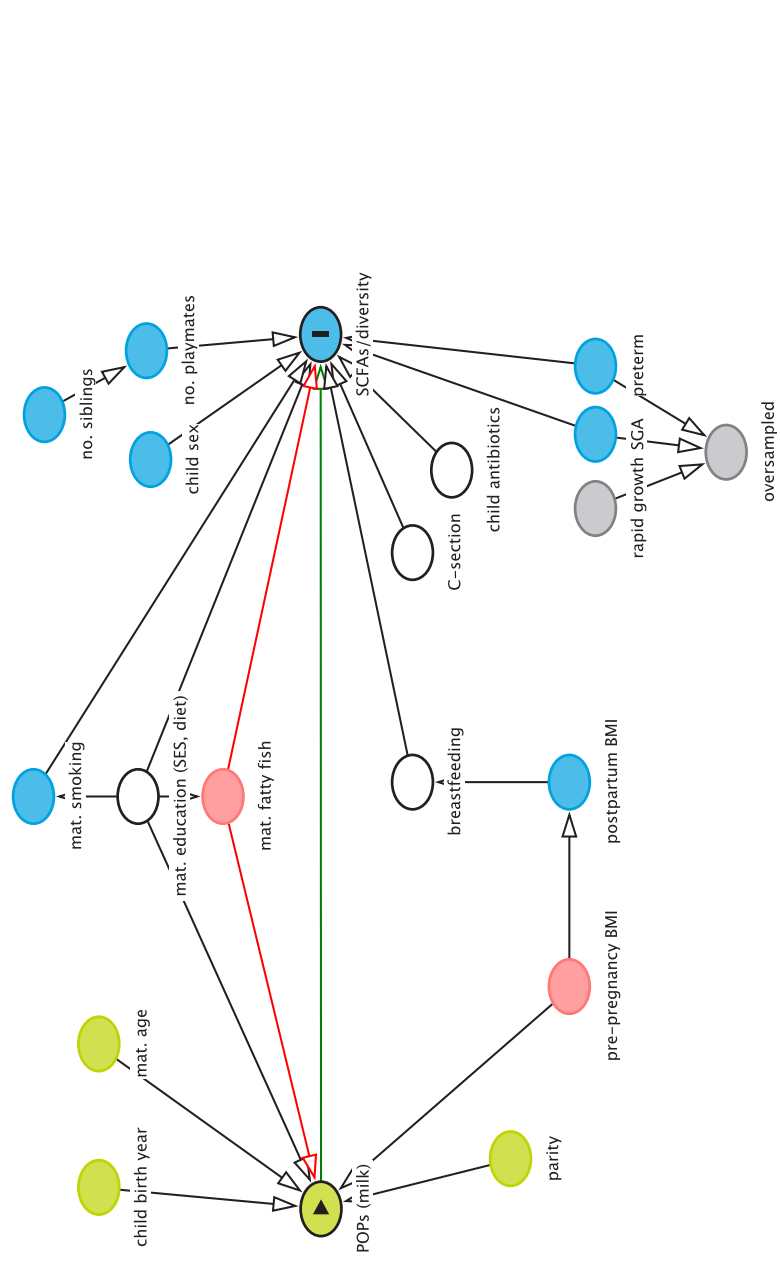


Figure S4. *Continued.*
For POP–asthma/LRTI models **(C)**, we considered the minimal sufficient adjustment set to include maternal education (as a proxy of SES and diet), breastfeeding, C-section, and child antibiotics. As these were linear regression models, we adjusted for some covariates likely to be strongly associated with only the outcome ('control variables'), which can increase precision of estimates.

Table S5. Associations between environmental toxicants and **registry-based asthma by 2014**: single-pollutant unpenalised logistic regression models and multi-pollutant elastic net penalised logistic regression models.

		Minimally adjusted[a]											Further adjusted[a]										
		Unpenalised logistic regression									ENET		Unpenalised logistic regression									ENET	
		Complete case					Multiple imputation (n=993)				(n=993)		Complete case					Multiple imputation (n=993)				(n=993)	
Exposure	Incr.[b]	n	OR	LCL	UCL	*p*-val	OR	LCL	UCL	*p*-val	*q*-val[c]	%	OR[d]	n	OR	LCL	UCL	*p*-val	OR	LCL	UCL	*p*-val	*q*-val[c]	%	OR[d]
PCB-74 (ng/g)	2.502	953	0.67	0.34	1.34	0.256	0.67	0.34	1.33	0.253	0.73	0	1	718	0.61	0.25	1.49	0.277	0.66	0.32	1.33	0.239	0.62	0	1
PCB-99 (ng/g)	2.423	953	0.67	0.36	1.26	0.215	0.68	0.36	1.26	0.220	0.72	0	1	718	0.60	0.27	1.34	0.211	0.63	0.32	1.21	0.164	0.58	0	1
PCB-105 (ng/g)	2.688	953	0.57	0.30	1.11	0.100	0.59	0.30	1.14	0.113	0.43	0	1	718	0.56	0.24	1.28	0.170	0.62	0.31	1.24	0.177	0.58	0	1
PCB-114 (ng/g)	5.520	953	1.09	0.56	2.13	0.803	1.11	0.57	2.16	0.765	0.92	6	1	718	0.79	0.26	2.39	0.679	1.19	0.57	2.46	0.642	1.00	7	1
PCB-118 (ng/g)	2.522	953	0.54	0.27	1.06	0.075	0.55	0.28	1.08	0.081	0.43	89	**0.91**	718	0.54	0.23	1.27	0.158	0.57	0.28	1.15	0.117	0.58	5	1
PCB-138 (ng/g)	2.518	953	**0.54**	**0.29**	**0.98**	**0.043**	0.55	0.30	1.00	0.050	0.43	91	**0.67**	718	**0.36**	**0.16**	**0.77**	**0.009**	**0.47**	**0.25**	**0.91**	**0.026**	0.56	80	**0.69**
PCB-153 (ng/g)	2.357	953	0.57	0.30	1.10	0.094	0.59	0.31	1.11	0.103	0.43	4	1	718	**0.38**	**0.15**	**0.93**	**0.034**	0.53	0.27	1.06	0.073	0.58	0	1
PCB-156 (ng/g)	2.749	953	0.73	0.37	1.45	0.369	0.75	0.38	1.46	0.396	0.92	0	1	718	0.64	0.25	1.61	0.342	0.78	0.38	1.59	0.492	0.95	0	1
PCB-157 (ng/g)	3.498	953	0.88	0.50	1.55	0.648	0.89	0.50	1.58	0.699	0.92	0	1	718	0.80	0.39	1.67	0.558	0.89	0.45	1.75	0.737	1.00	0	1
PCB-167 (ng/g)	2.608	929	0.61	0.30	1.22	0.162	0.57	0.29	1.15	0.115	0.43	1	1	698	0.49	0.19	1.30	0.154	0.57	0.27	1.20	0.139	0.58	0	1
PCB-170 (ng/g)	5.271	953	0.94	0.52	1.69	0.824	0.96	0.53	1.73	0.880	0.92	0	1	718	0.79	0.34	1.81	0.574	0.76	0.37	1.52	0.431	0.95	0	1
PCB-180 (ng/g)	2.523	953	0.54	0.26	1.12	0.097	0.58	0.30	1.14	0.115	0.43	8	1	718	**0.33**	**0.12**	**0.92**	**0.034**	0.57	0.29	1.15	0.116	0.58	0	1
PCB-189 (ng/g)	4.851	953	1.06	0.53	2.13	0.866	1.05	0.53	2.11	0.886	0.92	9	1	718	0.82	0.37	1.81	0.617	0.98	0.44	2.17	0.964	1.00	2	1
PCB-194 (ng/g)	3.313	953	0.81	0.45	1.43	0.460	0.82	0.46	1.45	0.490	0.92	0	1	718	0.70	0.37	1.31	0.260	0.79	0.41	1.50	0.466	0.95	0	1
HCB (ng/g)	2.093	953	1.27	0.67	2.40	0.459	1.26	0.68	2.35	0.464	0.92	12	1	718	1.31	0.55	3.11	0.542	1.25	0.64	2.42	0.511	0.95	12	1
β-HCH (ng/g)	3.886	953	**1.91**	**1.06**	**3.44**	**0.032**	**1.91**	**1.06**	**3.42**	**0.031**	0.43	91	**1.65**	718	**2.27**	**1.02**	**5.04**	**0.045**	**1.95**	**1.02**	**3.72**	**0.043**	0.56	80	**1.45**
oxychlordane (ng/g)	2.668	844	0.68	0.34	1.36	0.275	1.04	0.46	2.35	0.934	0.93	17	1	633	0.56	0.22	1.43	0.228	1.00	0.43	2.33	0.992	1.00	6	1
DDE (ng/g)	3.783	953	1.06	0.57	1.98	0.847	1.06	0.57	1.97	0.863	0.92	1	1	718	0.67	0.29	1.56	0.359	1.02	0.51	2.01	0.965	1.00	1	1
BDE-28 (ng/g)	5.834	916	1.20	0.66	2.18	0.553	1.15	0.61	2.15	0.665	0.92	9	1	690	1.27	0.58	2.74	0.551	1.08	0.54	2.17	0.826	1.00	2	1
BDE-47 (ng/g)	5.083	917	1.11	0.61	2.02	0.740	1.05	0.57	1.92	0.888	0.92	0	1	690	0.91	0.42	1.97	0.803	1.01	0.53	1.94	0.971	1.00	0	1

Table S5. *Continued.*

		Minimally adjusted[a]											Further adjusted[a]										
		Unpenalised logistic regression									ENET		Unpenalised logistic regression									ENET	
		Complete case					Multiple imputation (n=993)				(n=993)		Complete case					Multiple imputation (n=993)				(n=993)	
Exposure	Incr.[b]	n	OR	LCL	UCL	*p*-val	OR	LCL	UCL	*p*-val	*q*-val[c]	%	OR[d]	n	OR	LCL	UCL	*p*-val	OR	LCL	UCL	*p*-val	*q*-val[c]	%	OR[d]
BDE-99 (ng/g)	5.079	917	1.27	0.70	2.28	0.432	1.18	0.64	2.17	0.591	0.92	4	1	690	0.99	0.46	2.12	0.969	1.14	0.59	2.22	0.695	1.00	4	1
BDE-100 (ng/g)	4.241	917	1.00	0.54	1.84	0.997	0.94	0.50	1.79	0.858	0.92	1	1	690	0.74	0.33	1.67	0.468	0.88	0.44	1.76	0.711	1.00	1	1
BDE-153 (ng/g)	3.172	914	1.13	0.61	2.09	0.705	1.13	0.58	2.19	0.720	0.92	22	1	688	0.84	0.40	1.74	0.631	1.08	0.54	2.17	0.827	1.00	9	1
BDE-154 (ng/g)	4.366	915	1.12	0.61	2.04	0.714	1.05	0.56	1.97	0.869	0.92	0	1	689	0.90	0.41	1.95	0.781	1.04	0.54	2.02	0.902	1.00	0	1
PFOA (ng/L)	4.101	846	1.25	0.59	2.65	0.559	1.15	0.51	2.60	0.729	0.92	19	1	634	1.42	0.53	3.80	0.485	1.00	0.43	2.31	0.997	1.00	13	1
PFOS (ng/L)	2.981	846	0.76	0.38	1.51	0.431	0.76	0.39	1.48	0.416	0.92	19	1	634	0.65	0.25	1.70	0.381	0.63	0.31	1.30	0.212	0.61	41	1
Σ_7DL-PCBs (pmol/g)	2.442	929	0.62	0.31	1.23	0.170	0.60	0.30	1.20	0.148				698	0.56	0.23	1.41	0.219	0.62	0.30	1.29	0.200			
Σ_7NDL-PCBs (pmol/g)	2.319	953	0.57	0.29	1.12	0.101	0.59	0.31	1.13	0.112				718	**0.38**	**0.15**	**0.95**	**0.039**	0.54	0.27	1.08	0.081			
Σ_{14}PCBs (pmol/g)	2.297	929	0.60	0.30	1.19	0.146	0.59	0.30	1.15	0.119				698	0.40	0.15	1.05	0.064	0.55	0.27	1.12	0.097			
Σ_4OCPs (pmol/g)	3.109	844	1.32	0.69	2.53	0.409	1.40	0.75	2.63	0.290				633	0.82	0.33	2.08	0.678	1.40	0.70	2.78	0.342			
Σ_6PBDEs (pmol/g)	4.015	913	1.14	0.63	2.06	0.666	1.06	0.58	1.94	0.845				688	0.92	0.42	2.00	0.837	1.03	0.54	1.98	0.927			
Σ_2PFASs (pmol/L)	2.813	846	0.93	0.45	1.91	0.844	0.93	0.46	1.90	0.844				634	0.92	0.35	2.39	0.859	0.78	0.37	1.64	0.506			

Abbreviations: (P)BDE, (poly)brominated diphenyl ether; BMI, body mass index; β-HCH, β-hexachlorocyclohexane; DDE, dichlorodiphenyldichloroethylene; ENET, elastic net; HCB, hexachlorobenzene; Incr., increment; OCP, organochlorine pesticides; PCB, polychlorinated biphenyl; PFAS, poly-and perfluoroalkyl substances; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.

Odds ratios per a 2-standard deviation increase in natural-log (ln)–transformed exposure concentration.

[a] Models were adjusted for as described in Figure S4.

[b] Exposures were ln-transformed, mean-centered and rescaled to 2 times their standard deviations (increment presented on the ln-scale).

[c] *q*-values for the single-pollutant models were determined: corrected for multiple comparisons with a false discovery rate controlled at <5% (Benjamini and Hochberg 1995).

[d] Averaged coefficients from the elastic net models are presented for those exposures which were picked up >50% of the 100 imputed datasets tested.

Table S6. Associations between environmental toxicants and **maternal-reported asthma at 2 years**: single-pollutant unpenalised logistic regression models and multi-pollutant elastic net penalised logistic regression models.

		Minimally adjusted[a]												Further adjusted[a]											
		Unpenalised logistic regression										ENET		Unpenalised logistic regression										ENET	
		Complete case					Multiple imputation (n=993)					(n=993)		Complete case					Multiple imputation (n=993)					(n=993)	
Exposure	Incr.[b]	n	OR	LCL	UCL	*p*-val	OR	LCL	UCL	*p*-val	*q*-val[c]	%	OR[d]	n	OR	LCL	UCL	*p*-val	OR	LCL	UCL	*p*-val	*q*-val[c]	%	OR[d]
PCB-74 (ng/g)	2.502	826	0.68	0.35	1.31	0.246	0.75	0.39	1.42	0.373	0.73	6	1	712	0.54	0.26	1.11	0.094	0.72	0.38	1.39	0.327	0.82	3	1
PCB-99 (ng/g)	2.423	826	0.66	0.36	1.20	0.170	0.73	0.41	1.32	0.301	0.73	8	1	712	**0.45**	**0.23**	**0.87**	**0.018**	0.68	0.37	1.27	0.226	0.82	1	1
PCB-105 (ng/g)	2.688	826	**0.51**	**0.28**	**0.96**	**0.037**	0.53	0.29	0.98	**0.042**	0.73	73	**0.75**	712	**0.39**	**0.19**	**0.79**	**0.009**	0.55	0.29	1.03	0.063	0.74	44	1
PCB-114 (ng/g)	5.520	826	0.72	0.43	1.21	0.213	0.79	0.49	1.28	0.338	0.73	33	1	712	**0.45**	**0.24**	**0.86**	**0.015**	0.76	0.44	1.33	0.335	0.82	21	1
PCB-118 (ng/g)	2.522	826	0.56	0.29	1.05	0.072	0.56	0.30	1.05	0.071	0.73	23	1	712	**0.43**	**0.21**	**0.88**	**0.021**	0.57	0.30	1.09	0.089	0.74	13	1
PCB-138 (ng/g)	2.518	826	**0.55**	**0.31**	**0.97**	**0.039**	0.66	0.39	1.13	0.131	0.73	56	**0.84**	712	**0.38**	**0.20**	**0.73**	**0.004**	0.60	0.34	1.07	0.085	0.74	28	1
PCB-153 (ng/g)	2.357	826	0.74	0.40	1.37	0.340	0.83	0.46	1.49	0.532	0.73	3	1	712	0.52	0.25	1.07	0.077	0.79	0.43	1.46	0.452	0.82	1	1
PCB-156 (ng/g)	2.749	826	0.68	0.36	1.30	0.240	0.75	0.41	1.37	0.355	0.73	0	1	712	0.61	0.29	1.29	0.198	0.77	0.40	1.46	0.416	0.82	0	1
PCB-157 (ng/g)	3.498	826	0.72	0.45	1.15	0.169	0.74	0.46	1.19	0.209	0.73	49	1	712	0.66	0.39	1.14	0.134	0.70	0.41	1.22	0.208	0.82	16	1
PCB-167 (ng/g)	2.608	804	0.61	0.32	1.17	0.136	0.61	0.33	1.12	0.107	0.73	31	1	692	0.47	0.22	1.01	0.054	0.59	0.31	1.13	0.114	0.74	12	1
PCB-170 (ng/g)	5.271	826	1.30	0.64	2.65	0.468	1.34	0.67	2.66	0.408	0.73	59	**1.33**	712	1.06	0.46	2.43	0.891	1.20	0.53	2.72	0.658	0.82	15	1
PCB-180 (ng/g)	2.523	826	0.66	0.33	1.30	0.226	0.81	0.44	1.50	0.506	0.73	10	1	712	0.52	0.24	1.15	0.108	0.80	0.42	1.53	0.504	0.82	3	1
PCB-189 (ng/g)	4.851	826	1.18	0.60	2.30	0.634	1.23	0.63	2.42	0.542	0.73	57	**1.51**	712	1.05	0.50	2.23	0.897	1.18	0.55	2.54	0.670	0.82	20	1
PCB-194 (ng/g)	3.313	826	0.80	0.48	1.33	0.381	0.87	0.51	1.49	0.618	0.77	5	1	712	0.76	0.41	1.40	0.382	0.86	0.46	1.59	0.623	0.82	1	1
HCB (ng/g)	2.093	826	0.65	0.35	1.21	0.176	0.74	0.41	1.35	0.322	0.73	27	1	712	0.56	0.27	1.15	0.116	0.69	0.36	1.33	0.269	0.82	11	1
β-HCH (ng/g)	3.886	826	1.31	0.73	2.36	0.367	1.27	0.72	2.24	0.401	0.73	63	**1.35**	712	1.35	0.65	2.81	0.421	1.24	0.65	2.37	0.513	0.82	27	1
oxychlordane (ng/g)	2.668	730	0.78	0.41	1.48	0.449	0.91	0.49	1.68	0.759	0.88	30	1	628	0.59	0.29	1.21	0.147	0.88	0.47	1.65	0.694	0.82	7	1
DDE (ng/g)	3.783	826	0.95	0.52	1.74	0.864	1.07	0.59	1.94	0.821	0.89	44	1	712	0.68	0.34	1.35	0.269	1.00	0.53	1.89	0.993	0.99	15	1
BDE-28 (ng/g)	5.834	795	1.57	0.92	2.67	0.098	1.30	0.75	2.25	0.348	0.73	48	1	684	1.23	0.66	2.30	0.510	1.26	0.70	2.29	0.442	0.82	20	1
BDE-47 (ng/g)	5.083	796	1.38	0.83	2.30	0.218	1.20	0.72	2.00	0.479	0.73	32	1	684	1.09	0.60	1.97	0.788	1.17	0.68	2.00	0.579	0.82	8	1

Table S6. *Continued.*

		Minimally adjusted[a]												Further adjusted[a]											
		Unpenalised logistic regression										ENET		Unpenalised logistic regression										ENET	
		Complete case					Multiple imputation (n=993)					(n=993)		Complete case					Multiple imputation (n=993)					(n=993)	
Exposure	Incr.[b]	n	OR	LCL	UCL	*p*-val	OR	LCL	UCL	*p*-val	*q*-val[c]	%	OR[d]	n	OR	LCL	UCL	*p*-val	OR	LCL	UCL	*p*-val	*q*-val[c]	%	OR[d]
BDE-99 (ng/g)	5.079	796	1.33	0.79	2.22	0.284	1.21	0.71	2.05	0.484	0.73	43	1	684	1.10	0.61	1.98	0.764	1.16	0.66	2.05	0.600	0.82	13	1
BDE-100 (ng/g)	4.241	796	1.04	0.61	1.79	0.882	0.99	0.57	1.72	0.973	0.97	18	1	684	0.85	0.45	1.60	0.609	0.97	0.55	1.73	0.927	0.96	6	1
BDE-153 (ng/g)	3.172	793	0.99	0.58	1.72	0.983	1.08	0.63	1.85	0.778	0.88	24	1	682	0.91	0.52	1.59	0.741	1.09	0.63	1.89	0.759	0.86	10	1
BDE-154 (ng/g)	4.366	794	0.74	0.42	1.30	0.292	0.81	0.46	1.42	0.465	0.73	57	**0.79**	683	0.63	0.34	1.19	0.156	0.83	0.48	1.46	0.526	0.82	22	1
PFOA (ng/L)	4.101	733	1.30	0.66	2.60	0.451	1.05	0.54	2.05	0.887	0.92	33	1	628	1.28	0.61	2.72	0.515	0.96	0.49	1.90	0.912	0.96	12	1
PFOS (ng/L)	2.981	733	0.94	0.49	1.80	0.849	0.83	0.45	1.55	0.564	0.73	35	1	628	0.69	0.32	1.46	0.326	0.69	0.35	1.35	0.277	0.82	27	1
Σ_7DL-PCBs (pmol/g)	2.442	804	0.57	0.30	1.10	0.093	0.58	0.31	1.09	0.090				692	**0.45**	**0.21**	**0.95**	**0.037**	0.59	0.31	1.14	0.114			
Σ_7NDL-PCBs (pmol/g)	2.319	826	0.67	0.36	1.27	0.220	0.79	0.44	1.42	0.423				712	**0.47**	**0.23**	**0.99**	**0.046**	0.74	0.40	1.39	0.350			
Σ_{14}PCBs (pmol/g)	2.297	804	0.68	0.36	1.28	0.230	0.75	0.41	1.36	0.335				692	0.48	0.23	1.02	0.056	0.71	0.38	1.34	0.291			
Σ_4OCPs (pmol/g)	3.109	730	1.03	0.53	1.99	0.933	1.09	0.57	2.08	0.788				628	0.66	0.31	1.45	0.302	1.01	0.50	2.06	0.970			
Σ_6PBDEs (pmol/g)	4.015	792	1.34	0.81	2.22	0.249	1.19	0.71	1.99	0.514				682	1.08	0.60	1.95	0.798	1.17	0.68	2.02	0.582			
Σ_2PFASs (pmol/L)	2.813	733	1.09	0.56	2.15	0.794	0.95	0.50	1.80	0.873				628	0.91	0.43	1.92	0.800	0.81	0.42	1.58	0.536			

Abbreviations: (P)BDE, (poly)brominated diphenyl ether; BMI, body mass index; β-HCH, β-hexachlorocyclohexane; DDE, dichlorodiphenyldichloroethylene; ENET, elastic net; HCB, hexachlorobenzene; Incr., increment; OCP, organochlorine pesticides; PCB, polychlorinated biphenyl; PFAS, poly-and perfluoroalkyl substances; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.

Odds ratios per a 2-standard deviation increase in natural-log (ln)–transformed exposure concentration.

[a] Models were adjusted for as described in Figure S4.

[b] Exposures were ln-transformed, mean-centered and rescaled to 2 times their standard deviations (increment presented on the ln-scale).

[c] *q*-values for the single-pollutant models were determined: corrected for multiple comparisons with a false discovery rate controlled at <5% (Benjamini and Hochberg 1995).

[d] Averaged coefficients from the elastic net models are presented for those exposures which were picked up >50% of the 100 imputed datasets tested.

Table S7. Associations between environmental toxicants and one or more **lower respiratory tract infections by 2 years of age**: single-pollutant unpenalised logistic regression models and multi-pollutant elastic net penalised logistic regression models.

		Minimally adjusted[a]												Further adjusted[a]											
		Unpenalised logistic regression										ENET		Unpenalised logistic regression										ENET	
		Complete case					Multiple imputation (n=993)					(n=993)		Complete case					Multiple imputation (n=993)					(n=993)	
Exposure	Incr.[b]	n	OR	LCL	UCL	*p*-val	OR	LCL	UCL	*p*-val	*q*-val[c]	%	OR[d]	n	OR	LCL	UCL	*p*-val	OR	LCL	UCL	*p*-val	*q*-val[c]	%	OR[d]
PCB-74 (ng/g)	2.502	755	1.14	0.75	1.75	0.542	1.09	0.73	1.64	0.674	0.94	2	1	661	1.13	0.71	1.77	0.612	1.05	0.70	1.59	0.816	0.93	6	1
PCB-99 (ng/g)	2.423	755	0.98	0.66	1.45	0.902	1.00	0.69	1.46	0.995	1.00	4	1	661	0.96	0.63	1.47	0.864	0.95	0.65	1.40	0.805	0.93	7	1
PCB-105 (ng/g)	2.688	755	1.40	0.94	2.09	0.096	1.34	0.92	1.97	0.132	0.94	36	1	661	1.29	0.84	1.98	0.238	1.32	0.89	1.95	0.168	0.93	24	1
PCB-114 (ng/g)	5.520	755	1.24	0.74	2.09	0.420	1.15	0.72	1.82	0.567	0.94	15	1	661	1.07	0.60	1.91	0.813	1.11	0.68	1.82	0.666	0.93	17	1
PCB-118 (ng/g)	2.522	755	1.44	0.96	2.15	0.076	1.34	0.91	1.98	0.133	0.94	27	1	661	1.34	0.88	2.07	0.177	1.32	0.89	1.96	0.162	0.93	22	1
PCB-138 (ng/g)	2.518	755	0.90	0.60	1.34	0.602	0.90	0.62	1.32	0.595	0.94	9	1	661	0.86	0.55	1.33	0.487	0.86	0.58	1.27	0.442	0.93	11	1
PCB-153 (ng/g)	2.357	755	0.96	0.64	1.46	0.862	0.96	0.65	1.42	0.846	0.94	0	1	661	0.94	0.60	1.47	0.771	0.92	0.61	1.39	0.694	0.93	2	1
PCB-156 (ng/g)	2.749	755	0.93	0.61	1.41	0.719	0.95	0.64	1.41	0.799	0.94	0	1	661	0.90	0.57	1.43	0.661	0.93	0.62	1.41	0.747	0.93	1	1
PCB-157 (ng/g)	3.498	755	0.80	0.56	1.13	0.204	0.84	0.60	1.18	0.310	0.94	29	1	661	0.78	0.53	1.13	0.189	0.82	0.57	1.17	0.270	0.93	18	1
PCB-167 (ng/g)	2.608	739	1.27	0.84	1.92	0.262	1.20	0.81	1.77	0.366	0.94	7	1	646	1.17	0.74	1.85	0.492	1.17	0.78	1.76	0.448	0.93	12	1
PCB-170 (ng/g)	5.271	755	0.94	0.67	1.32	0.732	0.94	0.68	1.29	0.691	0.94	2	1	661	0.87	0.58	1.31	0.513	0.84	0.58	1.22	0.364	0.93	6	1
PCB-180 (ng/g)	2.523	755	0.77	0.49	1.21	0.259	0.82	0.54	1.24	0.340	0.94	16	1	661	0.78	0.47	1.28	0.318	0.80	0.52	1.22	0.294	0.93	13	1
PCB-189 (ng/g)	4.851	755	0.81	0.59	1.12	0.206	0.82	0.60	1.13	0.226	0.94	25	1	661	0.86	0.60	1.24	0.417	0.80	0.57	1.11	0.182	0.93	19	1
PCB-194 (ng/g)	3.313	755	0.74	0.51	1.06	0.101	0.74	0.52	1.06	0.104	0.94	47	1	661	0.72	0.49	1.06	0.093	0.71	0.49	1.02	0.067	0.93	31	1
HCB (ng/g)	2.093	755	1.11	0.73	1.68	0.621	1.21	0.82	1.81	0.337	0.94	24	1	661	1.10	0.70	1.73	0.670	1.21	0.80	1.84	0.364	0.93	20	1
β-HCH (ng/g)	3.886	755	1.15	0.77	1.73	0.486	1.21	0.83	1.76	0.321	0.94	22	1	661	1.07	0.66	1.72	0.798	1.17	0.77	1.76	0.468	0.93	15	1
oxychlordane (ng/g)	2.668	665	1.11	0.72	1.72	0.624	0.95	0.62	1.45	0.812	0.94	14	1	580	1.03	0.65	1.64	0.905	0.95	0.62	1.45	0.794	0.93	13	1
DDE (ng/g)	3.783	755	1.02	0.69	1.50	0.926	1.05	0.72	1.53	0.801	0.94	4	1	661	0.99	0.65	1.51	0.966	0.98	0.67	1.45	0.934	0.94	10	1
BDE-28 (ng/g)	5.834	731	0.95	0.66	1.37	0.791	0.99	0.68	1.44	0.963	1.00	6	1	640	0.95	0.63	1.42	0.790	0.93	0.63	1.38	0.733	0.93	11	1
BDE-47 (ng/g)	5.083	732	0.93	0.65	1.34	0.694	0.94	0.66	1.35	0.749	0.94	9	1	640	1.00	0.68	1.48	0.995	0.90	0.62	1.31	0.567	0.93	12	1

Table S7. *Continued.*

		Minimally adjusted[a]												Further adjusted[a]										
		Unpenalised logistic regression										ENET		Unpenalised logistic regression									ENET	
		Complete case					Multiple imputation (n=993)					(n=993)		Complete case					Multiple imputation (n=993)				(n=993)	
Exposure	Incr.[b]	n	OR	LCL	UCL	*p*-val	OR	LCL	UCL	*p*-val	*q*-val[c]	%	OR[d]	n	OR	LCL	UCL	*p*-val	OR	LCL	UCL	*p*-val	*q*-val[c]	%	OR[d]
BDE-99 (ng/g)	5.079	732	1.09	0.77	1.55	0.630	1.03	0.72	1.47	0.868	0.94	7	1	640	1.17	0.80	1.71	0.431	0.99	0.68	1.43	0.940	0.94	9	1
BDE-100 (ng/g)	4.241	732	1.00	0.70	1.43	0.978	0.96	0.68	1.37	0.821	0.94	2	1	640	1.11	0.75	1.63	0.614	0.93	0.64	1.33	0.678	0.93	4	1
BDE-153 (ng/g)	3.172	729	0.98	0.68	1.41	0.915	0.97	0.66	1.41	0.857	0.94	7	1	638	1.04	0.71	1.52	0.856	0.97	0.66	1.41	0.856	0.93	9	1
BDE-154 (ng/g)	4.366	730	1.02	0.72	1.46	0.899	0.97	0.68	1.39	0.872	0.94	5	1	639	1.13	0.77	1.65	0.538	0.96	0.67	1.38	0.834	0.93	9	1
PFOA (ng/L)	4.101	672	1.10	0.72	1.67	0.676	1.06	0.69	1.61	0.803	0.94	11	1	587	1.13	0.72	1.80	0.592	1.04	0.68	1.60	0.860	0.93	12	1
PFOS (ng/L)	2.981	672	0.97	0.64	1.48	0.900	0.93	0.63	1.37	0.722	0.94	10	1	587	0.82	0.51	1.33	0.427	0.84	0.55	1.28	0.413	0.93	21	1
Σ_7DL-PCBs (pmol/g)	2.442	739	1.23	0.82	1.86	0.316	1.21	0.82	1.78	0.345				646	1.15	0.74	1.79	0.537	1.19	0.80	1.78	0.398			
Σ_7NDL-PCBs (pmol/g)	2.319	755	0.90	0.60	1.37	0.632	0.92	0.62	1.36	0.659				661	0.87	0.55	1.38	0.561	0.87	0.58	1.31	0.508			
Σ_{14}PCBs (pmol/g)	2.297	739	0.93	0.61	1.42	0.722	0.96	0.65	1.43	0.848				646	0.87	0.54	1.39	0.556	0.92	0.61	1.39	0.693			
Σ_4OCPs (pmol/g)	3.109	665	1.05	0.68	1.60	0.840	1.05	0.71	1.55	0.802				580	0.98	0.62	1.56	0.943	1.00	0.67	1.50	1.000			
Σ_6PBDEs (pmol/g)	4.015	728	0.96	0.67	1.37	0.805	0.94	0.66	1.34	0.731				638	1.03	0.70	1.52	0.871	0.90	0.62	1.30	0.572			
Σ_2PFASs (pmol/L)	2.813	672	1.06	0.69	1.62	0.802	1.00	0.67	1.49	0.997				587	0.96	0.59	1.56	0.871	0.93	0.61	1.40	0.713			

Abbreviations: (P)BDE, (poly)brominated diphenyl ether; BMI, body mass index; β-HCH, β-hexachlorocyclohexane; DDE, dichlorodiphenyldichloroethylene; ENET, elastic net; HCB, hexachlorobenzene; Incr., increment; OCP, organochlorine pesticides; PCB, polychlorinated biphenyl; PFAS, poly-and perfluoroalkyl substances; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.

Odds ratios per a 2-standard deviation increase in natural-log (ln)–transformed exposure concentration.

[a] Models were adjusted for as described in Figure S4.

[b] Exposures were ln-transformed, mean-centered and rescaled to 2 times their standard deviations (increment presented on the ln-scale).

[c] *q*-values for the single-pollutant models were determined: corrected for multiple comparisons with a false discovery rate controlled at <5% (Benjamini and Hochberg 1995).

[d] Averaged coefficients from the elastic net models are presented for those exposures which were picked up >50% of the 100 imputed datasets tested.

Table S8. Unpenalised multi-pollutant models of the selected subset, summed chemical groups, and assessment of potential effect modification by child sex of the associations between contaminant exposures and respiratory health outcomes.

Outcome/ Selected exposures (2SD (ln ng/g))	Overall					Stratified by child sex				
	Complete case			Multiple imputation (n=993)		Girl (n=375/359/339)		Boy (n=468/436/415)		
	N	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	$p_{interaction}$
Asthma by 2014										
ENET-selected subset	953					*Reduced subset (full selected subset VIFs >3)*				
PCB-118 (DL)		0.64	0.27, 1.51	0.65	0.27, 1.55	–		–		
PCB-138 (NDL)		**0.42**	**0.19, 0.89**	**0.43**	**0.20, 0.91**	0.42	0.15, 1.16	**0.31**	**0.13, 0.74**	0.34
β-HCH		**3.02**	**1.66, 5.50**	**2.99**	**1.65, 5.43**	**3.35**	**1.50, 7.51**	2.34	0.90, 6.05	0.47
		VIFs: 1.39–1.85								
Summed groups	714									
Σ_{14}PCBs		**0.36**	**0.13, 0.99**	**0.39**	**0.18, 0.82**					
Σ_{4}OCPs		**2.42**	**1.01, 5.78**	**2.05**	**1.06, 3.94**					
Σ_{6}PBDEs		0.99	0.44, 2.21	1.05	0.55, 2.02					
Σ_{2}PFASs		1.05	0.44, 2.50	1.06	0.50, 2.25					
		VIFs:[a] 1.12–2.00								
Asthma at 2 years										
ENET-selected subset	794					*Reduced subset (full selected subset VIFs >3)*				
PCB-105 (DL)		0.49	0.21, 1.15	0.49	0.22, 1.10	–		–		
PCB-138 (NDL)		0.39	0.13, 1.17	**0.42**	**0.18, 0.97**	1.62	0.33, 8.03	**0.26**	**0.11, 0.59**	0.05
PCB-170 (NDL)		2.12	0.51, 8.78	2.03	0.54, 7.67	–		–		
PCB-189 (DL)		1.98	0.51, 7.69	2.01	0.57, 7.05	–				
β-HCH		**2.09**	**1.04, 4.22**	**1.96**	**1.03, 3.73**	1.54	0.38, 6.27	**2.41**	**1.05, 5.52**	0.40
BDE-154		0.85	0.49, 1.50	0.88	0.51, 1.53	–		–		

Table S8. *Continued.*

Outcome/ Selected exposures (2SD (ln ng/g))	Overall					Stratified by child sex				
		Complete case		Multiple imputation (n=993)		Girl (n=375/359/339)		Boy (n=468/436/415)		
	N	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	$p_{interaction}$
		VIFs: 1.08–3.56								
Summed groups	617									
Σ_{14}PCBs		0.66	0.22, 2.01	0.59	0.28, 1.25					
Σ_{4}OCPs		1.47	0.49, 4.43	1.35	0.64, 2.87					
Σ_{6}PBDEs		1.48	0.80, 2.75	1.23	0.72, 2.08					
Σ_{2}PFASs		1.06	0.49, 2.33	1.01	0.52, 1.94					
		VIFs: 1.09–2.87								
LRTI by 2 years										
Summed groups	564									
Σ_{14}PCBs		1.26	0.60, 2.65	0.90	0.54, 1.51					
Σ_{4}OCPs		0.81	0.38, 1.71	1.13	0.68, 1.87					
Σ_{6}PBDEs		0.96	0.61, 1.51	0.93	0.64, 1.34					
Σ_{2}PFASs		1.05	0.64, 1.74	1.02	0.68, 1.52					
		VIFs: 1.11–3.01								

Abbreviations: β-HCH, β-hexachlorocyclohexane; DL, dioxin-like; NDL, non-dioxin-like; (P)BDE, (poly)brominated diphenyl ether; OCP, organochlorine pesticides; PCB, polychlorinated biphenyl; PFAS, poly-and perfluoroalkyl substances; VIF, variance inflation factor.
Odds ratios per a 2-standard deviation increase in natural-log (ln)–transformed exposure concentration. Models were adjusted for maternal age, education, pre-pregnancy BMI, and parity.
[a] Of the 4 summed chemical groups, VIFs were highest for the Σ_{14}PCBs and Σ_{4}OCPs.

Table S9. Associations between Shannon diversity and SCFAs and the three respiratory health outcomes: unpenalised logistic regression models.

		Unadjusted					Minimally adjusted[a]									Further adjusted[a]								
		Complete case					Complete case					Multiple imp. (n=438)				Complete case					Multiple imp. (n=438)			
	Incr.[b]	n	OR	LCL	UCL	*p*-val	n	OR	LCL	UCL	*p*-val	OR	LCL	UCL	*p*-val	n	OR	LCL	UCL	*p*-val	OR	LCL	UCL	*p*-val
Asthma by 2014 (median 10.4 years of age), registry-based																								
4 months																								
Diversity	1.30	390	**2.76**	**1.01**	**7.53**	**0.047**	254	**3.73**	**1.02**	**13.60**	**0.046**	2.67	0.94	7.55	0.065	251	**4.12**	**1.05**	**16.23**	**0.043**	2.98	0.96	9.24	0.059
∑SCFAs	145.89	140	0.34	0.04	2.66	0.302	86	0.07	0.00	2.07	0.124	0.63	0.14	2.90	0.550	86	0.01	0.00	3.99	0.133	0.63	0.13	3.15	0.571
Acetic	125.41	140	0.33	0.04	2.72	0.300	86	0.06	0.00	2.01	0.117	0.59	0.12	2.75	0.494	86	0.02	0.00	5.00	0.159	0.58	0.12	2.90	0.503
Propionic	29.00	140	0.66	0.10	4.27	0.662	86	0.23	0.01	5.09	0.350	0.92	0.24	3.52	0.906	86	0.11	0.00	4.61	0.247	1.00	0.24	4.21	0.994
n-butyric	10.93	140	0.39	0.04	3.80	0.414	86	0.45	0.02	12.85	0.638	0.62	0.11	3.38	0.579	86	0.23	0.00	36.04	0.566	0.63	0.11	3.72	0.607
i-butyric	2.11	140	1.23	0.29	5.20	0.775	86	0.23	0.01	4.40	0.326	1.49	0.47	4.71	0.498	86	0.14	0.00	4.54	0.266	1.59	0.44	5.73	0.480
n-valeric	0.77	140	0.34	0.01	13.25	0.567	86	0.96	0.01	66.44	0.984	0.62	0.07	5.93	0.676	86	1.01	0.01	169.29	0.997	0.58	0.06	6.07	0.644
i-valeric	3.32	140	1.08	0.25	4.72	0.921	86	0.30	0.02	5.89	0.428	1.40	0.45	4.36	0.562	86	0.15	0.00	8.67	0.359	1.57	0.43	5.72	0.490
12 months																								
Diversity	1.47	301	1.38	0.46	4.11	0.563	193	1.38	0.35	5.45	0.649	1.10	0.37	3.27	0.863	191	1.37	0.34	5.54	0.662	1.09	0.36	3.31	0.875
∑SCFAs	129.78	324	1.08	0.38	3.04	0.883	201	1.64	0.56	4.87	0.369	1.07	0.39	2.95	0.895	197	1.71	0.54	5.39	0.359	1.06	0.38	2.97	0.915
Acetic	94.37	324	0.95	0.31	2.85	0.923	201	1.31	0.45	3.83	0.624	0.94	0.31	2.81	0.909	197	1.35	0.43	4.22	0.610	0.91	0.29	2.85	0.875
Propionic	28.21	324	0.98	0.33	2.90	0.977	201	1.58	0.39	6.47	0.522	1.01	0.35	2.91	0.987	197	1.66	0.40	6.81	0.485	1.07	0.38	3.06	0.896
n-butyric	26.37	324	1.31	0.50	3.39	0.585	201	1.92	0.67	5.49	0.224	1.22	0.47	3.17	0.679	197	1.82	0.65	5.11	0.255	1.16	0.45	2.97	0.755
i-butyric	2.84	324	1.94	0.81	4.65	0.139	201	2.51	0.90	6.98	0.078	2.07	0.91	4.73	0.085	197	2.56	0.92	7.12	0.071	2.01	0.88	4.57	0.098
n-valeric	2.76	324	1.39	0.63	3.05	0.417	201	1.80	0.67	4.84	0.248	1.35	0.63	2.91	0.444	197	1.89	0.66	5.42	0.234	1.35	0.61	2.96	0.458
i-valeric	4.19	324	2.18	0.94	5.05	0.069	201	**2.91**	**1.09**	**7.78**	**0.034**	**2.29**	**1.04**	**5.05**	**0.041**	197	**3.02**	**1.12**	**8.14**	**0.029**	**2.25**	**1.02**	**4.98**	**0.045**
24 months																								
Diversity	1.44	127	0.62	0.16	2.46	0.497	83	1.08	0.09	12.56	0.949	0.83	0.21	3.25	0.782	82	1.19	0.14	10.27	0.875	0.84	0.21	3.38	0.802
∑SCFAs	120.20	253	0.71	0.17	2.98	0.635	168	0.96	0.16	5.76	0.968	0.77	0.22	2.66	0.674	164	0.93	0.15	5.74	0.937	0.80	0.22	2.90	0.734
Acetic	73.19	253	0.94	0.26	3.44	0.929	168	1.42	0.26	7.83	0.687	0.93	0.28	3.04	0.899	164	1.14	0.22	5.85	0.878	0.96	0.28	3.23	0.941
Propionic	25.18	253	0.46	0.09	2.43	0.359	168	0.55	0.08	3.58	0.530	0.59	0.15	2.37	0.455	164	0.64	0.09	4.71	0.664	0.61	0.15	2.51	0.493
n-butyric	31.50	253	0.62	0.13	3.02	0.551	168	0.70	0.10	5.22	0.730	0.68	0.17	2.67	0.577	164	0.68	0.08	5.95	0.724	0.75	0.18	3.12	0.691
i-butyric	2.95	253	0.48	0.10	2.31	0.363	168	0.81	0.15	4.47	0.813	0.73	0.19	2.81	0.650	164	0.96	0.18	5.15	0.965	0.70	0.19	2.63	0.593
n-valeric	3.19	253	0.46	0.09	2.30	0.341	168	0.41	0.06	2.85	0.366	0.49	0.11	2.19	0.346	164	0.54	0.07	4.22	0.558	0.50	0.11	2.25	0.360
i-valeric	4.38	253	0.46	0.09	2.32	0.346	168	0.78	0.14	4.32	0.775	0.65	0.16	2.69	0.552	164	0.97	0.19	4.99	0.970	0.63	0.15	2.58	0.518

Table S9. *Continued.*

		Unadjusted					Minimally adjusted[a]									Further adjusted[a]								
		Complete case					Complete case					Multiple imp. (n=438)				Complete case					Multiple imp. (n=438)			
	Incr.[b]	n	OR	LCL	UCL	*p*-val	n	OR	LCL	UCL	*p*-val	OR	LCL	UCL	*p*-val	n	OR	LCL	UCL	*p*-val	OR	LCL	UCL	*p*-val
Asthma at 2 years, maternal-reported																								
4 months																								
Diversity	1.30	**308**	**2.30**	**1.02**	**5.19**	**0.045**	215	2.64	0.96	7.26	0.060	1.96	0.91	4.22	0.086	212	2.84	0.99	8.19	0.053	2.30	0.99	5.33	0.052
∑SCFAs	145.89	113	0.16	0.02	1.62	0.121	73	0.11	0.00	4.99	0.258	0.73	0.31	1.70	0.456	73	0.01	0.00	3.01	0.114	0.75	0.30	1.83	0.519
Acetic	125.41	113	0.15	0.01	1.65	0.121	73	0.07	0.00	4.77	0.212	0.74	0.31	1.75	0.487	73	0.01	0.00	5.08	0.148	0.74	0.30	1.84	0.518
Propionic	29.00	113	0.18	0.01	2.48	0.202	73	0.70	0.06	8.34	0.777	0.72	0.31	1.68	0.448	73	0.22	0.01	7.67	0.401	0.77	0.32	1.86	0.556
n-butyric	10.93	113	1.30	0.37	4.63	0.681	73	1.37	0.18	10.58	0.761	1.00	0.41	2.45	0.997	73	0.75	0.07	8.06	0.810	1.10	0.44	2.74	0.839
i-butyric	2.11	113	0.53	0.10	2.89	0.464	73	0.35	0.02	5.98	0.467	0.65	0.25	1.72	0.383	73	0.13	0.00	3.80	0.236	0.69	0.25	1.90	0.464
n-valeric	0.77	113	1.47	0.23	9.43	0.688	73	0.06	0.00	106.14	0.467	1.53	0.42	5.61	0.514	73	0.03	0.00	47.27	0.343	1.55	0.40	6.07	0.521
i-valeric	3.32	113	0.73	0.15	3.57	0.695	73	0.65	0.06	7.61	0.730	0.85	0.37	1.95	0.703	73	0.26	0.01	7.57	0.436	0.95	0.40	2.30	0.914
12 months																								
Diversity	1.47	255	0.77	0.35	1.73	0.531	171	0.58	0.21	1.59	0.289	0.68	0.31	1.48	0.324	169	0.57	0.20	1.64	0.297	0.67	0.29	1.55	0.349
∑SCFAs	129.78	280	1.44	0.62	3.33	0.400	182	1.19	0.34	4.19	0.790	1.53	0.76	3.06	0.229	178	1.44	0.38	5.47	0.595	1.54	0.75	3.18	0.238
Acetic	94.37	280	1.42	0.62	3.24	0.406	182	0.99	0.28	3.47	0.990	1.51	0.76	3.03	0.242	178	1.15	0.30	4.49	0.840	1.55	0.74	3.26	0.245
Propionic	28.21	280	1.06	0.45	2.52	0.896	182	1.13	0.31	4.18	0.856	1.18	0.56	2.49	0.655	178	1.26	0.34	4.63	0.731	1.22	0.57	2.62	0.609
n-butyric	26.37	280	1.21	0.54	2.71	0.653	182	1.47	0.51	4.26	0.481	1.28	0.64	2.55	0.487	178	1.52	0.55	4.23	0.418	1.22	0.61	2.45	0.565
i-butyric	2.84	280	1.81	0.84	3.92	0.130	182	1.45	0.51	4.11	0.481	1.59	0.83	3.04	0.164	178	1.54	0.56	4.25	0.408	1.57	0.81	3.04	0.178
n-valeric	2.76	280	1.04	0.40	2.68	0.939	182	1.53	0.59	3.95	0.383	1.10	0.50	2.40	0.812	178	1.61	0.58	4.41	0.359	1.06	0.47	2.40	0.888
i-valeric	4.19	280	1.86	0.89	3.88	0.101	182	1.43	0.51	3.98	0.493	1.47	0.76	2.85	0.253	178	1.55	0.57	4.20	0.391	1.48	0.76	2.89	0.252
24 months																								
Diversity	1.44	114	0.62	0.20	1.95	0.410	81	0.71	0.16	3.22	0.657	0.91	0.30	2.80	0.865	80	0.62	0.11	3.37	0.578	0.89	0.28	2.79	0.838
∑SCFAs	120.20	237	0.55	0.19	1.60	0.269	162	1.40	0.44	4.42	0.566	0.81	0.35	1.88	0.615	158	1.57	0.48	5.10	0.454	0.87	0.36	2.10	0.758
Acetic	73.19	237	0.49	0.17	1.45	0.196	162	1.23	0.37	4.12	0.735	0.76	0.33	1.76	0.517	158	1.29	0.37	4.44	0.689	0.80	0.34	1.90	0.611
Propionic	25.18	237	0.71	0.27	1.87	0.484	162	1.42	0.50	4.00	0.510	0.90	0.39	2.06	0.801	158	1.63	0.55	4.84	0.383	0.96	0.40	2.26	0.917
n-butyric	31.50	237	0.88	0.34	2.24	0.785	162	1.56	0.53	4.61	0.420	1.00	0.46	2.18	0.996	158	2.01	0.61	6.67	0.255	1.17	0.53	2.62	0.695
i-butyric	2.95	237	0.51	0.18	1.43	0.199	162	0.97	0.28	3.37	0.966	0.80	0.32	1.97	0.622	158	1.03	0.30	3.51	0.965	0.76	0.31	1.87	0.551
n-valeric	3.19	237	0.54	0.19	1.52	0.246	162	0.98	0.31	3.07	0.976	0.71	0.29	1.74	0.451	158	1.18	0.37	3.72	0.778	0.71	0.29	1.77	0.463
i-valeric	4.38	237	0.51	0.18	1.47	0.210	162	0.94	0.27	3.27	0.922	0.77	0.32	1.88	0.564	158	1.04	0.30	3.54	0.956	0.74	0.31	1.80	0.509

Table S9. *Continued.*

		Unadjusted					Minimally adjusted[a]									Further adjusted[a]								
		Complete case					Complete case					Multiple imp. (n=438)				Complete case					Multiple imp. (n=438)			
	Incr.[b]	n	OR	LCL	UCL	*p*-val	n	OR	LCL	UCL	*p*-val	OR	LCL	UCL	*p*-val	n	OR	LCL	UCL	*p*-val	OR	LCL	UCL	*p*-val
LRTI by 2 years, maternal-reported																								
4 months																								
Diversity	1.30	292	1.38	0.79	2.42	0.263	205	1.16	0.56	2.39	0.687	1.45	0.84	2.50	0.181	203	1.19	0.57	2.48	0.648	1.49	0.85	2.62	0.166
∑SCFAs	145.89	111	1.39	0.60	3.25	0.442	73	1.16	0.25	5.37	0.847	1.08	0.59	1.98	0.802	73	1.17	0.22	6.30	0.853	1.09	0.59	2.02	0.779
Acetic	125.41	111	1.52	0.66	3.52	0.329	73	1.20	0.26	5.53	0.819	1.16	0.62	2.14	0.645	73	1.20	0.23	6.17	0.830	1.17	0.63	2.19	0.623
Propionic	29.00	111	1.17	0.51	2.71	0.714	73	1.54	0.51	4.64	0.448	1.00	0.55	1.82	0.993	73	1.77	0.52	6.06	0.364	1.01	0.55	1.87	0.973
n-butyric	10.93	111	0.55	0.18	1.72	0.305	73	0.06	0.00	1.69	0.099	0.64	0.30	1.38	0.248	73	0.02	0.00	1.08	0.054	0.62	0.28	1.39	0.242
i-butyric	2.11	111	0.39	0.13	1.25	0.113	73	0.43	0.09	2.10	0.298	0.55	0.25	1.22	0.141	73	0.36	0.06	2.01	0.242	0.53	0.24	1.21	0.130
n-valeric	0.77	111	**0.01**	**0.00**	**0.70**	**0.035**	73	0.00	0.00	3.42	0.074	0.59	0.20	1.75	0.340	73	0.00	0.00	1.53	0.057	0.57	0.19	1.75	0.323
i-valeric	3.32	111	0.52	0.17	1.60	0.251	73	0.54	0.11	2.65	0.446	0.72	0.36	1.45	0.354	73	0.45	0.08	2.65	0.380	0.71	0.34	1.45	0.342
12 months																								
Diversity	1.47	244	0.62	0.35	1.09	0.097	163	**0.38**	**0.17**	**0.81**	**0.012**	0.64	0.36	1.15	0.138	162	**0.37**	**0.17**	**0.80**	**0.011**	0.63	0.35	1.15	0.131
∑SCFAs	129.78	270	1.15	0.65	2.03	0.631	175	0.85	0.34	2.16	0.733	1.18	0.71	1.99	0.521	172	0.92	0.36	2.35	0.861	1.17	0.70	1.97	0.549
Acetic	94.37	270	0.98	0.55	1.77	0.954	175	0.64	0.24	1.69	0.365	1.02	0.60	1.74	0.934	172	0.68	0.25	1.80	0.433	1.01	0.59	1.72	0.985
Propionic	28.21	270	1.67	0.92	3.02	0.092	175	1.37	0.54	3.50	0.512	1.51	0.83	2.72	0.173	172	1.50	0.58	3.90	0.401	1.52	0.84	2.76	0.164
n-butyric	26.37	270	1.15	0.66	2.00	0.624	175	1.42	0.68	2.99	0.354	1.23	0.73	2.07	0.429	172	1.50	0.70	3.21	0.295	1.23	0.73	2.06	0.438
i-butyric	2.84	270	1.64	0.94	2.87	0.084	175	1.40	0.64	3.07	0.403	1.48	0.87	2.52	0.146	172	1.42	0.64	3.15	0.393	1.48	0.87	2.53	0.147
n-valeric	2.76	270	0.80	0.42	1.52	0.491	175	0.55	0.18	1.70	0.297	0.81	0.44	1.50	0.500	172	0.58	0.19	1.77	0.334	0.81	0.44	1.50	0.498
i-valeric	4.19	270	1.56	0.90	2.70	0.116	175	1.26	0.58	2.76	0.556	1.36	0.80	2.32	0.250	172	1.27	0.57	2.82	0.553	1.36	0.80	2.32	0.251
24 months																								
Diversity	1.44	110	0.72	0.30	1.70	0.449	76	0.75	0.27	2.14	0.593	0.97	0.42	2.27	0.949	75	0.67	0.23	1.99	0.474	0.98	0.41	2.37	0.970
∑SCFAs	120.20	225	0.59	0.29	1.24	0.163	154	0.45	0.15	1.30	0.138	0.87	0.49	1.54	0.631	151	0.46	0.15	1.40	0.171	0.87	0.49	1.55	0.634
Acetic	73.19	225	0.81	0.42	1.58	0.535	154	0.70	0.27	1.84	0.470	0.99	0.56	1.75	0.972	151	0.77	0.28	2.11	0.610	0.99	0.56	1.76	0.973
Propionic	25.18	225	**0.38**	**0.17**	**0.86**	**0.020**	154	**0.30**	**0.10**	**0.96**	**0.041**	0.67	0.36	1.27	0.220	151	**0.28**	**0.09**	**0.93**	**0.037**	0.67	0.36	1.27	0.222
n-butyric	31.50	225	0.60	0.28	1.29	0.189	154	0.33	0.09	1.18	0.088	0.88	0.49	1.57	0.659	151	0.34	0.09	1.25	0.104	0.88	0.49	1.58	0.663
i-butyric	2.95	225	**0.35**	**0.15**	**0.78**	**0.011**	154	**0.30**	**0.10**	**0.92**	**0.036**	0.60	0.29	1.24	0.165	151	**0.24**	**0.07**	**0.82**	**0.022**	0.60	0.29	1.24	0.164
n-valeric	3.19	225	0.61	0.29	1.25	0.174	154	0.46	0.16	1.27	0.133	0.91	0.49	1.68	0.761	151	0.41	0.14	1.20	0.104	0.92	0.50	1.71	0.789
i-valeric	4.38	225	**0.43**	**0.19**	**0.94**	**0.033**	154	0.40	0.14	1.18	0.097	0.75	0.38	1.48	0.405	151	0.32	0.10	1.04	0.058	0.75	0.38	1.48	0.405

Table S9. *Continued.*

Abbreviations: CI, confidence interval; LRTI, lower respiratory tract infection; OR, odds ratio; SCFAs, short-chain fatty acids.
Regression coefficients were estimated from unpenalised logistic regression models. Odds ratios per a 2-standard deviation increase in untransformed diversity or absolute SCFA levels (mmol/kg).
[a] Models were adjusted for as described in Figure S4.
[b] Exposures were mean-centered and rescaled to 2 times their standard deviations (increment).

Table S10. Associations between individual SCFAs and the three respiratory health outcomes: penalised logistic regression models.

	Penalised ENET estimation and selection over imputed datasets (n=438)								
	4 months			12 months			24 months		
	% >0	mean OR	mean OR >50% sel.	% >0	mean OR	mean OR >50% sel.	% >0	mean OR	mean OR >50% sel.
Asthma at ~ 10 years									
Acetic	40	0.848	NA	3	0.993	NA	7	1.041	NA
Propionic	29	0.99	NA	10	0.967	NA	18	0.96	NA
n-butyric	39	0.858	NA	2	1.012	NA	9	0.984	NA
i-butyric	37	1.595	NA	5	1.01	NA	8	1.864	NA
n-valeric	37	0.88	NA	1	1.002	NA	35	0.876	NA
i-valeric	29	1.235	NA	40	1.324	NA	10	0.972	NA
Asthma at 2 years									
Acetic	58	0.942	0.942	50	1.21	1.21	42	0.852	NA
Propionic	52	0.922	0.922	21	0.955	NA	31	1.111	NA
n-butyric	58	1.12	1.12	20	1.005	NA	32	1.17	NA
i-butyric	62	0.685	0.685	48	1.457	NA	30	1.695	NA
n-valeric	79	1.727	1.727	26	1.02	NA	47	0.894	NA
i-valeric	43	1.66	NA	17	1	NA	33	1.014	NA
LRTI by 2 years									
Acetic	68	1.342	1.342	51	0.905	0.905	69	1.541	1.541
Propionic	63	1.173	1.173	80	1.437	1.437	75	0.707	0.707
n-butyric	81	0.764	0.764	55	1.091	1.091	63	0.893	0.893
i-butyric	83	0.522	0.522	71	2.575	2.575	80	0.481	0.481
n-valeric	83	0.793	0.793	82	0.688	0.688	66	1.45	1.45
i-valeric	55	2.204	2.204	34	0.868	NA	61	3.383	3.383

Models were minimally adjusted (see Figure S4).

Table S11. Associations between summed groups of environmental chemicals and Shannon diversity and SCFAs: unpenalised single- and multi-pollutant linear regression models.

Exposure (ln-2SD)	Unadjusted, single-pollutant					Adjusted,[a] single-pollutant									Adjusted,[a] multi-pollutant								
	Complete case					Complete case					Multiple imputation[b] (n=298)				Complete case[c]					Multiple imp.[b] (n=298)			
	n	*β*	LCL	UCL	*p*-val	n	*β*	LCL	UCL	*p*-val	*β*	LCL	UCL	*p*-val	n	*β*	LCL	UCL	*p*-val	*β*	LCL	UCL	*p*-val
Diversity																							
4 months																							
Σ_7DL-PCBs	271	0.022	-0.095	0.138	0.717	226	-0.023	-0.155	0.108	0.728	0.002	-0.116	0.120	0.975									
Σ_7NDL-PCBs	273	0.058	-0.058	0.175	0.330	228	0.026	-0.105	0.156	0.700	0.028	-0.090	0.146	0.637									
Σ_{14}PCBs	271	0.051	-0.065	0.168	0.390	226	0.016	-0.116	0.147	0.816	0.023	-0.095	0.141	0.705	177	-0.071	-0.280	0.138	0.508	-0.007	-0.176	0.162	0.936
Σ_4OCPs	261	0.083	-0.036	0.201	0.172	217	0.044	-0.089	0.177	0.518	0.038	-0.083	0.160	0.533		0.077	-0.143	0.297	0.494	0.038	-0.141	0.217	0.677
Σ_6PBDEs	263	0.012	-0.107	0.132	0.839	221	0.022	-0.104	0.148	0.732	0.034	-0.086	0.154	0.579		0.067	-0.075	0.210	0.355	0.025	-0.105	0.155	0.704
Σ_2PFASs	226	0.032	-0.098	0.163	0.628	189	0.007	-0.131	0.144	0.922	0.001	-0.131	0.133	0.987		0.001	-0.153	0.155	0.989	-0.010	-0.150	0.130	0.888
12 months																							
Σ_7DL-PCBs	204	-0.108	-0.242	0.026	0.116	179	-0.107	-0.258	0.045	0.168	-0.122	-0.262	0.018	0.087									
Σ_7NDL-PCBs	207	**-0.141**	**-0.276**	**-0.006**	**0.042**	182	**-0.160**	**-0.308**	**-0.012**	**0.035**	-0.113	-0.247	0.022	0.099									
Σ_{14}PCBs	204	-0.109	-0.242	0.024	0.109	179	-0.121	-0.269	0.028	0.112	-0.117	-0.252	0.018	0.090	143	-0.008	-0.265	0.249	0.952	-0.025	-0.235	0.185	0.812
Σ_4OCPs	200	**-0.166**	**-0.296**	**-0.035**	**0.014**	175	**-0.191**	**-0.333**	**-0.048**	**0.010**	**-0.149**	**-0.278**	**-0.019**	**0.024**		-0.124	-0.381	0.132	0.343	-0.125	-0.335	0.085	0.241
Σ_6PBDEs	198	-0.041	-0.166	0.083	0.515	175	-0.057	-0.188	0.074	0.395	-0.047	-0.180	0.086	0.485		-0.004	-0.147	0.139	0.954	0.002	-0.138	0.142	0.973
Σ_2PFASs	176	-0.050	-0.195	0.095	0.499	155	-0.102	-0.255	0.051	0.194	-0.066	-0.213	0.082	0.381		-0.048	-0.211	0.114	0.561	-0.028	-0.181	0.124	0.713
24 months																							
Σ_7DL-PCBs	88	**-0.309**	**-0.502**	**-0.116**	**0.002**	85	**-0.321**	**-0.521**	**-0.121**	**0.002**	-0.169	-0.377	0.040	0.111									
Σ_7NDL-PCBs	89	**-0.320**	**-0.503**	**-0.138**	**0.001**	86	**-0.317**	**-0.503**	**-0.131**	**0.001**	**-0.210**	**-0.400**	**-0.021**	**0.030**									
Σ_{14}PCBs	88	**-0.319**	**-0.502**	**-0.136**	**0.001**	85	**-0.316**	**-0.503**	**-0.130**	**0.001**	**-0.207**	**-0.400**	**-0.015**	**0.035**	68	**-0.545**	**-0.789**	**-0.301**	**0.000**	**-0.285**	**-0.530**	**-0.041**	**0.022**
Σ_4OCPs	84	-0.059	-0.249	0.131	0.543	81	-0.040	-0.244	0.165	0.704	-0.081	-0.274	0.112	0.405		**0.393**	**0.079**	**0.708**	**0.017**	0.105	-0.142	0.352	0.399
Σ_6PBDEs	87	0.084	-0.101	0.269	0.377	84	0.086	-0.104	0.276	0.376	0.000	-0.168	0.169	0.997		0.036	-0.179	0.250	0.746	0.035	-0.142	0.212	0.697
Σ_2PFASs	77	-0.139	-0.354	0.076	0.209	74	-0.161	-0.382	0.059	0.157	-0.050	-0.233	0.133	0.590		-0.137	-0.346	0.071	0.202	-0.010	-0.201	0.181	0.917
Total SCFAs																							
4 months																							
Σ_7DL-PCBs	87	0.002	-0.205	0.208	0.988	74	-0.045	-0.263	0.172	0.683	0.016	-0.140	0.173	0.835									
Σ_7NDL-PCBs	89	0.071	-0.123	0.264	0.476	76	0.050	-0.150	0.250	0.623	0.048	-0.103	0.199	0.529									
Σ_{14}PCBs	87	0.060	-0.140	0.259	0.559	74	0.026	-0.181	0.233	0.807	0.044	-0.107	0.195	0.566	65	0.202	-0.070	0.473	0.151	0.098	-0.112	0.308	0.359
Σ_4OCPs	89	-0.051	-0.276	0.173	0.654	76	-0.198	-0.439	0.044	0.113	-0.013	-0.165	0.139	0.868		**-0.452**	**-0.812**	**-0.092**	**0.017**	-0.086	-0.306	0.134	0.439

Table S11. *Continued.*

Exposure (ln-2SD)	Unadjusted, single-pollutant					Adjusted,[a] single-pollutant									Adjusted,[a] multi-pollutant								
	Complete case					Complete case					Multiple imputation[b] (n=298)				Complete case[c]					Multiple imp.[b] (n=298)			
	n	*β*	LCL	UCL	*p*-val	n	*β*	LCL	UCL	*p*-val	*β*	LCL	UCL	*p*-val	n	*β*	LCL	UCL	*p*-val	*β*	LCL	UCL	*p*-val
Σ_6PBDEs	84	-0.068	-0.326	0.189	0.605	71	-0.120	-0.376	0.137	0.363	0.005	-0.141	0.151	0.948		0.035	-0.248	0.317	0.812	0.007	-0.150	0.165	0.926
Σ_3PFASs	79	0.067	-0.180	0.313	0.598	69	0.042	-0.193	0.277	0.726	0.018	-0.146	0.183	0.825		0.063	-0.198	0.323	0.639	0.015	-0.156	0.186	0.862
12 months																							
Σ_7DL-PCBs	221	0.097	-0.035	0.229	0.149	196	0.079	-0.053	0.211	0.243	0.069	-0.058	0.196	0.285									
Σ_7NDL-PCBs	224	0.126	-0.003	0.255	0.057	199	0.096	-0.031	0.222	0.139	0.105	-0.020	0.231	0.100									
Σ_{14}PCBs	221	**0.135**	**0.004**	**0.265**	**0.044**	196	0.107	-0.022	0.236	0.104	0.102	-0.024	0.227	0.113	157	**0.339**	**0.147**	**0.531**	**0.001**	0.127	-0.045	0.299	0.146
Σ_4OCPs	213	0.067	-0.065	0.199	0.320	188	-0.044	-0.176	0.088	0.513	0.044	-0.082	0.171	0.490		**-0.274**	**-0.478**	**-0.069**	**0.010**	-0.056	-0.234	0.122	0.539
Σ_6PBDEs	215	0.018	-0.108	0.144	0.785	191	-0.005	-0.123	0.113	0.937	0.022	-0.105	0.149	0.733		0.054	-0.075	0.183	0.411	0.003	-0.131	0.137	0.964
Σ_3PFASs	191	0.079	-0.050	0.209	0.232	173	0.026	-0.112	0.164	0.710	0.067	-0.080	0.214	0.372		-0.014	-0.162	0.134	0.853	0.049	-0.107	0.204	0.536
24 months																							
Σ_7DL-PCBs	184	-0.099	-0.243	0.045	0.179	176	-0.128	-0.276	0.020	0.092	-0.079	-0.214	0.056	0.251									
Σ_7NDL-PCBs	187	-0.084	-0.227	0.059	0.250	179	-0.109	-0.254	0.037	0.146	-0.062	-0.196	0.072	0.364									
Σ_{14}PCBs	184	-0.089	-0.231	0.053	0.220	176	-0.117	-0.262	0.028	0.115	-0.065	-0.200	0.069	0.338	134	0.070	-0.153	0.293	0.540	0.040	-0.154	0.233	0.688
Σ_4OCPs	173	**-0.156**	**-0.296**	**-0.016**	**0.030**	165	**-0.176**	**-0.321**	**-0.031**	**0.018**	-0.115	-0.250	0.021	0.096		-0.116	-0.363	0.132	0.360	-0.110	-0.312	0.091	0.281
Σ_6PBDEs	180	-0.128	-0.264	0.008	0.068	172	-0.125	-0.264	0.013	0.078	-0.066	-0.196	0.065	0.324		-0.068	-0.225	0.088	0.395	-0.027	-0.166	0.113	0.707
Σ_3PFASs	156	-0.148	-0.299	0.002	0.056	151	-0.138	-0.294	0.017	0.082	-0.113	-0.254	0.028	0.116		-0.100	-0.272	0.072	0.256	-0.092	-0.242	0.059	0.230
Acetic																							
4 months																							
Σ_7DL-PCBs	87	-0.039	-0.245	0.168	0.714	74	-0.074	-0.293	0.145	0.511	-0.025	-0.178	0.128	0.749									
Σ_7NDL-PCBs	89	0.016	-0.178	0.210	0.870	76	0.004	-0.196	0.205	0.967	0.002	-0.146	0.150	0.981									
Σ_{14}PCBs	87	0.014	-0.185	0.214	0.888	74	-0.008	-0.216	0.200	0.939	-0.003	-0.151	0.146	0.973	65	0.182	-0.093	0.457	0.200	0.045	-0.162	0.252	0.670
Σ_4OCPs	89	-0.121	-0.344	0.102	0.291	76	**-0.260**	**-0.499**	**-0.022**	**0.036**	-0.038	-0.185	0.110	0.615		**-0.407**	**-0.772**	**-0.042**	**0.033**	-0.047	-0.263	0.168	0.663
Σ_6PBDEs	84	-0.199	-0.453	0.055	0.128	71	**-0.260**	**-0.511**	**-0.008**	**0.047**	-0.075	-0.219	0.068	0.302		-0.131	-0.417	0.156	0.375	-0.072	-0.226	0.082	0.356
Σ_3PFASs	79	0.058	-0.195	0.312	0.653	69	0.028	-0.214	0.270	0.819	-0.001	-0.161	0.160	0.994		0.081	-0.183	0.344	0.552	0.011	-0.154	0.177	0.895
12 months																							
Σ_7DL-PCBs	221	0.117	-0.015	0.249	0.083	196	0.108	-0.024	0.240	0.112	0.073	-0.053	0.199	0.256									
Σ_7NDL-PCBs	224	**0.136**	**0.007**	**0.265**	**0.040**	199	0.126	-0.001	0.252	0.053	0.106	-0.019	0.230	0.096									
Σ_{14}PCBs	221	**0.147**	**0.017**	**0.277**	**0.028**	196	**0.140**	**0.011**	**0.268**	**0.034**	0.102	-0.023	0.227	0.108	157	**0.360**	**0.173**	**0.547**	**0.000**	0.088	-0.083	0.259	0.312
Σ_4OCPs	213	0.105	-0.027	0.238	0.120	188	-0.005	-0.138	0.128	0.937	0.075	-0.051	0.201	0.242		**-0.235**	**-0.434**	**-0.035**	**0.022**	0.003	-0.174	0.181	0.969

Table S11. *Continued.*

Exposure (ln-2SD)	Unadjusted, single-pollutant					Adjusted,[a] single-pollutant									Adjusted,[a] multi-pollutant								
	Complete case					Complete case					Multiple imputation[b] (n=298)				Complete case[c]					Multiple imp.[b] (n=298)			
	n	*β*	LCL	UCL	*p*-val	n	*β*	LCL	UCL	*p*-val	*β*	LCL	UCL	*p*-val	n	*β*	LCL	UCL	*p*-val	*β*	LCL	UCL	*p*-val
Σ_6PBDEs	215	0.021	-0.106	0.148	0.746	191	0.000	-0.120	0.119	0.994	0.020	-0.105	0.144	0.756		0.044	-0.082	0.169	0.494	-0.010	-0.143	0.122	0.877
Σ_2PFASs	191	0.104	-0.020	0.229	0.101	173	0.048	-0.086	0.181	0.486	0.080	-0.072	0.232	0.300		-0.005	-0.150	0.139	0.946	0.059	-0.102	0.220	0.471
24 months																							
Σ_7DL-PCBs	184	-0.035	-0.176	0.107	0.634	176	-0.055	-0.197	0.088	0.451	-0.036	-0.169	0.098	0.597									
Σ_7NDL-PCBs	187	-0.029	-0.172	0.115	0.694	179	-0.048	-0.192	0.095	0.512	-0.018	-0.152	0.115	0.786									
Σ_{14}PCBs	184	-0.032	-0.171	0.107	0.652	176	-0.054	-0.193	0.085	0.447	-0.021	-0.155	0.113	0.758	134	0.114	-0.108	0.335	0.316	0.070	-0.118	0.257	0.464
Σ_4OCPs	173	-0.114	-0.258	0.030	0.124	165	-0.134	-0.282	0.013	0.075	-0.072	-0.208	0.064	0.297		-0.125	-0.370	0.121	0.322	-0.081	-0.277	0.115	0.416
Σ_6PBDEs	180	-0.128	-0.262	0.005	0.062	172	-0.123	-0.256	0.010	0.071	-0.064	-0.201	0.072	0.352		-0.073	-0.228	0.082	0.360	-0.041	-0.185	0.104	0.581
Σ_2PFASs	156	-0.147	-0.302	0.008	0.065	151	-0.127	-0.283	0.030	0.114	-0.111	-0.251	0.028	0.118		-0.096	-0.266	0.075	0.274	-0.103	-0.254	0.048	0.181
Propionic																							
4 months																							
Σ_7DL-PCBs	87	0.101	-0.098	0.300	0.324	74	0.040	-0.172	0.252	0.712	0.098	-0.074	0.270	0.259									
Σ_7NDL-PCBs	89	**0.203**	**0.014**	**0.392**	**0.038**	76	0.157	-0.047	0.361	0.135	0.134	-0.033	0.301	0.116									
Σ_{14}PCBs	87	0.156	-0.035	0.347	0.112	74	0.098	-0.102	0.298	0.341	0.131	-0.036	0.298	0.123	65	0.116	-0.131	0.363	0.363	0.174	-0.066	0.414	0.154
Σ_4OCPs	89	0.203	-0.018	0.423	0.075	76	0.108	-0.145	0.361	0.407	0.057	-0.130	0.244	0.547		-0.273	-0.601	0.055	0.108	-0.106	-0.375	0.164	0.438
Σ_6PBDEs	84	**0.312**	**0.071**	**0.553**	**0.013**	71	**0.309**	**0.068**	**0.551**	**0.015**	0.157	-0.074	0.389	0.179		**0.444**	**0.187**	**0.702**	**0.001**	0.155	-0.091	0.400	0.211
Σ_2PFASs	79	-0.007	-0.231	0.217	0.952	69	0.024	-0.213	0.262	0.841	0.008	-0.188	0.204	0.934		-0.061	-0.298	0.176	0.617	-0.031	-0.241	0.180	0.772
12 months																							
Σ_7DL-PCBs	221	0.038	-0.095	0.171	0.573	196	0.008	-0.122	0.137	0.909	0.051	-0.080	0.182	0.444									
Σ_7NDL-PCBs	224	0.082	-0.047	0.212	0.214	199	0.019	-0.104	0.143	0.760	0.081	-0.048	0.209	0.217									
Σ_{14}PCBs	221	0.078	-0.053	0.210	0.244	196	0.017	-0.110	0.143	0.795	0.078	-0.051	0.207	0.234	157	0.119	-0.085	0.323	0.256	0.160	-0.013	0.333	0.069
Σ_4OCPs	213	-0.024	-0.156	0.108	0.722	188	-0.041	-0.169	0.087	0.532	-0.011	-0.137	0.116	0.870		-0.128	-0.346	0.089	0.249	-0.117	-0.293	0.058	0.188
Σ_6PBDEs	215	-0.021	-0.150	0.109	0.755	191	-0.019	-0.136	0.098	0.751	-0.010	-0.136	0.115	0.873		0.000	-0.137	0.137	0.997	-0.011	-0.143	0.121	0.872
Σ_2PFASs	191	-0.003	-0.140	0.134	0.967	173	-0.017	-0.155	0.121	0.806	0.012	-0.122	0.147	0.855		-0.024	-0.182	0.134	0.767	0.004	-0.137	0.144	0.960
24 months																							
Σ_7DL-PCBs	184	**-0.180**	**-0.326**	**-0.034**	**0.017**	176	**-0.208**	**-0.362**	**-0.054**	**0.009**	-0.106	-0.253	0.041	0.156									
Σ_7NDL-PCBs	187	**-0.166**	**-0.307**	**-0.024**	**0.023**	179	**-0.180**	**-0.328**	**-0.032**	**0.018**	-0.105	-0.248	0.038	0.150									
Σ_{14}PCBs	184	**-0.172**	**-0.316**	**-0.028**	**0.020**	176	**-0.190**	**-0.341**	**-0.039**	**0.015**	-0.107	-0.251	0.037	0.143	134	-0.026	-0.257	0.205	0.826	-0.029	-0.238	0.179	0.780
Σ_4OCPs	173	**-0.176**	**-0.316**	**-0.036**	**0.015**	165	**-0.181**	**-0.330**	**-0.032**	**0.019**	-0.127	-0.270	0.017	0.083		-0.096	-0.352	0.161	0.466	-0.090	-0.309	0.130	0.421

Table S11. *Continued.*

Exposure (ln-2SD)	Unadjusted, single-pollutant					Adjusted,[a] single-pollutant									Adjusted,[a] multi-pollutant								
	Complete case					Complete case					Multiple imputation[b] (n=298)				Complete case[c]					Multiple imp.[b] (n=298)			
	n	*β*	LCL	UCL	*p*-val	n	*β*	LCL	UCL	*p*-val	*β*	LCL	UCL	*p*-val	n	*β*	LCL	UCL	*p*-val	*β*	LCL	UCL	*p*-val
Σ_6PBDEs	180	-0.097	-0.235	0.040	0.168	172	-0.105	-0.249	0.038	0.152	-0.053	-0.193	0.088	0.462		-0.039	-0.201	0.123	0.639	-0.009	-0.161	0.143	0.909
Σ_3PFASs	156	-0.135	-0.286	0.016	0.081	151	-0.146	-0.304	0.012	0.072	-0.088	-0.241	0.065	0.258		-0.090	-0.268	0.088	0.322	-0.057	-0.221	0.106	0.489
***n*-butyric**																							
4 months																							
Σ_7DL-PCBs	87	0.074	-0.131	0.279	0.483	74	0.071	-0.150	0.291	0.531	0.048	-0.162	0.257	0.651									
Σ_7NDL-PCBs	89	0.048	-0.146	0.242	0.628	76	0.079	-0.122	0.281	0.443	0.035	-0.159	0.230	0.717									
Σ_{14}PCBs	87	0.068	-0.130	0.266	0.504	74	0.087	-0.121	0.296	0.415	0.039	-0.157	0.234	0.695	65	0.208	-0.080	0.496	0.162	0.048	-0.246	0.343	0.744
Σ_4OCPs	89	-0.020	-0.245	0.204	0.860	76	-0.029	-0.278	0.219	0.819	0.005	-0.204	0.214	0.965		**-0.447**	**-0.829**	**-0.065**	**0.025**	-0.123	-0.433	0.187	0.428
Σ_6PBDEs	84	**0.296**	**0.046**	**0.545**	**0.023**	71	**0.302**	**0.049**	**0.554**	**0.022**	0.203	-0.017	0.423	0.070		**0.445**	**0.145**	**0.744**	**0.005**	0.217	-0.015	0.450	0.066
Σ_2PFASs	79	0.177	-0.079	0.434	0.179	69	0.112	-0.141	0.366	0.389	0.107	-0.093	0.307	0.291		0.064	-0.212	0.340	0.653	0.094	-0.122	0.311	0.387
12 months																							
Σ_7DL-PCBs	221	0.021	-0.112	0.153	0.760	196	0.017	-0.126	0.161	0.812	0.012	-0.120	0.145	0.855									
Σ_7NDL-PCBs	224	0.039	-0.091	0.169	0.560	199	0.021	-0.117	0.158	0.769	0.029	-0.103	0.162	0.662									
Σ_{14}PCBs	221	0.043	-0.089	0.175	0.522	196	0.029	-0.112	0.169	0.691	0.027	-0.105	0.159	0.686	157	**0.244**	**0.035**	**0.452**	**0.023**	0.104	-0.078	0.285	0.260
Σ_4OCPs	213	-0.023	-0.153	0.108	0.735	188	-0.127	-0.267	0.012	0.075	-0.043	-0.177	0.091	0.528		**-0.339**	**-0.561**	**-0.116**	**0.003**	-0.132	-0.322	0.059	0.174
Σ_6PBDEs	215	0.024	-0.102	0.150	0.708	191	-0.004	-0.131	0.122	0.946	0.025	-0.107	0.157	0.710		0.091	-0.049	0.231	0.205	0.040	-0.098	0.179	0.567
Σ_2PFASs	191	0.011	-0.127	0.149	0.874	173	-0.022	-0.175	0.131	0.780	0.015	-0.125	0.156	0.829		-0.025	-0.186	0.137	0.766	0.017	-0.131	0.164	0.825
24 months																							
Σ_7DL-PCBs	184	-0.101	-0.248	0.046	0.181	176	-0.137	-0.288	0.015	0.079	-0.075	-0.217	0.067	0.297									
Σ_7NDL-PCBs	187	-0.074	-0.217	0.069	0.311	179	-0.104	-0.250	0.042	0.166	-0.060	-0.200	0.081	0.402									
Σ_{14}PCBs	184	-0.080	-0.225	0.065	0.282	176	-0.114	-0.262	0.035	0.135	-0.064	-0.205	0.077	0.373	134	0.059	-0.152	0.271	0.584	0.030	-0.179	0.239	0.779
Σ_4OCPs	173	**-0.143**	**-0.276**	**-0.010**	**0.036**	165	**-0.166**	**-0.304**	**-0.028**	**0.020**	-0.112	-0.253	0.029	0.119		-0.117	-0.352	0.118	0.330	-0.125	-0.343	0.093	0.257
Σ_6PBDEs	180	-0.069	-0.209	0.071	0.336	172	-0.065	-0.208	0.077	0.372	-0.034	-0.169	0.101	0.622		-0.020	-0.169	0.128	0.788	0.004	-0.141	0.149	0.956
Σ_2PFASs	156	-0.066	-0.201	0.070	0.345	151	-0.060	-0.200	0.080	0.401	-0.056	-0.208	0.097	0.473		-0.029	-0.192	0.134	0.730	-0.033	-0.192	0.127	0.688
***i*-butyric**																							
4 months																							
Σ_7DL-PCBs	87	0.180	-0.022	0.383	0.084	74	0.111	-0.107	0.329	0.321	0.131	-0.074	0.337	0.207									
Σ_7NDL-PCBs	89	0.176	-0.014	0.367	0.073	76	0.131	-0.069	0.332	0.204	0.121	-0.075	0.318	0.221									
Σ_{14}PCBs	87	0.175	-0.021	0.371	0.083	74	0.117	-0.090	0.324	0.271	0.125	-0.072	0.323	0.210	65	0.076	-0.215	0.367	0.610	0.142	-0.147	0.432	0.329

Table S11. *Continued.*

Exposure (ln-2SD)	Unadjusted, single-pollutant					Adjusted,[a] single-pollutant									Adjusted,[a] multi-pollutant								
	Complete case					Complete case					Multiple imputation[b] (n=298)				Complete case[c]					Multiple imp.[b] (n=298)			
	n	*β*	LCL	UCL	*p*-val	n	*β*	LCL	UCL	*p*-val	*β*	LCL	UCL	*p*-val	n	*β*	LCL	UCL	*p*-val	*β*	LCL	UCL	*p*-val
Σ_4OCPs	89	0.183	-0.038	0.404	0.108	76	0.091	-0.157	0.340	0.473	0.068	-0.152	0.289	0.537		-0.118	-0.504	0.268	0.552	-0.037	-0.353	0.278	0.814
Σ_6PBDEs	84	0.175	-0.078	0.427	0.179	71	0.193	-0.064	0.450	0.146	0.031	-0.203	0.264	0.793		0.230	-0.073	0.533	0.142	0.005	-0.241	0.252	0.966
Σ_2PFASs	79	0.118	-0.115	0.352	0.324	69	0.110	-0.135	0.354	0.383	0.054	-0.166	0.275	0.624		0.083	-0.196	0.363	0.560	0.027	-0.208	0.263	0.817
12 months																							
Σ_7DL-PCBs	221	-0.017	-0.150	0.116	0.798	196	-0.090	-0.231	0.051	0.210	-0.005	-0.134	0.125	0.945									
Σ_7NDL-PCBs	224	-0.003	-0.133	0.127	0.966	199	-0.091	-0.225	0.044	0.189	0.009	-0.118	0.135	0.892									
Σ_{14}PCBs	221	-0.002	-0.134	0.130	0.976	196	-0.091	-0.229	0.047	0.196	0.007	-0.120	0.134	0.914	157	-0.039	-0.249	0.170	0.713	0.013	-0.162	0.187	0.887
Σ_4OCPs	213	-0.012	-0.144	0.120	0.854	188	-0.071	-0.211	0.069	0.324	0.002	-0.124	0.128	0.978		-0.089	-0.312	0.134	0.437	-0.019	-0.198	0.160	0.835
Σ_6PBDEs	215	0.032	-0.091	0.155	0.611	191	0.029	-0.092	0.151	0.639	0.053	-0.079	0.184	0.433		0.058	-0.083	0.198	0.422	0.059	-0.080	0.199	0.402
Σ_2PFASs	191	-0.029	-0.168	0.111	0.687	173	-0.029	-0.179	0.122	0.709	-0.022	-0.154	0.110	0.743		0.011	-0.151	0.173	0.898	-0.029	-0.168	0.110	0.682
24 months																							
Σ_7DL-PCBs	184	-0.142	-0.289	0.006	0.062	176	-0.146	-0.296	0.004	0.059	-0.103	-0.248	0.041	0.160									
Σ_7NDL-PCBs	187	-0.093	-0.236	0.050	0.203	179	-0.113	-0.257	0.032	0.128	-0.067	-0.208	0.074	0.351									
Σ_{14}PCBs	184	-0.099	-0.245	0.047	0.185	176	-0.118	-0.265	0.029	0.118	-0.074	-0.216	0.068	0.303	134	-0.108	-0.335	0.119	0.351	-0.072	-0.278	0.135	0.494
Σ_4OCPs	173	-0.068	-0.209	0.072	0.342	165	-0.077	-0.222	0.068	0.301	-0.046	-0.187	0.096	0.525		0.145	-0.106	0.397	0.260	0.030	-0.188	0.248	0.787
Σ_6PBDEs	180	-0.121	-0.260	0.018	0.090	172	-0.111	-0.251	0.030	0.124	-0.053	-0.198	0.092	0.473		-0.120	-0.279	0.039	0.143	-0.038	-0.196	0.121	0.640
Σ_2PFASs	156	-0.111	-0.266	0.043	0.160	151	-0.134	-0.292	0.023	0.097	-0.073	-0.217	0.071	0.317		-0.152	-0.327	0.023	0.092	-0.058	-0.212	0.096	0.461
***n*-valeric**																							
4 months																							
Σ_7DL-PCBs	87	-0.048	-0.254	0.158	0.650	74	-0.050	-0.299	0.200	0.698	0.077	-0.134	0.287	0.469									
Σ_7NDL-PCBs	89	-0.050	-0.244	0.144	0.612	76	-0.083	-0.311	0.145	0.477	0.073	-0.129	0.275	0.474									
Σ_{14}PCBs	87	-0.042	-0.241	0.157	0.679	74	-0.067	-0.304	0.169	0.578	0.076	-0.128	0.279	0.461	65	-0.097	-0.363	0.169	0.477	0.130	-0.152	0.413	0.360
Σ_4OCPs	89	0.049	-0.175	0.273	0.667	76	0.016	-0.265	0.298	0.910	0.011	-0.213	0.235	0.921		0.060	-0.293	0.412	0.742	-0.086	-0.397	0.226	0.584
Σ_6PBDEs	84	0.189	-0.066	0.444	0.149	71	0.206	-0.089	0.501	0.176	0.038	-0.199	0.274	0.750		0.226	-0.050	0.503	0.114	0.037	-0.213	0.288	0.765
Σ_2PFASs	79	0.017	-0.184	0.217	0.871	69	-0.022	-0.245	0.200	0.847	-0.001	-0.226	0.224	0.993		-0.042	-0.297	0.213	0.750	-0.018	-0.252	0.217	0.880
12 months																							
Σ_7DL-PCBs	221	-0.032	-0.165	0.100	0.632	196	-0.035	-0.172	0.103	0.622	0.028	-0.102	0.158	0.669									
Σ_7NDL-PCBs	224	0.007	-0.123	0.137	0.915	199	-0.021	-0.153	0.111	0.758	0.051	-0.080	0.182	0.444									
Σ_{14}PCBs	221	0.002	-0.130	0.134	0.978	196	-0.026	-0.160	0.109	0.710	0.049	-0.082	0.179	0.461	157	-0.107	-0.288	0.074	0.250	0.144	-0.040	0.329	0.125

Table S11. *Continued.*

Exposure (ln-2SD)	Unadjusted, single-pollutant					Adjusted,[a] single-pollutant									Adjusted,[a] multi-pollutant								
	Complete case					Complete case					Multiple imputation[b] (n=298)				Complete case[c]					Multiple imp.[b] (n=298)			
	n	*β*	LCL	UCL	*p*-val	n	*β*	LCL	UCL	*p*-val	*β*	LCL	UCL	*p*-val	n	*β*	LCL	UCL	*p*-val	*β*	LCL	UCL	*p*-val
Σ_4OCPs	213	-0.061	-0.192	0.070	0.362	188	-0.056	-0.191	0.079	0.418	-0.046	-0.174	0.082	0.482		0.115	-0.078	0.307	0.246	-0.165	-0.354	0.025	0.089
Σ_6PBDEs	215	-0.028	-0.154	0.099	0.669	191	-0.044	-0.165	0.077	0.478	0.027	-0.108	0.162	0.694		-0.029	-0.151	0.092	0.636	0.042	-0.101	0.184	0.565
Σ_2PFASs	191	0.048	-0.079	0.175	0.463	173	0.002	-0.130	0.135	0.972	0.028	-0.117	0.174	0.702		-0.039	-0.179	0.101	0.583	0.028	-0.123	0.178	0.719
24 months																							
Σ_7DL-PCBs	184	-0.134	-0.282	0.014	0.078	176	-0.143	-0.298	0.011	0.071	-0.080	-0.231	0.071	0.298									
Σ_7NDL-PCBs	187	**-0.148**	**-0.290**	**-0.006**	**0.042**	179	**-0.164**	**-0.311**	**-0.018**	**0.029**	-0.093	-0.241	0.056	0.219									
Σ_{14}PCBs	184	**-0.147**	**-0.292**	**-0.002**	**0.048**	176	**-0.165**	**-0.316**	**-0.015**	**0.032**	-0.092	-0.242	0.057	0.223	134	-0.112	-0.328	0.104	0.311	-0.034	-0.247	0.179	0.753
Σ_4OCPs	173	**-0.161**	**-0.300**	**-0.021**	**0.025**	165	**-0.175**	**-0.321**	**-0.028**	**0.021**	-0.111	-0.258	0.037	0.139		0.051	-0.189	0.290	0.679	-0.103	-0.321	0.115	0.352
Σ_6PBDEs	180	-0.065	-0.205	0.075	0.364	172	-0.082	-0.226	0.062	0.267	-0.001	-0.142	0.139	0.987		-0.034	-0.186	0.117	0.658	0.038	-0.110	0.186	0.610
Σ_2PFASs	156	-0.097	-0.242	0.049	0.195	151	-0.107	-0.258	0.045	0.169	-0.017	-0.175	0.140	0.829		-0.085	-0.252	0.081	0.317	0.011	-0.155	0.177	0.897
***i*-valeric**																							
4 months																							
Σ_7DL-PCBs	87	0.156	-0.048	0.359	0.137	74	0.087	-0.138	0.311	0.451	0.140	-0.054	0.335	0.155									
Σ_7NDL-PCBs	89	0.166	-0.025	0.357	0.092	76	0.123	-0.084	0.330	0.247	0.145	-0.042	0.333	0.127									
Σ_{14}PCBs	87	0.157	-0.040	0.353	0.121	74	0.098	-0.114	0.311	0.368	0.147	-0.041	0.336	0.123	65	0.077	-0.224	0.378	0.618	0.147	-0.155	0.449	0.333
Σ_4OCPs	89	0.181	-0.040	0.402	0.112	76	0.090	-0.166	0.346	0.492	0.092	-0.117	0.301	0.382		-0.184	-0.583	0.215	0.371	-0.066	-0.397	0.265	0.691
Σ_6PBDEs	84	0.224	-0.025	0.473	0.082	71	0.239	-0.022	0.499	0.078	0.168	-0.076	0.411	0.173		0.294	-0.019	0.607	0.071	0.148	-0.112	0.409	0.258
Σ_2PFASs	79	0.126	-0.116	0.368	0.312	69	0.101	-0.156	0.359	0.443	0.079	-0.138	0.295	0.468		0.085	-0.204	0.373	0.567	0.037	-0.192	0.267	0.746
12 months																							
Σ_7DL-PCBs	221	-0.042	-0.175	0.091	0.537	196	-0.119	-0.262	0.024	0.106	-0.021	-0.151	0.110	0.753									
Σ_7NDL-PCBs	224	-0.034	-0.164	0.096	0.611	199	-0.123	-0.260	0.013	0.079	-0.015	-0.142	0.113	0.819									
Σ_{14}PCBs	221	-0.034	-0.165	0.098	0.619	196	-0.125	-0.265	0.015	0.081	-0.016	-0.143	0.112	0.811	157	-0.092	-0.304	0.121	0.399	-0.024	-0.199	0.152	0.789
Σ_4OCPs	213	-0.017	-0.150	0.115	0.798	188	-0.073	-0.217	0.071	0.322	-0.002	-0.129	0.125	0.976		-0.044	-0.270	0.182	0.704	0.005	-0.174	0.184	0.957
Σ_6PBDEs	215	0.024	-0.099	0.147	0.702	191	0.016	-0.107	0.140	0.797	0.047	-0.085	0.180	0.480		0.043	-0.099	0.186	0.554	0.056	-0.083	0.195	0.428
Σ_2PFASs	191	-0.047	-0.186	0.093	0.512	173	-0.037	-0.190	0.115	0.632	-0.032	-0.166	0.102	0.638		0.002	-0.162	0.166	0.984	-0.035	-0.176	0.105	0.620
24 months																							
Σ_7DL-PCBs	184	-0.112	-0.260	0.036	0.140	176	-0.111	-0.261	0.040	0.153	-0.077	-0.228	0.073	0.312									
Σ_7NDL-PCBs	187	-0.074	-0.217	0.069	0.312	179	-0.089	-0.234	0.055	0.227	-0.047	-0.195	0.100	0.528									
Σ_{14}PCBs	184	-0.077	-0.223	0.069	0.303	176	-0.091	-0.238	0.057	0.230	-0.053	-0.202	0.095	0.479	134	-0.090	-0.312	0.132	0.428	-0.040	-0.251	0.171	0.708

Table S11. *Continued.*

Exposure (ln-2SD)	Unadjusted, single-pollutant					Adjusted,[a] single-pollutant									Adjusted,[a] multi-pollutant								
	Complete case					Complete case					Multiple imputation[b] (n=298)				Complete case[c]					Multiple imp.[b] (n=298)			
	n	*β*	LCL	UCL	*p*-val	n	*β*	LCL	UCL	*p*-val	*β*	LCL	UCL	*p*-val	n	*β*	LCL	UCL	*p*-val	*β*	LCL	UCL	*p*-val
$\sum_4$OCPs	173	-0.066	-0.208	0.076	0.362	165	-0.063	-0.209	0.083	0.399	-0.041	-0.188	0.107	0.586		0.162	-0.084	0.408	0.199	0.010	-0.206	0.225	0.930
$\sum_6$PBDEs	180	-0.116	-0.255	0.023	0.104	172	-0.111	-0.251	0.029	0.123	-0.048	-0.190	0.094	0.505		-0.149	-0.305	0.007	0.063	-0.035	-0.185	0.115	0.645
$\sum_2$PFASs	156	-0.099	-0.250	0.051	0.199	151	-0.119	-0.272	0.035	0.131	-0.058	-0.209	0.093	0.446		-0.145	-0.316	0.026	0.099	-0.046	-0.206	0.114	0.570

Abbreviations: PBDE, polybrominated diphenyl ether; CI, confidence interval; LRTI, lower respiratory tract infection; OCP, organochlorine pesticides; PCB, polychlorinated biphenyls; PFAS, poly-and perfluoroalkyl substances.

Regression coefficients were estimated from unpenalised linear regression models; *β* (95% CI) represent a 2-standard deviation change in untransformed diversity or absolute SCFA levels (mmol/kg) per 2-standard deviation increase in ln-transformed contaminant levels (refer to Tables S5 and S9 for the increments). Summed-PBDEs, PCBs, OCPs and PBDEs are in units of pmol/g and summed-PFASs in pmol/L.

[a] Models were adjusted for maternal education, breastfeeding duration, C-section, and recent antibiotic use (refer to Figure S4).

[b] Multiple imputation models: n=100 imputed datasets (n=298). Data was missing for 32.6–43.2% of the contaminant data; 11.0–31.3% of the Shannon diversity and 26.0–68.0% of the SCFA data; and 0.0–18.9% of the confounder data.

[c] For multi-pollutant models, VIFs for exposures were <3, but highest (~2.3) for the $\sum_{14}$PCBs and $\sum_4$OCPs and lower (~1.2) for $\sum_6$PBDEs and $\sum_2$PFASs.

Table S12. Associations between individual environmental chemicals and Shannon diversity and SCFAs: penalised elastic net regression models.

	Diversity						Total SCFAs						Acetic						Propionic					
	4 mo.		12 mo.		24 mo.		4 mo.		12 mo.		24 mo.		4 mo.		12 mo.		24 mo.		4 mo.		12 mo.		24 mo.	
Exposure	%	β[a]	%	β	%	OR	%	β	%	β	%	β	%	β	%	β	%	β	%	β	%	β	%	β
PCB-74	14	0	29	0	66	-0.149	8	0	0	0	42	0	9	0	0	0	54	-5.278	39	0	3	0	42	0
PCB-99	12	0	4	0	31	0	3	0	0	0	10	0	2	0	0	0	29	0	16	0	1	0	2	0
PCB-105	17	0	17	0	50	-0.133	9	0	1	0	1	0	19	0	0	0	3	0	23	0	2	0	2	0
PCB-114	35	0	**77**	**-0.069**	**72**	**-0.012**	19	0	25	0	17	0	21	0	12	0	25	0	52	-0.305	3	0	21	0
PCB-118	11	0	1	0	39	0	9	0	0	0	10	0	7	0	0	0	11	0	29	0	0	0	3	0
PCB-138	8	0	30	0	67	-0.342	7	0	0	0	3	0	2	0	0	0	10	0	**77**	5.500	3	0	0	0
PCB-153	29	0	1	0	23	0	1	0	0	0	0	0	2	0	0	0	0	0	14	0	0	0	1	0
PCB-156	23	0	15	0	34	0	7	0	0	0	0	0	4	0	0	0	0	0	15	0	2	0	3	0
PCB-157	31	0	32	0	27	0	5	0	3	0	2	0	12	0	0	0	12	0	47	0	1	0	6	0
PCB-167	24	0	23	0	34	0	4	0	0	0	1	0	4	0	0	0	1	0	30	0	4	0	2	0
PCB-170	4	0	13	0	45	0	21	0	44	0	1	0	20	0	22	0	4	0	13	0	27	0	3	0
PCB-180	31	0	2	0	13	0	6	0	15	0	4	0	9	0	18	0	2	0	29	0	0	0	10	0
PCB-189	25	0	30	0	66	0.065	15	0	10	0	11	0	14	0	0	0	34	0	45	0	29	0	10	0
PCB-194	30	0	51	0.049	58	-0.001	33	0	26	0	17	0	16	0	0	0	52	1.509	41	0	31	0	2	0
HCB	33	0	31	0	65	0.005	15	0	0	0	17	0	21	0	0	0	40	0	50	-1.179	3	0	10	0
β-HCH	30	0	**84**	**-0.072**	**75**	**0.171**	13	0	0	0	2	0	14	0	0	0	14	0	36	0	2	0	23	0
oxychlor.	32	0	30	0	48	0	17	0	1	0	17	0	18	0	0	0	26	0	44	0	3	0	8	0
DDE	16	0	**71**	**-0.091**	48	0	13	0	0	0	41	0	14	0	0	0	21	0	60	-1.800	9	0	29	0
BDE-28	35	0	26	0	61	0.038	15	0	1	0	25	0	8	0	0	0	44	0	41	0	1	0	4	0
BDE-47	29	0	38	0	42	0	8	0	1	0	7	0	17	0	0	0	14	0	69	2.491	1	0	7	0
BDE-99	19	0	19	0	48	0	12	0	0	0	46	0	13	0	0	0	60	-2.626	47	0	3	0	23	0
BDE-100	16	0	10	0	48	0	13	0	0	0	6	0	29	0	0	0	14	0	34	0	8	0	1	0
BDE-153	24	0	36	0	58	-0.065	15	0	1	0	8	0	21	0	0	0	24	0	47	0	3	0	5	0
BDE-154	26	0	42	0	53	0.020	8	0	0	0	13	0	16	0	0	0	20	0	40	0	3	0	27	0
PFOA	42	0	67	-0.085	**83**	**0.181**	26	0	18	0	59	-3.732	37	0	5	0	**80**	**-5.095**	53	0.534	19	0	17	0
PFOS	39	0	25	0	**78**	**-0.153**	36	0	1	0	18	0	38	0	6	0	19	0	60	-1.019	8	0	19	0

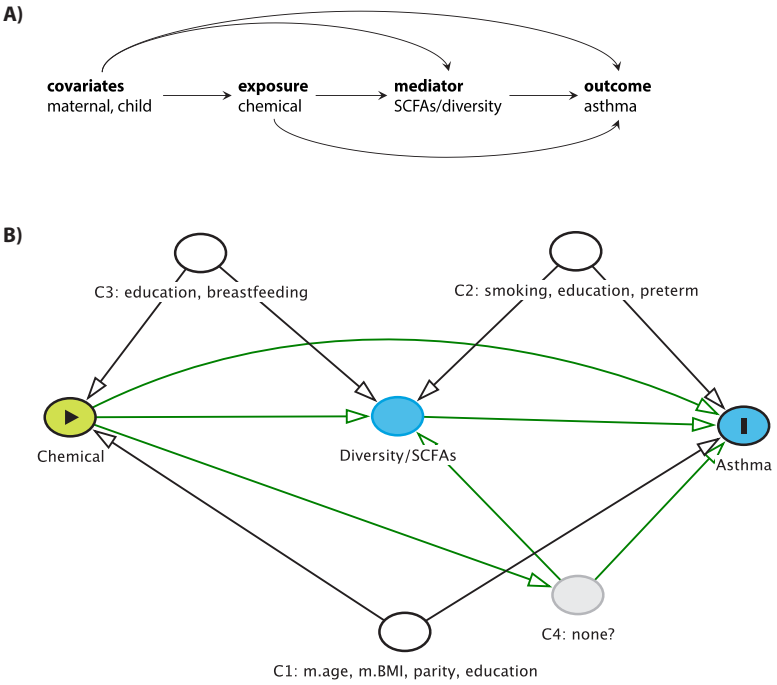
Table S12. *Continued*

	n-butyric						*i*-butyric						*n*-valeric						*i*-valeric					
	4 mo.		12 mo.		24 mo.		4 mo.		12 mo.		24 mo.		4 mo.		12 mo.		24 mo.		4 mo.		12 mo.		24 mo.	
Exposure	%	β[a]	%	β	%	OR	%	β	%	β	%	β	%	β	%	β	%	β	%	β	%	β	%	β
PCB-74	45	0	6	0	4	0	48	0	1	0	30	0	42	0	44	0	42	0	42	0	0	0	40	0
PCB-99	38	0	0	0	1	0	19	0	0	0	7	0	24	0	27	0	1	0	29	0	0	0	10	0
PCB-105	43	0	1	0	0	0	45	0	2	0	2	0	32	0	34	0	12	0	38	0	0	0	6	0
PCB-114	56	-0.355	8	0	18	0	38	0	8	0	28	0	52	-0.017	54	-0.226	23	0	53	-0.100	7	0	27	0
PCB-118	24	0	0	0	7	0	23	0	0	0	20	0	26	0	6	0	0	0	16	0	0	0	3	0
PCB-138	34	0	0	0	2	0	48	0	1	0	0	0	35	0	7	0	2	0	29	0	0	0	3	0
PCB-153	31	0	0	0	0	0	19	0	0	0	3	0	15	0	14	0	3	0	12	0	0	0	18	0
PCB-156	32	0	0	0	4	0	23	0	1	0	7	0	22	0	44	0	10	0	19	0	0	0	3	0
PCB-157	46	0	3	0	2	0	47	0	0	0	8	0	**75**	0.130	59	0.353	4	0	36	0	0	0	6	0
PCB-167	41	0	1	0	3	0	35	0	2	0	11	0	31	0	49	0	0	0	43	0	0	0	3	0
PCB-170	44	0	1	0	0	0	18	0	1	0	0	0	28	0	19	0	2	0	18	0	0	0	2	0
PCB-180	31	0	0	0	3	0	26	0	0	0	6	0	33	0	24	0	15	0	21	0	0	0	3	0
PCB-189	66	0.628	3	0	5	0	52	0.038	2	0	28	0	45	0	35	0	13	0	60	0.115	3	0	31	0
PCB-194	52	0.343	16	0	1	0	69	0.134	1	0	5	0	65	0.020	41	0	14	0	**85**	**0.236**	0	0	14	0
HCB	68	0.479	1	0	3	0	47	0	0	0	13	0	51	0.015	38	0	23	0	47	0	0	0	18	0
β-HCH	52	-0.171	8	0	3	0	47	0	5	0	14	0	37	0	42	0	9	0	33	0	0	0	17	0
oxychlor.	65	0.151	7	0	10	0	55	0.002	0	0	14	0	60	0.001	41	0	14	0	46	0	0	0	19	0
DDE	**77**	-1.118	4	0	53	-0.984	60	-0.021	1	0	5	0	58	-0.019	47	0	29	0	53	-0.090	0	0	10	0
BDE-28	**75**	**1.333**	4	0	10	0	61	0.100	2	0	10	0	65	-0.021	35	0	21	0	54	0.127	3	0	21	0
BDE-47	41	0	1	0	8	0	43	0	2	0	12	0	44	0	24	0	3	0	44	0	2	0	14	0
BDE-99	91	2.906	1	0	8	0	43	0	3	0	31	0	53	0.005	24	0	6	0	66	0.159	2	0	29	0
BDE-100	47	0	2	0	2	0	35	0	2	0	3	0	32	0	30	0	11	0	41	0	0	0	7	0
BDE-153	47	0	3	0	24	0	48	0	3	0	20	0	39	0	32	0	7	0	46	0	0	0	21	0
BDE-154	44	0	2	0	6	0	41	0	2	0	8	0	41	0	24	0	6	0	37	0	0	0	12	0
PFOA	67	0.343	6	0	6	0	64	0.105	4	0	11	0	60	-0.004	44	0	19	0	64	0.192	6	0	20	0
PFOS	78	0.846	2	0	12	0	56	-0.041	4	0	39	0	60	-0.007	41	0	11	0	45	0	8	0	37	0

Regression coefficients were estimated from penalised elastic net linear regression models; β (95% CI) represent a 2-standard deviation change in untransformed diversity or absolute SCFA levels (mmol/kg) per 2-standard deviation increase in ln-transformed contaminant levels (refer to Tables S5 and S9 for the increments). Models were adjusted for maternal education, breast-feeding duration, C-section, and recent antibiotic use (refer to Figure S4).

[a] Averaged coefficients from the elastic net models are presented for those exposures which were picked up >50% of the 100 imputed datasets tested (n=298).

Figure S5. Mediation analysis: **(A)** a simplistic schematic; **(B)** confounding model; and **(C)** results for the two investigated exposure-mediator-outcome pathways.



Assumptions: Sufficient control for C1) exposure-outcome, C2) mediator-outcome, C3) exposure-mediator confounding, and that C4) none of the mediator-outcome confounders are themselves affected by the exposure.

C) Mediated (i.e. indirect) natural effects assessed in the mediation analysis [with 95% confidence intervals based on bootstrapping with 1000 resamplings, where the proportion mediated = natural indirect effect/ (natural direct effect + natural indirect effect)]

1. β-HCH → total SCFAs at 4 months → asthma at 10 years

	Estimate	95% CI		*p*-value
Total Effect	-0.012	-0.023	0.005	0.11
Average natural indirect effect	0.000	-0.001	0.001	0.89
Average natural direct effect	-0.012	-0.023	0.005	0.11
Proportion mediated	0.000	-0.138	0.143	0.93

2. ∑PCBs → Shannon diversity age 4 months → asthma at 10 years

	Estimate	95% CI		*p*-value
Total Effect	-0.015	-0.024	-0.008	0.00
Average natural indirect effect	0.000	-0.001	0.001	0.66
Average natural direct effect	-0.015	-0.024	-0.008	0.00
Proportion mediated	-0.004	-0.083	0.032	0.66

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Chapter 6

Performance of variable selection methods for assessing the health effects of correlated exposures in case-control studies

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Abstract

Objectives: There is growing recognition that simultaneously assessing multiple exposures may reduce false positive discoveries and improve epidemiological effect estimates. We evaluated the performance of statistical methods for identifying exposure–outcome associations across various data structures typical of environmental and occupational epidemiology analyses.

Methods: We simulated a case-control study, generating 100 datasets for each of 270 different simulation scenarios; varying the number of exposure variables, the correlation between exposures, samples size, the number of effective exposures, and the magnitude of effect estimates. We compared conventional analytical approaches, i.e., univariable (with and without multiplicity adjustment), multivariable, and stepwise logistic regression, with variable selection methods: sparse partial least squares discriminant analysis, boosting, and frequentist and Bayesian penalised regression approaches.

Results: The variable selection methods consistently yielded more precise effect estimates and generally improved selection accuracy compared to conventional logistic regression methods, especially for scenarios with higher correlation levels. Penalised lasso and elastic net regression both seemed to perform particularly well, specifically when statistical inference based on a balanced weighting of high sensitivity and a low proportion of false discoveries is sought.

Conclusions: In this extensive simulation study with multicollinear data, we found that most variable selection methods consistently outperformed conventional approaches, and demonstrated how performance is influenced by the structure of the data and underlying model.

Introduction

To better estimate the modifiable components of disease risk, environmental and occupational epidemiologists are increasing their efforts to capture the myriad of exposures humans come in contact with. Researchers are using a variety of tools to perform multi-faceted exposure assessment in observational epidemiology studies, including using data from questionnaires, sensors, geospatial modelling, job-exposure matrices, biomonitoring, and high-throughput molecular analyses. As researchers gather increasingly richer datasets, they may find themselves confronted with complex exposure profiles that render identification of exposure–outcome associations using conventional statistical analyses challenging.

Studies often assess one exposure, or sometimes one chemical class or closely related group of exposures, in relation to a health outcome. A shift from single-exposure modelling to multiple-exposure (multi-pollutant or chemical mixture) modelling has been advocated to better identify and estimate the independent effects of exposures.[1] Selection and estimation using conventional methods for multi-pollutant modelling, such as stepwise regression and multiple regression, may suffer from locally rather than globally optimal models, influenced by the order variables enter the model, or may yield unstable estimates if exposures are multicollinear. Use of more advanced statistical methods has gained traction in the past few years, particularly in air pollution and chemical contaminant epidemiology.[2-6] Several (families of) variable selection methods have more recently been developed (see Chadeau-Hyam et al.[7] for a review). However, there is limited data on the relative efficacy of these methods for analysis of data generally relevant for occupational and environmental epidemiologists; i.e., with a limited number of observations, relatively few true associations, complex correlation structures, and modest effect sizes.

Recently published simulation analyses have evaluated methods for the analysis of continuous outcomes considering data structures inspired by the pregnancy exposome[8] and air pollution epidemiology.[9] We extended this work by *i)* studying a binary outcome, as much of epidemiological research deals with data from case-control studies and presence/absence of disease, *ii)* focusing on a larger set of simulation scenarios, and *iii)* by exploring in what way different characteristics of the simulated exposure matrix and the strength of the exposure–outcome association affect the performance of variable selection methods.

We evaluated dimension reduction, penalisation, and boosting approaches which are readily implemented in standard software, and pursued the scenario in which there are no prior hypotheses regarding candidate associations, or where this information is ignored; so following a data-driven approach to variable selection.

Methods

Variable selection methods

We briefly describe each method used to estimate associations between simulated exposures and a binary health outcome. Additional details are provided in Table 1 and the Supplemental

Table 1. Methods applied for analysis of multiple exposures and a binary health outcome.

Method	R package: function(s)	Implementation
Univariable logistic regression	stats: glm	Selection: $p < 0.05$.
Univariable-FDR logistic regression[10]	stats: glm, p.adjust	Selection: FDR < 0.05.
Multivariable logistic regression	stats: glm	Selection: $p < 0.05$.
Stepwise logistic regression	stats: step, glm	Selection: smallest Akaike information criterion.
sPLS-DA[11,12]	spls: cv.splsda, splsda	Model (K, η) tuned via CV. Selection: $\beta \neq 0$.
Lasso regression[13,14]	glmnet: cv.glmnet, glmnet	Model (λ) tuned via CV. Selection: $\beta \neq 0$.
Elastic net regression[14,15]	glmnet: cv.glmnet, glmnet	Model (α, λ) tuned via CV. Selection: $\beta \neq 0$.
Bayesian lasso regression[16,17]	reglogit: reglogit	500 burn-in plus 1,000 iterations. Selection: If 95% highest posterior density interval did not include 0.
Boosted regression[18,19]	mboost: cvrisk, glmboost	≤ 1,500 iterations; CV to determine the stopping iteration. Selection: $\beta \neq 0$.

Abbreviations: CV, cross-validation; FDR, false discovery rate; sPLS-DA, sparse partial least squares discriminant analysis.

Methods. We used R software, version 3.2.1 (R Foundation for Statistical Computing, Vienna, Austria), to simulate and analyse our data.

Univariable(-FDR) and multivariable. The most frequently used strategy for binary classification in epidemiology is to assess separate single-exposure logistic regression models (possibly adjusted for a limited set of known or suspected confounders), referred to as univariable (or univariate) regression. Another strategy is multivariable (or multiple) regression, in which all exposure variables are included in one logistic regression model. We fit generalised linear models with the logit link function and maximum likelihood estimation. Selection of exposures was based on p-values, with a value of 0.05 considered as the cut-off. We also evaluated univariable regression with selection based on controlling the false discovery rate (FDR <0.05, using the Benjamini–Hochberg method[10]). This approach is increasingly being applied to adjust for multiplicity, and has been applied in the so-called environment-wide association study approach.[20]

Stepwise. We implemented forward–backward stepwise logistic regression in which one variable is successively added or deleted at each step, based on minimizing the Akaike information criterion. This is a widely-used automated selection procedure.

sPLS-DA. Partial least squares (PLS) regression[21,22] is a dimension reduction method which projects the outcome and exposure variables onto a smaller number of orthogonal latent variables, called components, maximizing their covariance. Sparse PLS (sPLS) regression[11] introduces variable selection by indirectly imposing a L_1 (lasso type) penalty on the direction (loading) vector defining the projection, so that only a subset of the most informative exposure variables contribute to the components. sPLS can be used for classification

problems by inputting the outcome as a dummy matrix in the PLS algorithm, and using the resulting component scores in a linear discriminant analysis (DA). Model complexity is determined by the number of components (K) and the level of penalisation (η), which were tuned using two-dimensional cross-validation. We selected the model which corresponded to the minimum misclassification error rate in the primary analyses, and modified the default cross-validation within the R package to include an intercept-only model.

Lasso. Penalisation approaches, also referred to as regularisation or shrinkage methods, shrink coefficients towards zero, with the amount of shrinkage inversely proportional to the contribution of the variable to the overall model fit (i.e., important variables are shrunken less). This shrinkage introduces bias but also decreases the variance and generally leads to more stable models, especially for multicollinear data. The most popular penalty is the lasso (least absolute shrinkage and selection operator) penalty, which is proportional to the sum of the absolute values of the coefficients.[13] Variable selection (sparsity) is achieved because coefficients for a subset of variables may be shrunk exactly to zero.

Elastic net. Elastic net regression is similar to lasso regression, but uses a weighted sum of lasso and ridge regression penalties.[15] The ridge regression penalty is proportional to the sum of the squared regression coefficients, which results in shrinkage of the coefficients towards zero, but not to zero exactly, and for coefficients for highly correlated variables towards a common value. Whereas lasso regression tends to select only one variable from a group of correlated variables, elastic net can co-select a group of correlated variables (due to the ridge penalty), while still performing variable selection (due to the lasso penalty). A second tuning parameter (α) controls the balance between the lasso and ridge penalties; we optimised α and the amount of penalisation (λ) using two-dimensional cross-validation. For both lasso and elastic net regression, we selected the most parsimonious model which corresponded to the minimum plus one standard error in binomial deviance in the primary analyses: a more stringent optimisation criterion than the minimum, and one that has been advocated for variable selection.[23]

Bayesian lasso. Variable selection in Bayesian models can be achieved using independent Laplace (or double exponential) prior distributions centred at zero to estimate regression coefficients, also referred to as regularised logistic regression.[16,24] A hyper-prior controls the shape of these Laplace priors, where the optimal amount of shrinkage is adaptively determined from the data itself, achieving approximate multiplicity control. A variable was considered to be selected if its 95% highest posterior density interval did not include zero.[25]

Boosting. We fitted generalised linear models using a component-wise gradient boosting algorithm,[18,26,27] which is an iterative technique that aims to optimise predictive accuracy by combining a set of weak classifiers. With each iteration step, the variable that results in the greatest reduction in error based on the negative binomial log-likelihood loss function is selected. A small proportion (here 10%) of the fit for the selected variable is added to the current estimate, and the residuals are then used in subsequent iterations. Estimates for the

variable selected at each iteration are aggregated. Variable selection is achieved by restricting the number of boosting iterations, and cross-validation with the minimum binomial deviance criterion was used to determine the optimal number of iterations.

Simulation study

We designed our simulation study to be representative of data structures that are common in environmental and occupational epidemiology. We performed a Monte Carlo simulation, varying five aspects of the data (listed in Table 2), resulting in a total of 270 different scenarios. For each scenario we simulated 100 datasets, yielding a total of 27 000 different datasets.

First, the matrix of continuous exposures X was simulated by sampling 100 000 observations from a multivariate normal distribution with a mean of zero, standard deviation of one, and covariance matrix with off-diagonal elements with a uniform correlation level of ρ. X was column mean-centred and standardised to unit variance. Second, the case/control status for the outcome vector Y was simulated from a Bernoulli distribution, with probability $\Pr(Y=1|X) = 1/\{1+\exp[-(\beta_0 + \beta_X X)]\}$. Parameter β_0 was chosen as to be sufficiently large to ensure that the simulation resulted in enough cases and controls for the scenario-specific study size, while β_X was fixed according to the magnitude of the desired effect estimate, the odds ratio (OR) = $\exp(\beta_X)$. An equal number of cases and controls were sampled without replacement and used to construct the final dataset for the analyses.

Table 2. Parameters tested in simulation scenarios[a].

n[b]	p[c]	ρ[d]	q[e]	OR[f]
200 (9000)	10 (9000)	0.0 (9000)	0 (2700)	1.0 (2700)
500 (9000)	20 (9000)	0.4 (9000)	1 (8100)	1.2 (8100)
1 000 (9000)	50 (9000)	0.8 (9000)	2 (8100)	1.5 (8100)
			5 (8100)	2.0 (8100)

Abbreviation: OR, odds ratio.
[a] Parameter levels and (the number of simulated datasets generated per level). 100 simulations were generated for each of the 270 [= 3 × 3 × 3 × (1 + 3 × 3)] simulation scenarios, where effect size 1.0 only applies to simulations with 0 effective variables.
[b] The sample sizes.
[c] The number of exposure or predictor variables.
[d] The correlation levels in a square diagonal matrix whose diagonal elements are 1.0 with uniform correlations between exposures (boxed correlations were simulated in a sensitivity analysis).
[e] The number of effective or truly associated exposures.
[f] The effect sizes.

Evaluation criteria

To assess the relative classification performance of the variable selection methods, we tallied the number of true positive, false positive, true negative, and false negative associations (see Supplemental Table S1) across simulations, and computed:

(1) The *sensitivity*: the proportion of true associations that were correctly identified, also

referred to as recall. No value for sensitivity was computed when the number of true associations equalled zero.

(2) The *false discovery proportion (FDP)*: the proportion of positive findings (associations identified as significant) that were incorrectly identified. FDP was defined as zero when the number of positive findings was zero. The complement of FDP (1–FDP) is equivalent to the positive predictive value, also referred to as precision.

The simulation design is imbalanced in that the number of true negatives exceeds the number of true positives. As such, we compare 1–FDP to the sensitivity, akin to a precision-recall curve,[28] rather than comparing the sensitivity to the false positive rate (the proportion of true null associations identified incorrectly as significant), as is shown in receiver-operating characteristic curves commonly used to assess the performance of classifiers.[29] We also consider various weightings of the *F*-measure, the weighted harmonic mean of sensitivity and 1–FDP, in which either metric can be assigned greater importance (differential weighting), or equal importance (balanced weighting).[30]

(3) The *mean squared error (MSE)*: to assess the estimation performance, we computed a combination of the (squared) bias of the estimated *coefficients* plus its variance across *s* simulations, $\frac{1}{s}\Sigma(\hat{\beta}-\beta)^2$.

We evaluated the overall performance of the methods, across all simulation scenarios, and across all parameter levels. We focus on the results obtained upon varying the three parameters that may be known or assessed in advance by the researcher (i.e., the sample size, the number of exposure variables, and the correlations between exposure variables), as opposed to the parameters that are estimated but remain uncertain in most real-life analyses (i.e., the number of true associations and the effect sizes).

As a sensitivity analysis we repeated all 27 000 simulations using a blocked correlation structure (instead of a single, uniform correlation structure), where we subdivided the correlation matrix into two equally sized sub-matrices that had the same uniform correlation between exposures within the *same* sub-matrix ($\rho = 0.4$ or $\rho = 0.8$), but where there was no correlation between exposures in *different* sub-matrices ($\rho = 0.0$). Additionally, we re-tested sPLS-DA and frequentist penalisation models with alternative optimisation criteria; the minimum plus one standard error and the minimum cross-validation error, respectively.

Results

Overall performance

We used nine different selection strategies to analyse the 27 000 simulated datasets. The overall results, averaged across all simulation scenarios, are presented in Figure 1A. A higher sensitivity and lower FDP indicate superior performance in selecting true associations and fewer false associations, thus if a researcher considers sensitivity to be equally as important as

FDP, then methods located in the upper-right quadrant of the plot (i.e., 1–FDP-sensitivity or precision-recall space) are more favourable. The area of the bubbles is proportional to the median MSE of the coefficient estimates, and smaller bubbles therefore reflect more stable and precise estimation of the true magnitude of associations. The lasso and elastic net regression methods appear to be the best performing methods overall for achieving a balanced sensitivity:FDP selection performance, with the elastic net achieving slightly higher sensitivity and lasso regression achieving a somewhat lower FDP, and both yielding nearly the lowest MSE values. When we examine these three performance criteria separately (Supplemental Figure S1), we note that the conventional modelling approach, univariable regression, has both the best sensitivity and worst FDP ranking. Univariable with FDR control yields an improved selection performance, although the estimation performance remains unchanged. A far greater proportion of MSE values are large for univariable regression compared to the other methods (the 75th percentile was 0.31 for univariable and univariable-FDR versus 0.06 for multivariable and 0.01 for elastic net regression).

Researchers may value sensitivity more highly than FDP or vice versa, depending on the focus of research question. If we consider a composite of sensitivity and 1–FDP, the median *F*-measure across simulation scenarios, then lasso and elastic net regression have the highest ranking whether sensitivity and 1–FDP have a balanced weighting (F_1) or whether sensitivity is a quarter or half as important ($F_{0.25}$, $F_{0.5}$) or twice or four times as important (F_2, F_4) as 1–FDP. The ranking of the methods across all simulations at F_1 (elastic net > lasso > sPLS-DA > boosted > stepwise > multivariable > univariable > univariable-FDR > Bayesian lasso) is fairly consistent across $F_{0.25}$ to F_4 differential weightings (with only boosted, stepwise, and multivariable swapping the middle-ranking positions; Supplemental Table S2).

Influence of parameters on performance

We examined the performance across varying levels of the simulation parameters, starting with those that are likely to be known by researchers before the statistical analyses (Figure 1B–D). Bayesian lasso consistently occupied the upper-left quadrant, displaying very low FDP, but poor overall sensitivity. The other methods clustered between a 1–FDP ranging from around 0.2 to 0.8, and sensitivity around 0.4 to 0.9. The relative position of the methods in the FDP and sensitivity space generally remained consistent upon varying the parameters. The penalised regression methods (lasso, elastic net, and Bayesian lasso) and boosting consistently displayed the smallest MSE values across the levels of the parameters (see also Supplemental Figure S3). Mean sensitivity improved substantially with increasing sample size for all methods, and most so for Bayesian lasso (+0.44 from n = 200 to n = 1000) and multivariable regression (+0.34). The FDP was less affected by sample size; most methods showed minor improvements (up to +0.11 in mean 1–FDP), and Bayesian lasso and univariable(-FDR) showed a minimal decline. The median MSE for multivariable regression at the smallest sample size (n = 200) was 1.8 times larger than the next largest MSE [univariable(-FDR) at n = 200].

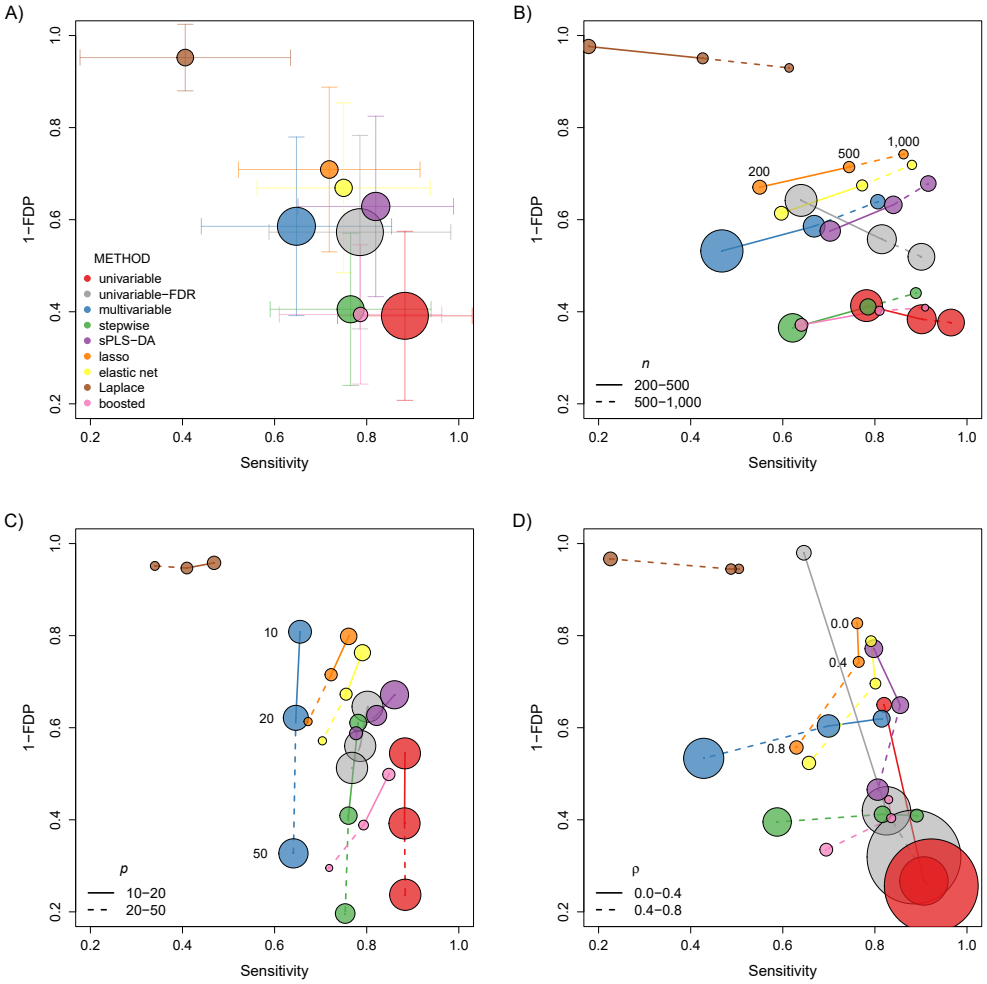

Figure 1. Performance of the variable selection methods **(A)** across all 270 simulation scenarios and stratified by **(B)** sample size, **(C)** number of exposure variables, and **(D)** correlation levels. The mean sensitivity is plotted against 1 minus the mean false discovery proportion (1–FDP), where the size of each bubble is proportional to the median mean squared error (MSE). Error bars represent half standard deviation values of sensitivity and 1–FDP. Note that the axes are truncated at 0.2, and the scaling of MSE values is relative and differs between plots.

Sensitivity was minimally affected by the number of exposure variables (≤–0.13 for an increase from 10 to 50), whereas the 1–FDP was strongly affected for conventional methods (–0.31 to –0.48) and moderately affected for univariable-FDR (–0.13) and most of the variable selection methods (–0.08 to –0.20; except for the Bayesian lasso, which was negligibly affected). This may have been expected since there was no direct multiplicity adjustment in variable selection methods, except for the adaptable prior in Bayesian lasso, and this clearly demonstrates that cross-validation is no substitute.

For increasing correlation levels, the patterns were less consistent across methods.

Most methods seemed tolerant to small-to-moderate correlations, with the sensitivity and/or FDP affected more seriously when the correlation levels increases from $\rho = 0.4$ to $\rho = 0.8$, as compared with an increase from $\rho = 0$ to $\rho = 0.4$. The exceptions were univariable and univariable-FDR, which showed a large drop in 1–FDP from the uncorrelated to correlated scenarios. With respect to the *F*-measure, elastic net and lasso had the highest $F_{0.25-4}$-measure rankings upon stratification by correlation level, with the exception of univariable-FDR in the uncorrelated scenarios (which had the highest ranking at $\rho = 0$; Supplemental Table S2).

We also examined the performance for parameters that may be estimated but remain uncertain (Supplemental Figure S2). As the number of effective (truly associated) exposures increased from one to five, there was a moderate increase in the proportion of false discoveries for most methods, except the penalised regression methods. The sensitivity consistently increased across methods as the strength of the association increased.

To gain more insight into the behaviour of the methods, we stratified by all three parameters which can be assessed in advance by the researcher. In Figure 2 we compare the performance of more conventional methods, univariable-FDR and stepwise regression, with the less-frequently applied elastic net regression (results for each method are presented in Supplemental Figure S3). Methods located at the top of the panel, with one panel for each performance metric, indicate superior performance. Elastic net clearly outperforms stepwise with respect to FDP, and also outperforms univariable-FDR in scenarios with a higher number of exposures ($p = 20$ and $p = 50$). All three methods exhibit a similar sensitivity with $p = 20$ exposures, whereas stepwise has the highest sensitivity with $p = 10$ and univariable-FDR with $p = 50$. The MSE values are lowest for elastic net, modestly reduced compared to stepwise and markedly reduced compared to univariable-FDR in scenarios with $p = 20$ and $p = 50$ exposures.

Sensitivity analyses

Upon repeating the simulation analysis using a blocked correlation structure, the selection and estimation performance was similar to the simulations with a uniform correlation structure, with the exception of a marked decrease in MSE for univariable(-FDR) with correlated exposures in a blocked structure (Supplemental Figure S4). Optimising the sPLS-DA model based on the minimum plus one standard error cross-validation prediction error, rather than the minimum error, led sPLS-DA to have a negligible FDP but poor sensitivity. The changes in performance for lasso and elastic net with a switch to optimisation based on the minimum error were less substantial; a moderate increase in FDP and minor increase in sensitivity was observed (Supplemental Figure S5). As a *post hoc* analysis, we evaluated multivariable regression with FDR control, a relatively infrequently applied approach. It showed a similar performance as Bayesian lasso. There was a pronounced improvement in FDP and a moderate drop in sensitivity compared to the primary multivariable analyses without correction for multiplicity (Supplemental Figure S6).

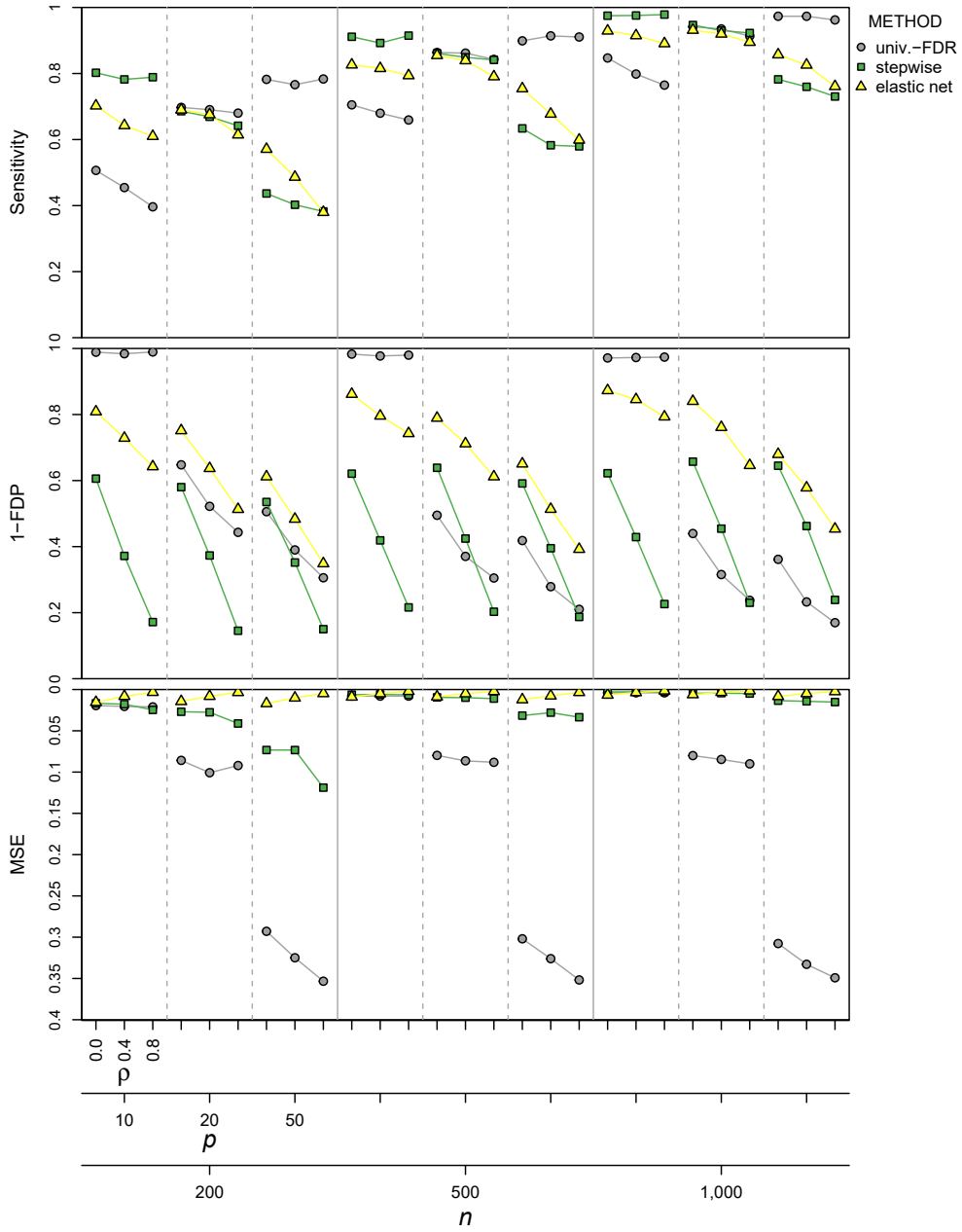

Figure 2. Sensitivity and false discovery proportion (FDP; mean values) and mean squared error (MSE; median values) stratified by the three *a priori* known simulation scenario parameters for univariable regression with false discovery rate (FDR) correction, stepwise regression, and elastic net regression.

Discussion

In this simulation study, based on a case-control design, modern variable selection approaches nearly always outperformed conventional logistic regression approaches in recovering the underlying causal model when exposures were correlated. Single-exposure models (univariable either with or without FDR-control) and stepwise regression showed high false discovery proportions and MSE values in many scenarios, partly attributable to lack of control for confounding by correlated exposures for the single-exposure models and overfitting for the stepwise regression models. Boosted regression also had a high FDP, although the average MSE was low. Full multivariable regression modelling yielded high MSE and moderately high FDP, especially with higher correlations among exposures. The Bayesian lasso had a very low FDP, albeit at the cost of greatly reduced sensitivity. Elastic net and lasso regression, and sPLS-DA performed best on average with respect to identifying associations across the wide array of simulation scenarios, for a balance of sensitivity and low FDP, and also across more lenient exploratory versus more stringent confirmatory statistical inference rankings (i.e., $F_{0.25}$ to F_4), although elastic net and lasso regression performed better with respect to estimation performance (i.e., lower MSE).

Compared to lasso regression, elastic net regression was slightly more sensitive but also had a slightly higher FDP. The two-dimensional cross-validation often resulted in α close to 1 (the lasso setting), so the general similarity is not surprising. Fixing α to a smaller value, such as 0.5, led to slightly higher sensitivity accompanied by greater increases in FDP for elastic net compared to lasso in scenarios with correlated exposures (data not shown). A more complex correlation structure may also have resulted in more divergent results between elastic net and lasso regression. The MSE values were smaller than for most other methods, which indicates that associations were estimated more precisely, even though penalisation results in estimates that are biased towards the null. Alternative penalties have been developed which reduce this shrinkage bias for larger coefficients, such as nonconvex and adaptive penalties (Supplemental Methods).

A drawback of frequentist penalisation methods is that these do not yield valid standard errors or other uncertainty measures. Inference based on *p*-values conditional on the selected subset (post-selection inference), sequential FDR-control, and selection based on subsampling-stability have recently been explored,[31-33] but are computationally demanding or are not yet available in standard statistical packages and were therefore not included in our simulation study. The Bayesian lasso does provide (samples from) the full posterior for effect estimates, which can be used to draft credible intervals.[34] The Bayesian lasso showed a very low sensitivity in the present simulation study, particularly in scenarios with a smaller sample size (n = 200) or a larger number of exposure variables (q = 50), reflecting that the prior corrects for multiplicity,[35] in line with multivariable regression with FDR control. Using a slightly lower threshold for variable selection resulted in behaviour more similar to the frequentist penalised methods (data not shown). In addition to selection criteria, the choice of

the sampling (i.e., Markov chain Monte Carlo) algorithm and (hyper)prior have been shown to influence performance of Bayesian variable selection approaches.[36-39] Other simulation studies with a higher range of effective variables[40] and high-dimensional scenarios[16] found that Bayesian lasso outperformed frequentist lasso.

sPLS-DA performed nearly as well at selecting associations in the present simulation study, although MSE values were somewhat larger than for the penalised regression models. The selection performance of sPLS-DA was much more sensitive to the model optimisation criteria than the penalised regression methods. The coefficient for each selected variable in a sPLS-DA model is calculated by summing over latent components, and this renders the resulting coefficient difficult to interpret unless the latent components have a direct interpretation. Another difficulty with sPLS-DA (and most other dimension reduction methods) is that there is no direct way to adjust for confounding. One option is to first pre-adjust the outcome and exposure variables for the confounding variables,[41] and use the residuals from these models as input for the sPLS-DA model. (s)PLS-DA may prove more useful for predictive modelling, pattern recognition, or delineating clusters than for variable selection.

Boosting exhibited an undesirably high FDP. This may be because we relied on minimisation of the cross-validated risk for that method, which is known to result in models that have good predictive ability, but that often include too many variables, especially when the signal-to-noise ratio is low. We demonstrated that several methods (lasso and elastic net and especially sPLS-DA) are sensitive to the model optimisation criteria. This deserves more attention in future simulation studies.[42,43]

There are many other methods for variable selection (also called model selection), and the battery of available approaches is expected to rapidly evolve; for instance, Bayesian kernel machine regression[44] has recently been proposed as a suitable approach. Selection methods have more frequently been evaluated for high-dimensional data ($p >> n$), as variable selection methods have proven particularly useful for these data structures, such as genetics and –omics data.[45] These simulations have limited generalizability because environmental and occupational exposure sets are often characterised by denser correlations than most genomics datasets.[46] There are also more evaluations of linear regression than of other link-functions, as they are generally computationally less demanding and have relatively high power. In a recent simulation based on a low-dimensional pregnancy exposome dataset (p = 237 exposures and q = 0–25 truly associated) and linear modelling, Agier et al.[8] found that deletion/substitution/addition[47] and a stochastic search algorithm, Graphical Unit Evolutionary Stochastic Search[48] performed best; we did not include these methods because either a current R package was not available or the method was too computationally demanding for a large simulation study, and the latter method cannot be applied in a logistic regression framework. They also found that elastic net performed reasonably well. In an evaluation of multi-pollutant linear modelling (p = 4–20), Sun et al.[9] also report that lasso regression performed well. We evaluated a relatively small number of exposures compared to the higher dimensional model-space that is expected

to be considered in future environment/exposome-wide association studies. Nevertheless, the patterns in performance—notably in the presence of correlated exposures—would apply for the conventional methods and also be expected to apply for the more novel variable selection methods in scenarios with many more exposures.

A limitation of the present study is that simulation results entail some uncertainty; however, increasing the number of simulations (presently 100 per scenario aggregated over various combinations of scenarios) would not be expected to alter the trends or overall inferences. Not unexpectedly, we found that performance was highly dependent on most of the parameters we included in the design of the simulations. We observed that the sample size and strength of the association had the greatest influence on the sensitivity of methods, and selection performance of modern variable selection methods was generally more robust to changes in correlation levels. We tested parameters that we hypothesised would be most influential. Future work could assess others that are highly relevant in environmental and occupational epidemiology, such as the effect of measurement error (due to e.g., limitations in the precision of analytical assays or error in exposure assessment models), skewed exposure distributions, interactions or effect modification by other exposure variables, non-linear associations, mixed models, and more complex correlation structures. It has been shown that measurement error, and correlated measurement error, usually attenuate effect estimation in two-exposure modelling of chemical biomarkers and air pollutants.[49,50] Furthermore, model misspecification, for instance due to inclusion of a variable that improves prediction irrespective of the causal structure can induce bias due to omitted variable bias and collider stratification bias. Although the magnitude of this bias amplification might often be expected to be relatively minor compared to the reduction in residual confounding bias from correlated co-exposures, the cumulative bias is a complex function of many factors including covariance and the strength of each association, and this is an area of methodological development that warrants further attention in variable selection modelling.[51,52]

Simultaneously assessing multiple exposures in an epidemiological analysis, as in the recently championed environment-wide association study approach, reduces selective reporting and publication bias. Variable selection approaches, which mitigate the problem of multicollinearity, can complement or perhaps replace the single-exposure approach to environment-wide association studies. This study offers some guidance for choosing among various modelling approaches considering the structure of the data and whether the aim of the analysis is exploratory with a preference for sensitivity or confirmatory with a preference for specificity. While no single method was infallible in the presence of correlations, we demonstrated that several variable selection approaches yielded improved effect estimation and selection accuracy, and that penalised regression approaches in particular were an attractive option across an array of analytical scenarios.

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Supplemental Material

Supplemental Methods

Here we provide additional details about the variable selection methods. We restrict our explanations to the methods as applied for analysis of binary outcomes.

Notation

Let $y = (y_1, \ldots y_n)$ denote a binary outcome coded as 0 or 1 (i.e. $y_i \in \{0,1\}$), such as case/control status, for a sample (study population) of $i = 1 \ldots n$ individuals. Suppose we have p exposure variables, for a $n \times p$ matrix $X = x_{ij} = (x_{i1} \ldots x_{ip})^T$. We estimate the probability π that $y = 1$, $\Pr(y = 1)$, where β_0 is the intercept and β_j the regression coefficient for the j^{th} exposure, yielding a vector $\beta = (\beta_0, \beta_1, \ldots \beta_p)^T$.

Data pre-processing

We assume that, as in the simulation, all exposure variables have been mean-centred (so the intercept is zero and common to all models), and also scaled to one standard deviation (unit variance). Scaling is important for some methods—sPLS-DA and the penalised methods (not gradient boosting)—in that it imparts variables with the same contrast, and therefore the same prior probability of being selected when simultaneously modelled.

Univariable, multivariable, and stepwise regression

For these three conventional modelling methods, we fit a generalised linear model with the logit link function:

$$\log\left(\frac{\pi_i}{1-\pi_i}\right) = \beta_0 + \sum_{j=1}^{p} \beta_j x_{ij}$$

which simplifies to $\text{logit}(\pi_j) = X\beta$. Models were estimated by maximising the log-likelihood function using the *glm* function, available in the *stats* package in R. In univariable regression (also commonly referred to univariate regression), a single variable was tested in one model and the p-value was evaluated. In multivariable regression (also commonly referred to multiple regression), all variables were simultaneously modelled in one model. As mentioned in the main text, we used a selection criterion of $p < 0.05$. For univariable regression, we also applied a Benjamini–Hochberg false discovery rate[1] adjusted $q < 0.05$. This has been applied in the 'environment-wide association study' approach.[2]

For stepwise regression, we applied bi-directional (forward–backward) selection using the default settings for the *step* function,[3] in which both forward selection (start will an intercept-only or null model and successively add the variable that most improves the model fit, here based on minimizing the Akaike Information Criterion) and backward elimination (successively exclude the variable that has the least impact on the model fit) is performed at each step. The set of models tested is a nested sequence of models (i.e., is influenced by the

order of variables entered or eliminated from the model), and as such, not all possible subsets are tested, and therefore a limitation of stepwise regression is that models may represent locally optimal rather than globally optimal models when not all variables are completely independent (i.e. $x_1 + x_3 + x_4$ may represent a better fit than $x_1 + x_2 + x_3$, but will not be identified if x_2 leads to a larger drop than x_3 when not mutually adjusted for subsequent exposures).

Sparse partial least squares discriminant analysis (sPLS-DA)

Partial least squares (PLS) decomposition is generalised (in matrix form) as[4,5]:

$$y = Tq^{\top} + f \quad = XWq^{\top} + f \quad = X\beta_{PLS} + f$$
$$X = TP^{\top} + E$$

where T represents the matrix ($n \times K$) of latent components or 'scores' of orthogonal (mutually uncorrelated), linear combinations of X for the K number of model components; q and P represent the vector of y- and matrix of X-loading coefficients or 'loadings'; and f and E the random errors. W is a matrix ($p \times K$) of direction vectors or 'loading weights' (w_K). Latent components are derived via successive optimisations (depending on the PLS1 algorithm), such that $\hat{T} = X\widehat{W}_K$ or $\hat{T} = X\widehat{W}(\hat{P}^{\top}\widehat{W})^{-1}$. PLS regression coefficients are computed as $\hat{\beta}_{PLS} = \widehat{W}_K\hat{q}^{\top}$.

In sparse partial least squares (sPLS) regression, penalisation is applied during the dimension reduction step. In brief, a penalty (η) is applied to a surrogate of the direction vector (w), which is a close analogue of the original direction vector, as elaborated in Chun and Keles.[6] The sPLS sparsity penalty (η) approximates the L_1 penalty of lasso[7]: $\min_{\beta} \| y - X\beta \|^2 + \lambda \|\beta\|_1$ where $\|\beta\|_1 = \sum_{j=1}^{p} |\beta_j|$. The univariate sPLS penalisation can be simplified to[6,8]:

$$\widehat{w} = \max\left(0, |\widetilde{w}| - \eta \max_{1 \le i \le p} |\widetilde{w}_i|\right) \cdot sign(\widetilde{w})$$

where $\widetilde{w} = \left(\widetilde{w}_1 \ldots . \widetilde{w}_p\right)^{\top}$ are the estimated PLS direction vectors with $\widetilde{w}_1 = X^{\top}y / \| X^{\top}y \|_2$ and $0 \le \eta \le 1$ (sparsity increases as η approaches 1, and if $\eta = 0$ then the model is equivalent to PLS). A fraction of each direction vector is retained. Thus, sPLS is a two-stage procedure; once sparsity has been applied on the direction vectors (and implicitly, a subset of X-variables selected), coefficients are derived from ordinary PLS regression.

In sPLS-DA, first sparse components are created using sPLS-regression with $\boldsymbol{y}$ input as a dummy matrix, and second, classification of $\boldsymbol{y}$ is performed using linear discriminant analysis (LDA) in this component space.[9]

Several other methods have been proposed to extend sPLS-regression to sPLS-DA, e.g., a one-stage sPLS-DA[10] and sparse generalised PLS (SGPLS) or sPLS with logistic regression as the classifier.[9] We applied the two-stage sPLS and LDA method proposed by Chung and Keles[9] and implemented in the *spls* package.[6,11] sPLS-DA models were fitted with the SIMPLS algorithm, described in detail elsewhere.[4,12] Refer to references[5,8] for reviews of PLS

and sparse PLS methods.

The number of components (K) and the level of penalisation (a.k.a. the thresholding parameter, η) were tuned using 20-fold cross-validation. Models with K values 1 through 5 (5 being the max number of possible effective predictors) and η values between the interval of 0.01 and 0.99 in steps of 0.05 were tested, in addition to a null (intercept only) model. We modified the default function *cv.splsda* to include an intercept-only model (no exposures selected). In the primary analysis, we selected the model which corresponded to the minimum deviance, as this is the default for this package. As a sensitivity analysis, to compare with alternatives suggested for penalised regression, we applied the one standard error rule, selecting the most parsimonious model within one standard error (SE) of the minimum deviance.[13,14]

Lasso and elastic net regression

For the frequentist penalisation methods considered, the model seeks to minimise the negative binomial log-likelihood subject to a penalty that is dependent on the magnitude of the estimated coefficients. The penalties differ for the lasso and elastic net models.

Lasso[7]:

$$\hat{\beta} = \underset{\beta}{\operatorname{argmin}} \sum_{i=1}^{n} \left(-y_i (x_i^{\top}\beta) + \log\left(1 + e^{x_i^{\top}\beta}\right)\right) + \lambda \sum_{j=1}^{p} \left|\beta_j\right|$$

Elastic net[15]:

$$\hat{\beta} = \underset{\beta}{\operatorname{argmin}} \sum_{i=1}^{n} \left(-y_i (x_i^{\top}\beta) + \log\left(1 + e^{x_i^{\top}\beta}\right)\right) + \lambda \sum_{j=1}^{p} \left(\frac{1}{2}(1-\alpha)\beta^2 + \alpha|\beta_j|\right)$$

where $0 \le \alpha \le 1$ and $\lambda \ge 0$. The amount of penalisation (shrinkage) increases as increases, implying that some coefficients may be shrunk exactly to zero (odds ratio = 1.0). If $\alpha = 1$, the elastic net model is equivalent to a lasso model, and if $\alpha = 0$, the model is equivalent to a ridge regression model (the ridge penalty is described in the main text).

Both lasso and elastic net regression were modelled using the *glmnet* package, which uses the coordinate descent algorithm.[16] Models were tuned via 20-fold cross-validation, using the function *cv.glmnet*. The folds were set to include the same set of observations across all simulations to ensure that the tuning of α and λ was not influenced by the composition of the folds. For lasso, we selected model with the largest λ which corresponds to the minimum plus 1 SE residual deviance of the binomial model for the primary analysis (and to the minimum in a sensitivity analysis). The default *cv.glmnet* function requires a value for α to be specified, and the function performs cross-validation of models over a range of λ values. We modified this to perform a purely data-driven '2D' cross-validation of both α and λ. For elastic net, a grid of α (values 0:1 in steps of 0.05) and λ (default set of 100 values tested within *cv.glmnet*)

were searched, and the sparsest model corresponding to the α–λ pairs with the minimum plus 1 SE cross-validation deviance was selected. We also present results for the minimum residual deviance.

Note that alternative penalties have been advocated which yield less biased coefficient estimates than lasso and elastic net, and possess the so-called oracle property—that is, the correct subset is selected with a probability approaching one, and, if the true underlying model were known and the sample size were large, unbiased coefficients would theoretically be estimated. The smoothly clipped absolute deviation (SCAD)[17] and minimax concave penalty (MCP)[18] are non-convex penalties, which although they require more computationally demanding algorithms[19] to solve than the popular convex optimisation lasso penalty, they yield less biased estimates because large coefficients are penalised less than small coefficients. The adaptive lasso[20] and adaptive lasso[21] behave similarly; coefficient penalisation is weighted, usually by the size of corresponding ridge regression coefficients. They tend to achieve more aggressive penalisation and sparser selection. It is also possible to pre-specify groups for selection using group lasso,[22,23] if for example, a set of chemical contaminants are suspected to all exert an effect via the same biological pathway.

Bayesian lasso regression (Laplace)

Tibshirani[7] noted that the frequentist Lasso penalty can be generalised as a maximum a posteriori (MAP) estimate of the linear regression model with a Laplace (double exponential) prior on regression coefficients, with density $\left(\frac{1}{2\tau}\right) e^{\left(-\frac{|\beta|}{\tau}\right)}$ and with (location) $\mu = 0$ and (rate) $\tau = \frac{1}{\lambda}$.

The Laplace priors can be represented as a scale mixture of normal priors on coefficients and independent exponential priors on their variances[24] in a hierarchical Bayesian approach.[25] Bayesian lasso modelling has been extended to logistic regression using a data augmentation approach. We used the *reglogit* function from the package *reglogit* ('Simulation-Based Regularised Logistic Regression') which implements a Gibbs sampling Markov chain Monte Carlo (MCMC) algorithm.[26,27]

The shape of the Bayesian Lasso prior controlling the amount of penalisation is tuned in a data-dependent way, using an inverse-gamma hyper-prior. In the present simulation, we used the *reglogit* package defaults for the parameters of this hyper-prior. The Bayesian Lasso does not penalise variables exactly to zero, so a variable selection criterion still needs to be applied. We selected variables whose 95% highest posterior density (HPD) interval did not include zero. Models were run with 1500 MCMC iterations, with a burn-in of 500 (discarded iterations), so results (posterior estimates) per simulation were based on a total of 1000 samples. Refer to Hamra et al.[28] for an introduction to MCMC methodology.

Boosting regression

In gradient boosting,[29-31] the optimal prediction function $f^*(\cdot)$ which relates X to y is estimated by minimizing the observed mean (a.k.a. the 'empirical risk'):

$$\hat{f}(\cdot) = \underset{f(\cdot)}{\operatorname{argmin}} \left\{ \frac{1}{n} \sum_{i=1}^{n} \rho\big(y_i, f(X_i)\big) \right\}$$

where $\rho(\cdot)$ is a loss function. For binary classification using boosting, we used the Binomial Boosting (a.k.a. LogitBoost) algorithm[29] which uses the negative of the binomial log-likelihood loss function.

The component-wise gradient boosting algorithm is an iterative forward stage-wise additive method, that uses an additive combination of "simple" function estimators (called base-learners) to approximate a more complex model. For the simulations we restricted the set of base-learners to be a linear function of a single predictor variable. In brief, the algorithm used to fit this model is implemented as follows (see also references[29,32] for a more extensive description):

1. Initialise the function estimate $(\hat{f}^{[0]})$ with the null (empty) model, i.e., $\hat{f}^{[0]} = \log\left(\frac{\pi}{1-\pi}\right)/2$ where $\pi = \Pr(y = 1)$. Compute the negative gradient vector $u^{[0]}$ of the loss function. Initialise the coefficients for each variable $\hat{\beta}^{[0]} = 0$.
2. Now iteratively fit each base-learner 1,2,..,P to the gradient $u^{[m]}$, yielding P vectors of predicted values. Select the base-learner that best fits $u^{[m]}$, yielding fitted values $\hat{u}^{[m]}$. When using only linear base-learners, this amounts to selecting the predictor variable that improves the prediction model most.
3. Update the current function estimate $\hat{f}^{[m]} = \hat{f}^{[m-1]} + \nu\hat{u}^{[m]}$, where $0 < \nu \leq 1$ is the step-length factor (or shrinkage factor or learning rate). Using this factor in combination with an early stopping rule (see next) avoids overfitting. Update the coefficient vector $\hat{\beta}^{[m]} = \hat{\beta}^{[m-1]} + \nu\hat{\beta}^{[m]}$.
4. Iterate steps 2 to 3 until the optimal stopping iteration $m = m_{\text{stop}}$ is reached.

Note that in each boosting iteration, the base-learners are fitting the errors ('deviance residuals') of the previous iteration. Only one base-learner (in this case, one x variable, hence 'component-wise') can be selected at each iteration (step 3). However, a base-learner may be selected again in subsequent iterations, and the final estimates will be based on the sum of the individual estimates from each iteration. If a base-learner was never selected, the coefficient estimate will be zero (hence performing variable selection or model choice, depending on the number of input variables in the base-learners).

Gradient boosting in effect performs regularisation by stopping the number of iterations before convergence in that effect estimates increase incrementally and in small steps (a function of the size of ν) with each iteration, and effect estimates are shrunken compared to OLS estimates. This shrinkage leads to increased bias but reduced variance of estimates, and

usually yields increased stability of the model estimates.

Using the *glmboost* function in the *mboost* package ('model-based boosting')[33] package, we set the hyper-parameters to 1500 initial boosting iterations, and a step-length of $\nu = 0.1$ (the package default). The size of v has been shown to be of minor importance as long as it is small, such as $\nu = 0.1$, i.e., 10% (smaller values of v yield more shrinkage).[31] We used the built in cross-validation procedure, cvrisk, with the default 25-fold bootstrapping, to determine the main tuning parameter—the optimum number of boosting iterations (m_{stop}) which optimises prediction (i.e., minimises the binomial deviance). The estimates are half of the log-odds ratio (due to the internal recoding of the outcome variable to -1 and +1 instead of 0 and 1), so estimates need to be doubled.

Note that other regression models and loss functions can be fitted within the gradient boosting framework. Some other forms of boosting, such as tree-based boosting, do not yield estimates of the association but only perform selection and are so called 'black-box' machine learning methods.

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Table S1. Binary confusion matrix used to derive performance metrics.

		Actual		
		Positive (H_0 is false = true association[a])	**Negative** (H_0 is true = no association)	**Metrics**
Predicted	**Positive findings (P)** (reject H_0 = declared significant)	True positive **(TP)**	False positive **(FP)** *Type I error*	**False discovery proportion (FDP)**[a] = FP / (TP + FP) | P > 0[b] **Positive predictive value (PPV)** (a.k.a. precision) = TP / (TP + FP) | P > 0[b] = 1 – FDP
	Negative findings (N) (accept H_0 = declared non-significant)	False negative **(FN)** *Type II error*	True negative **(TN)**	
Metrics		**Sensitivity** (a.k.a. recall or true positive rate) = TP / (TP + FN) | (TP + FN >0)[b]	**False positive rate (FPR)** = FP / (FP + TN)	$F_\beta = \frac{(1+\beta^2) \times TP}{(1+\beta^2) \times TP + \beta^2 \times FN + FP} = (1+\beta^2)\frac{PPV \times sensitivity}{\beta^2 \times PPV + sensitivity}$[d] **Mean squared error (MSE)** $= \frac{1}{\#\ simulations}(\hat{\beta} - \beta)^2$

Abbreviations: a.k.a., also known as; H_0, null hypothesis.

[a] Note that different terminology is used to represent the same performance measures in the multiple testing literature (e.g., '*m*' hypothesis tests, '*V*' false discoveries instead of FP, '*R*' total H_0 rejections instead of P, where FDP = V / R).

[b] Sensitivity is set to missing when the number of true positive associations is zero (this applied to 2,700 out of 27,000 simulations in the simulation design); FDP is set to zero (and PPV to one) when the number of positive findings (associations identified as significant) is zero (the number of simulations this applied to varied across methods).

[c] FDP is referred to as false discovery rate (FDR) in some papers; we follow the notation that considers FDR to be the expected FDP.

[d] F_β, where the weighting by β means that if $\beta > 1$, sensitivity (recall) is β times more important than PPV = 1 – FDP (precision), and if $\beta < 1$, then PPV is β times more important than sensitivity. *F*-measure (a.k.a. *F*-score) values are always in the interval [0,1], with 1 indicating a perfect selection or prediction.

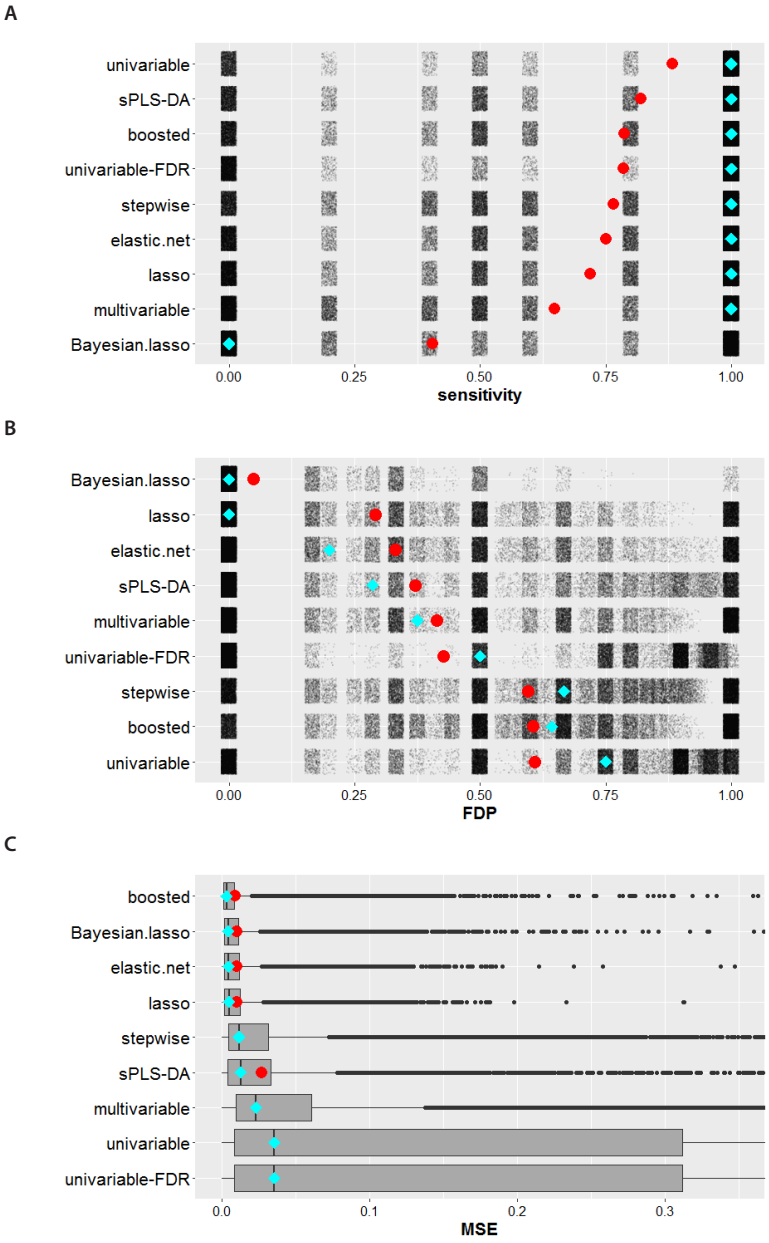


Figure S1. Overall performance across the simulations stratified by performance metric. Results for all simulations (100 datasets simulated for each of the 270 data structure scenarios) for each method are plotted per row (rows of jitter or box plots). Methods are ordered from best to worst (top to bottom rows) performing by **(A)** mean sensitivity, **(B)** mean false discovery proportion (FDP), and **(C)** median mean squared error (MSE) values. Red dots denote the mean values, and blue diamonds the median values. The *x*-axis has been truncated for MSE (the mean MSE values for stepwise, multivariable and univariable(-FDR) regression were 8667, 5.12 x 10^{25} and 0.49, respectively). The jitter plots show a distinctive, discrete pattern which reflects the design of the simulation study; note that the dots have been jittered to give a sense of density.

Table S2. Median *F*-measure values[a] across all simulation scenarios and stratified by correlation levels.

			1-FDP ⟷ sensitivity				
Scenarios		**Method**	$F_{0.25}$	$F_{0.5}$	F_1	F_2	F_4
All scenarios		univariable	0.21	0.24	0.33	0.53	0.71
		univariable-FDR	0.15	0.17	0.25	0.40	0.65
		multivariable	0.52	0.56	0.50	0.63	0.77
		stepwise	0.52	0.56	0.50	0.63	0.77
		sPLS-DA	0.52	0.56	0.67	0.77	0.89
		lasso	0.67	0.68	0.67	0.83	0.94
		elastic net	0.68	0.69	0.67	0.83	0.94
		Bayesian lasso	0.00	0.00	0.00	0.00	0.00
		boosted	0.37	0.40	0.50	0.71	0.85
Correlation	0.0	univariable	0.68	0.69	0.67	0.83	0.94
		univariable-FDR	1.00	1.00	1.00	1.00	1.00
		multivariable	0.57	0.56	0.67	0.83	0.94
		stepwise	0.57	0.56	0.67	0.83	0.94
		sPLS-DA	0.84	0.80	0.80	0.91	0.97
		lasso	0.94	0.86	0.91	0.96	0.99
		elastic net	0.96	0.88	0.91	0.96	0.99
		Bayesian lasso	0.52	0.56	0.67	0.65	0.61
		boosted	0.41	0.45	0.57	0.71	0.89
	0.4	univariable	0.11	0.12	0.19	0.36	0.65
		univariable-FDR	0.11	0.12	0.18	0.36	0.65
		multivariable	0.52	0.56	0.59	0.67	0.81
		stepwise	0.52	0.56	0.59	0.67	0.81
		sPLS-DA	0.66	0.68	0.67	0.83	0.93
		lasso	0.68	0.71	0.77	0.83	0.94
		elastic net	0.73	0.71	0.80	0.86	0.96
		Bayesian lasso	0.68	0.59	0.57	0.56	0.52
		boosted	0.41	0.44	0.50	0.71	0.89

Table S2. *Continued.*

Scenarios	Method	1-FDP ⟷ sensitivity				
		$F_{0.25}$	$F_{0.5}$	F_1	F_2	F_4
0.8	univariable	0.11	0.12	0.18	0.36	0.65
	univariable-FDR	0.11	0.12	0.18	0.36	0.65
	multivariable	0.26	0.29	0.29	0.24	0.21
	stepwise	0.26	0.29	0.29	0.24	0.21
	sPLS-DA	0.28	0.31	0.40	0.56	0.76
	lasso	0.49	0.49	0.50	0.63	0.77
	elastic net	0.50	0.50	0.55	0.63	0.75
	Bayesian lasso	0.00	0.00	0.00	0.00	0.00
	boosted	0.34	0.36	0.44	0.59	0.76

[a] F_1 is a balanced weighting (harmonic mean) of sensitivity and the complement of the false discovery proportion (1 – FDP, or the positive predictive value); $F_{0.25}$ and $F_{0.5}$ favor low FDP, and F_2 and F_4 favor high sensitivity, weighting sensitivity a quarter, half, twice and four-times as much as 1 – FDP. The intensity of the shading reflects increasing *F*-measure values (better performance).

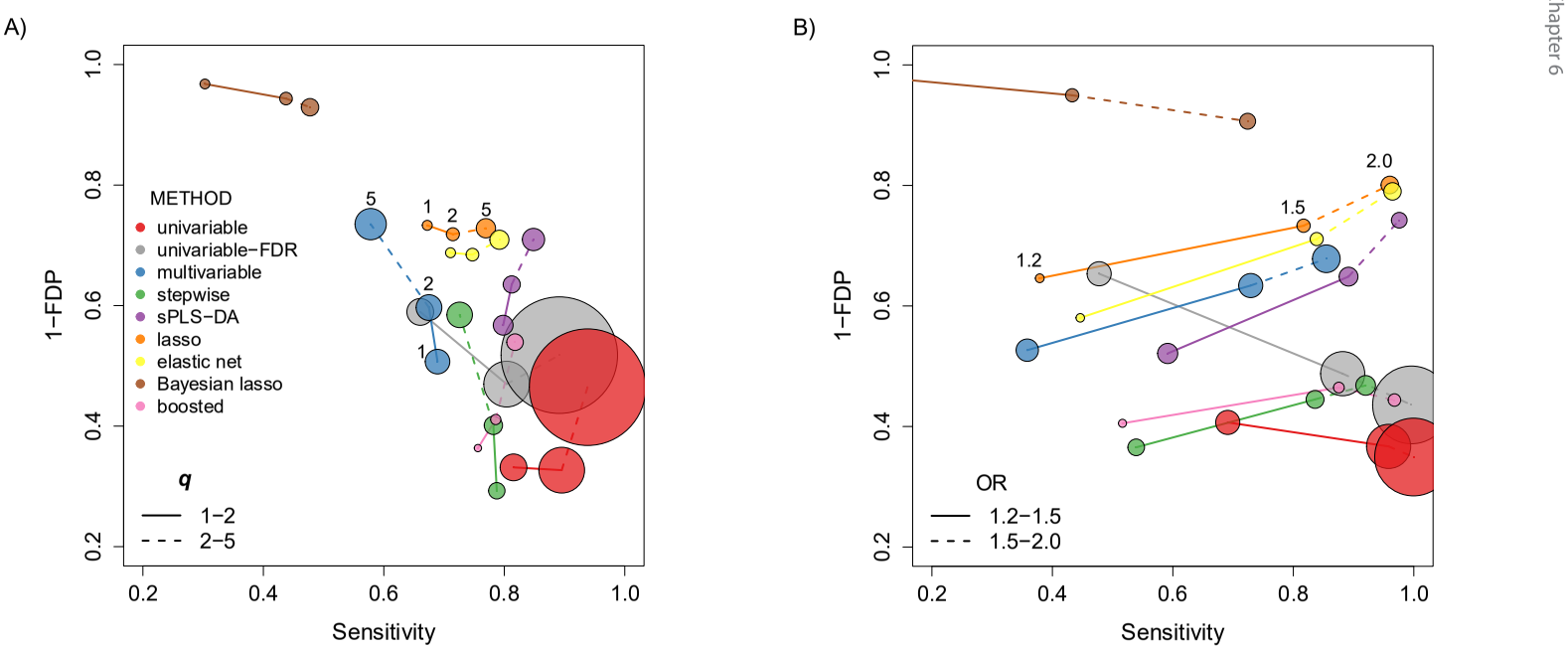


Figure S2. Performance of the variable selection methods stratified by the estimated simulation scenario parameters, **(A)** the number of truly associated, effective variables (*q*), and **(B)** the effect size of the association, the magnitude of the odds ratio (OR). The mean sensitivity is plotted against the mean of one minus the false discovery proportion (FDP), where the size of each bubble is proportional to the median MSE. Results presented are restricted to simulation scenarios with at least one effective predictor variables and OR > 1.0. The axes are truncated at 0.2 to be consistent with the other plots; sensitivity = 0.06 (outside the plot area) for OR = 1.2 for Bayesian lasso.

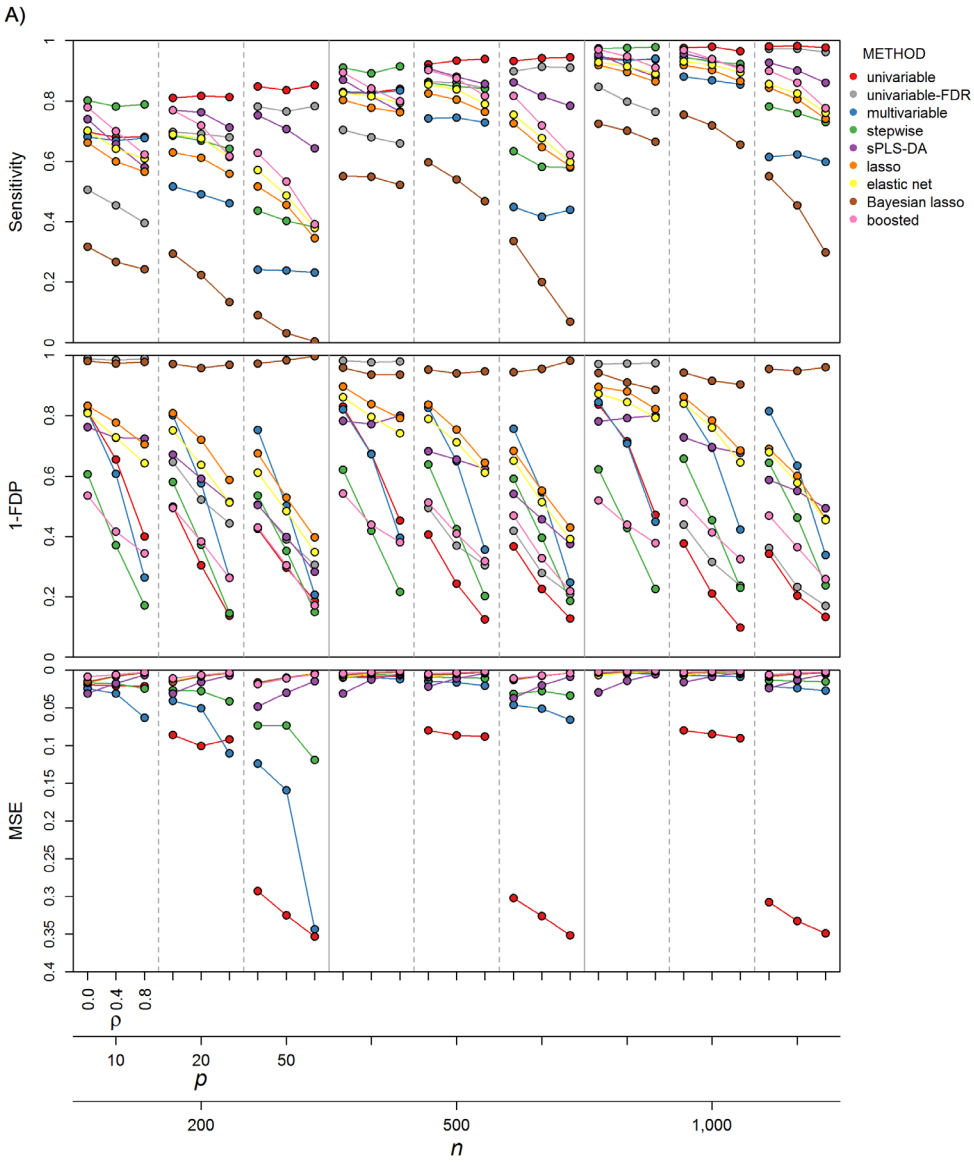
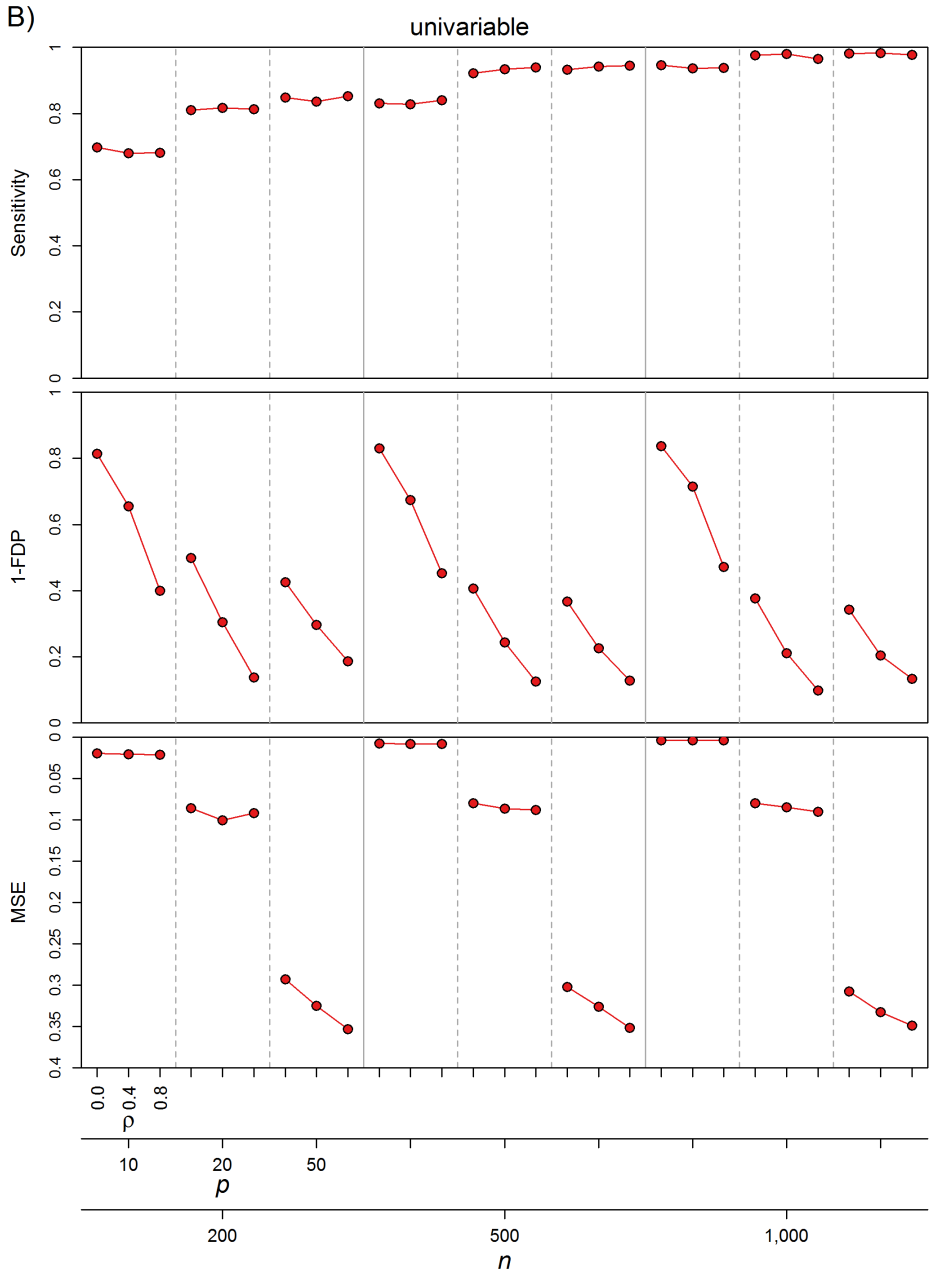


Figure S3. Sensitivity, 1 – false discovery proportion (FDP), and mean squared error (MSE) values stratified by all three *a priori* known simulation scenario parameters: correlation levels (ρ), the number of simulated exposure variables (p), and the sample sizes (n). Mean values of sensitivity and 1 – FDP and median values of MSE are shown, where a higher position within each panel indicates better performance. All methods are shown in one plot **(A)**, and additionally, per method **(B–J)**. The median MSE values are identical for univariable and univariable-FDR, and are only visible for the former in plot A. Note that the *y*-axis for MSE truncated to 0.05 in plots F–J (methods: sPLS-DA to boosted).



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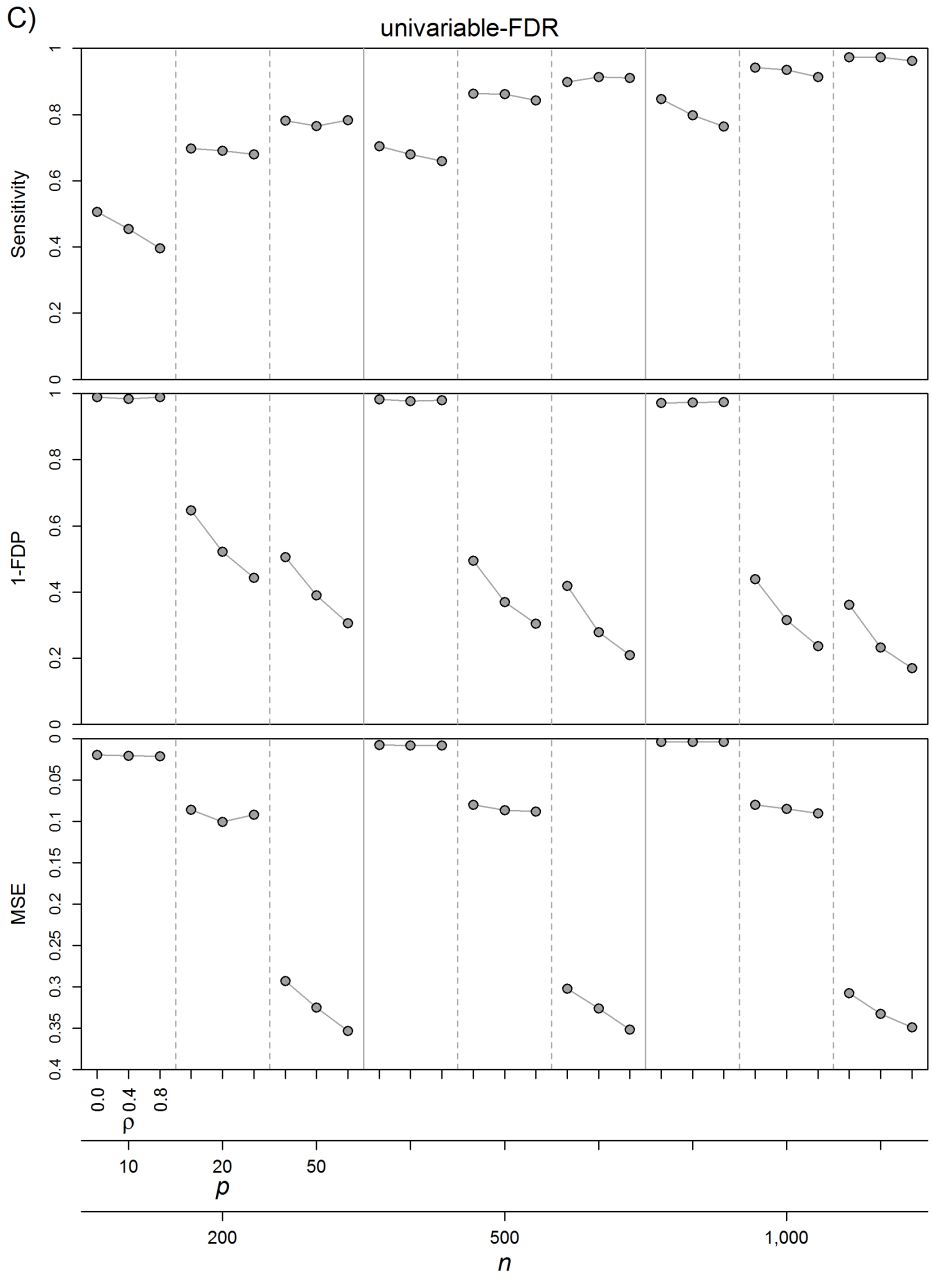

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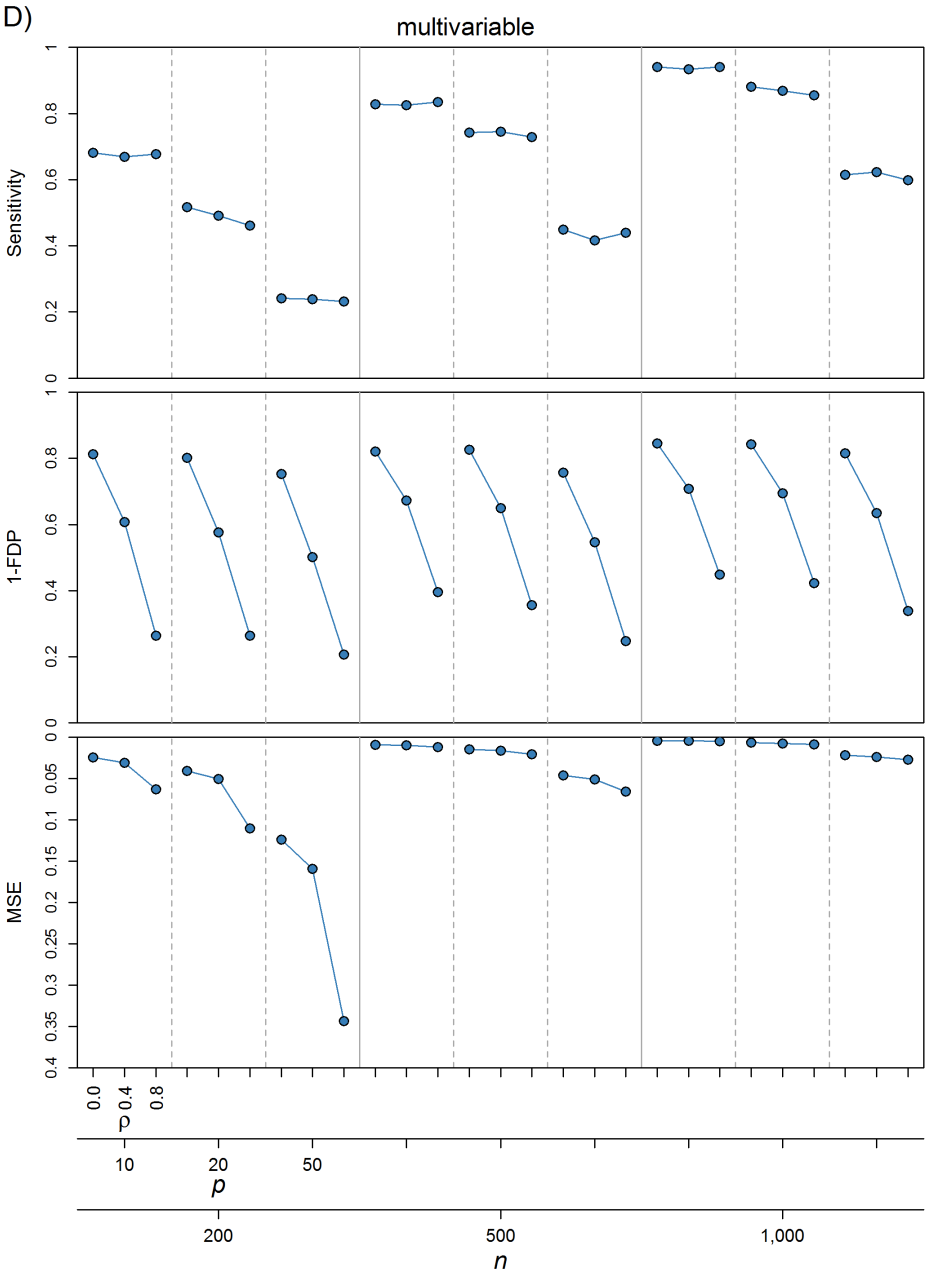

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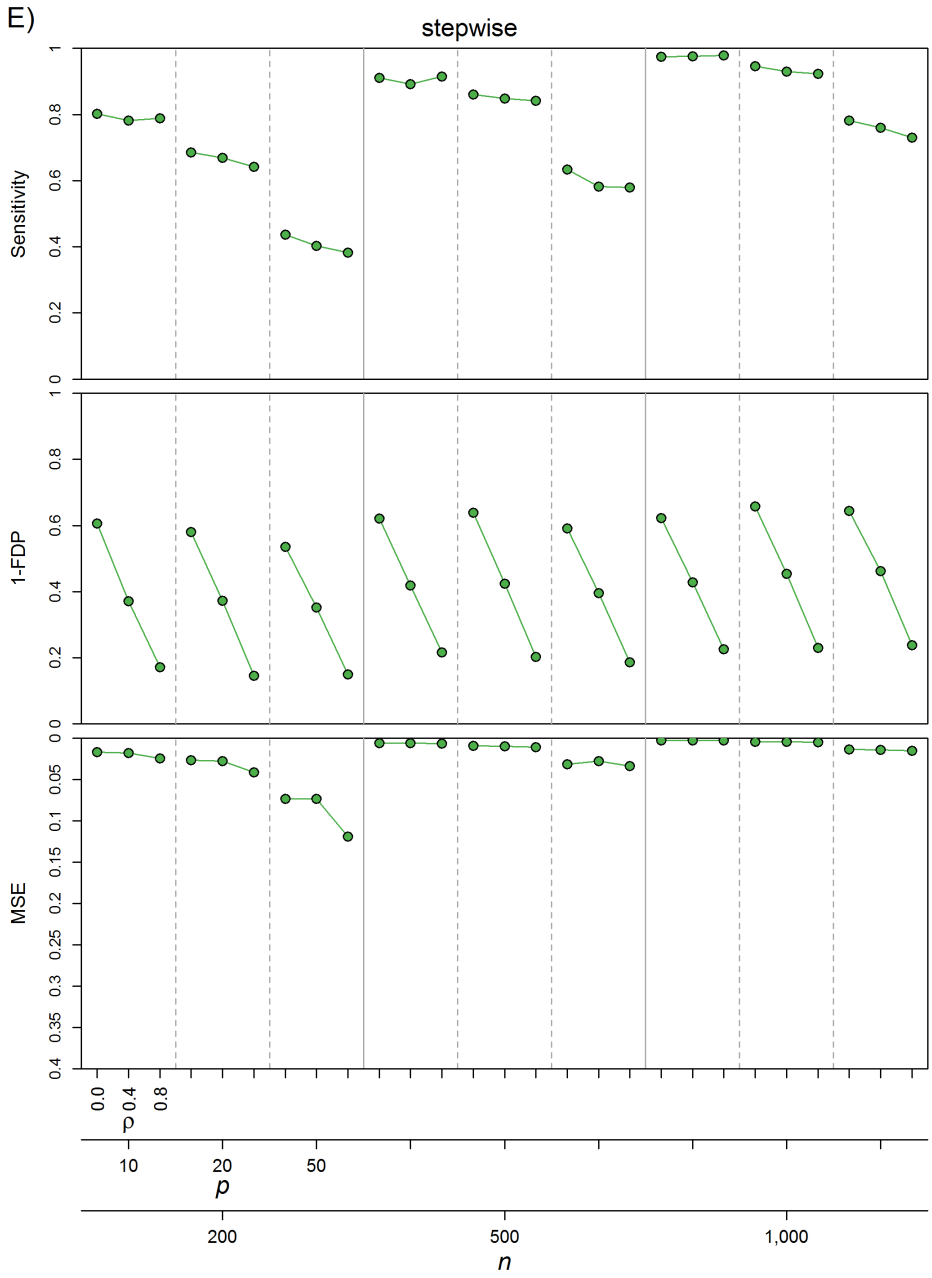

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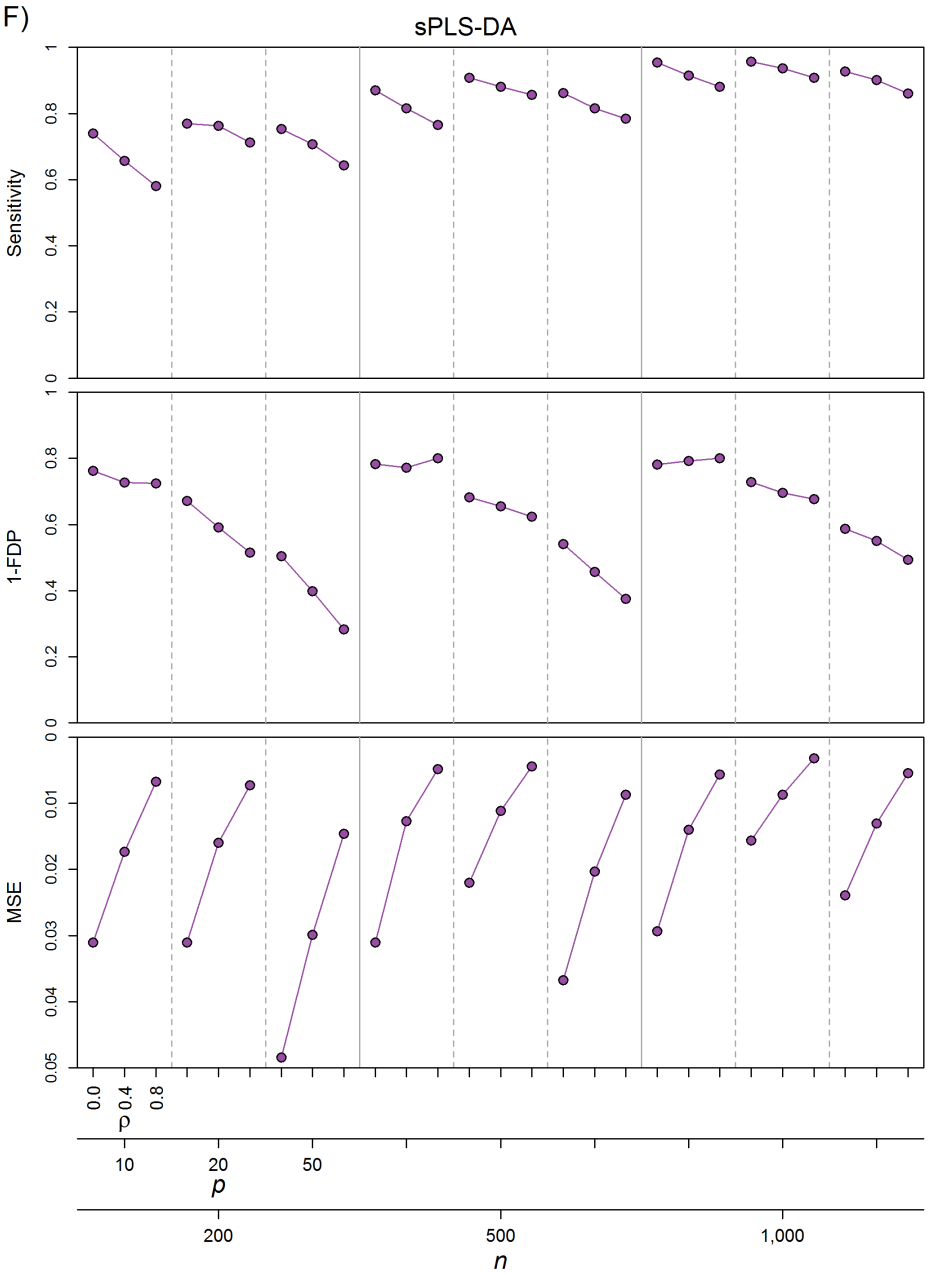

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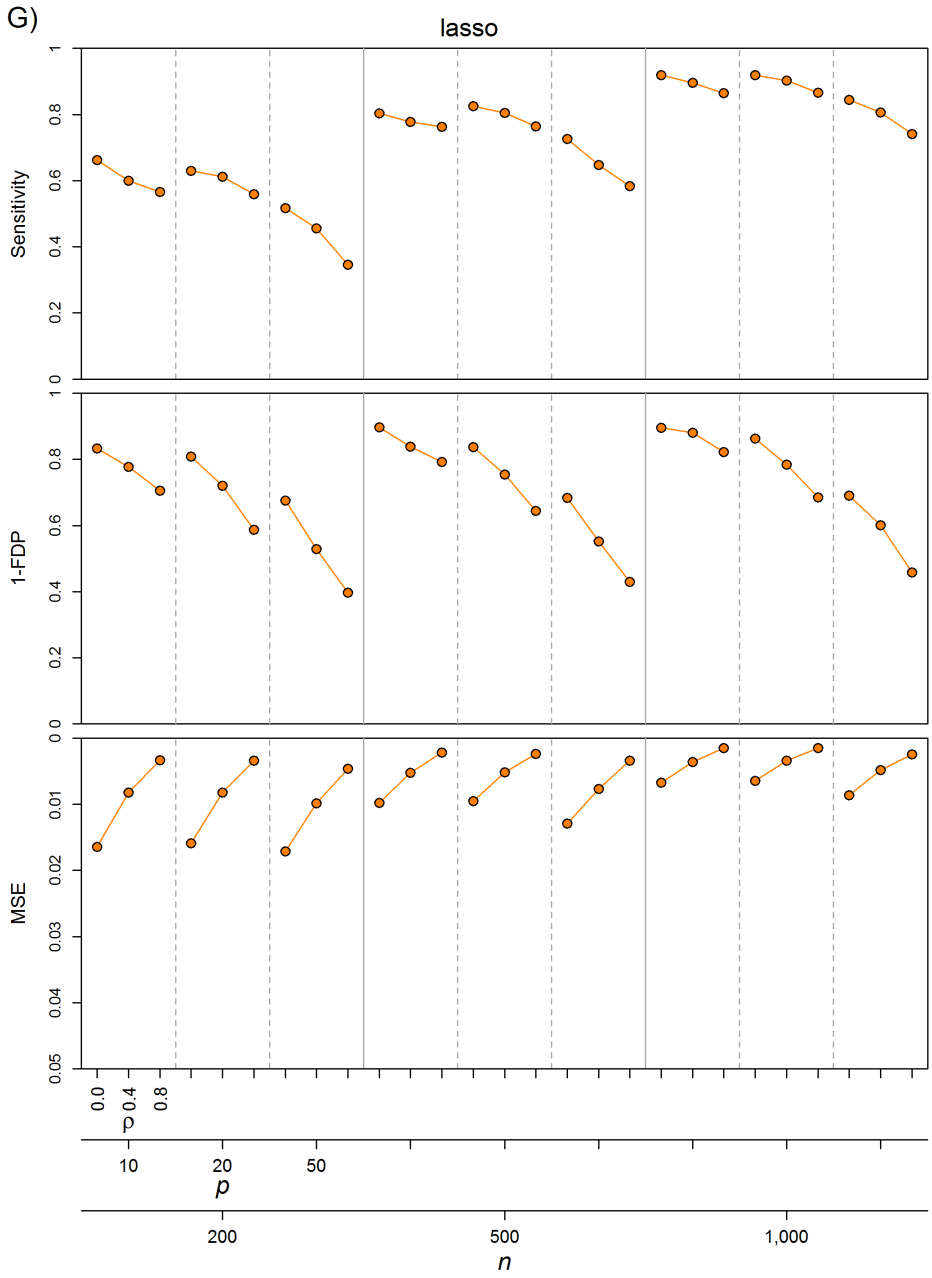

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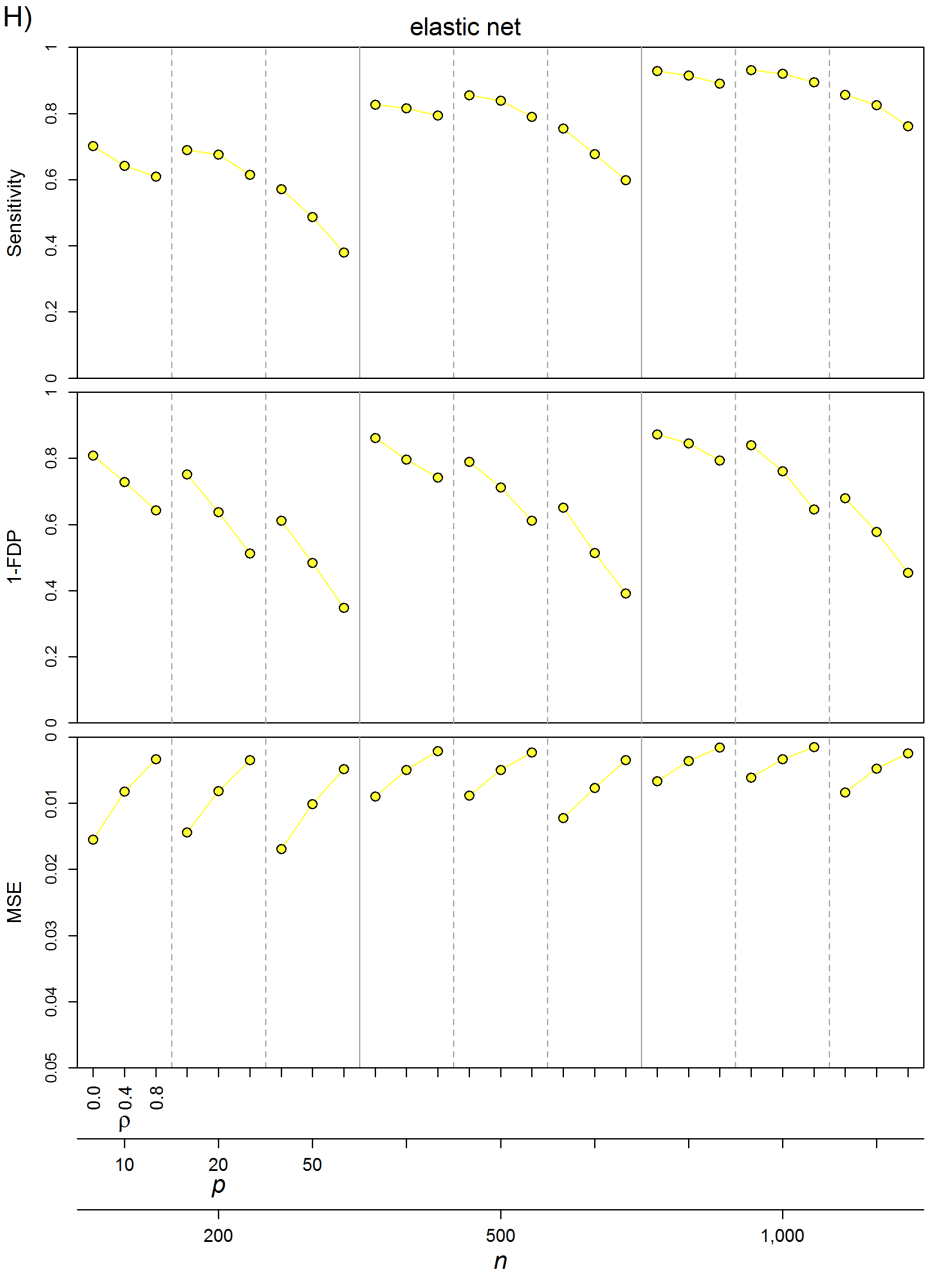

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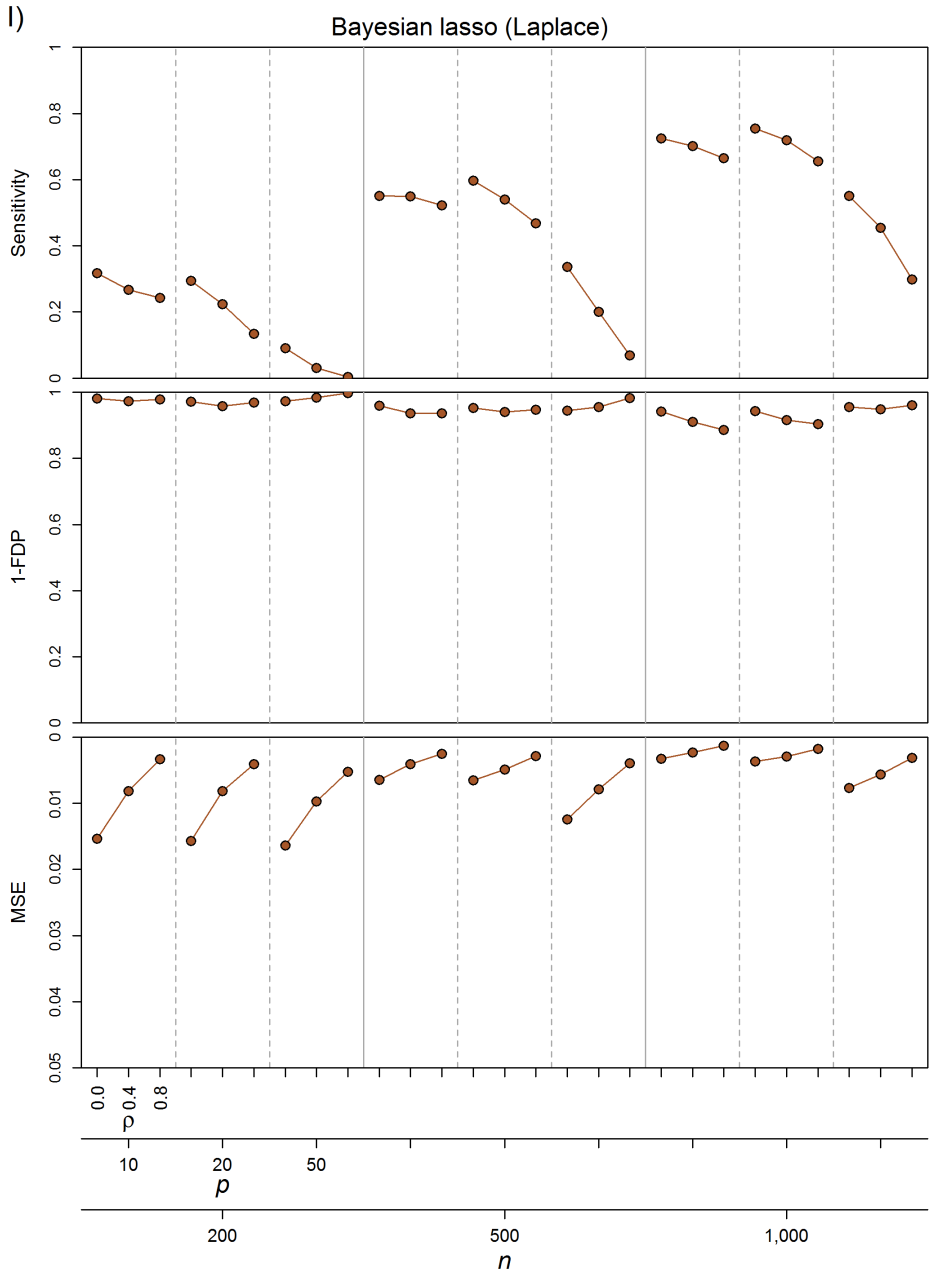

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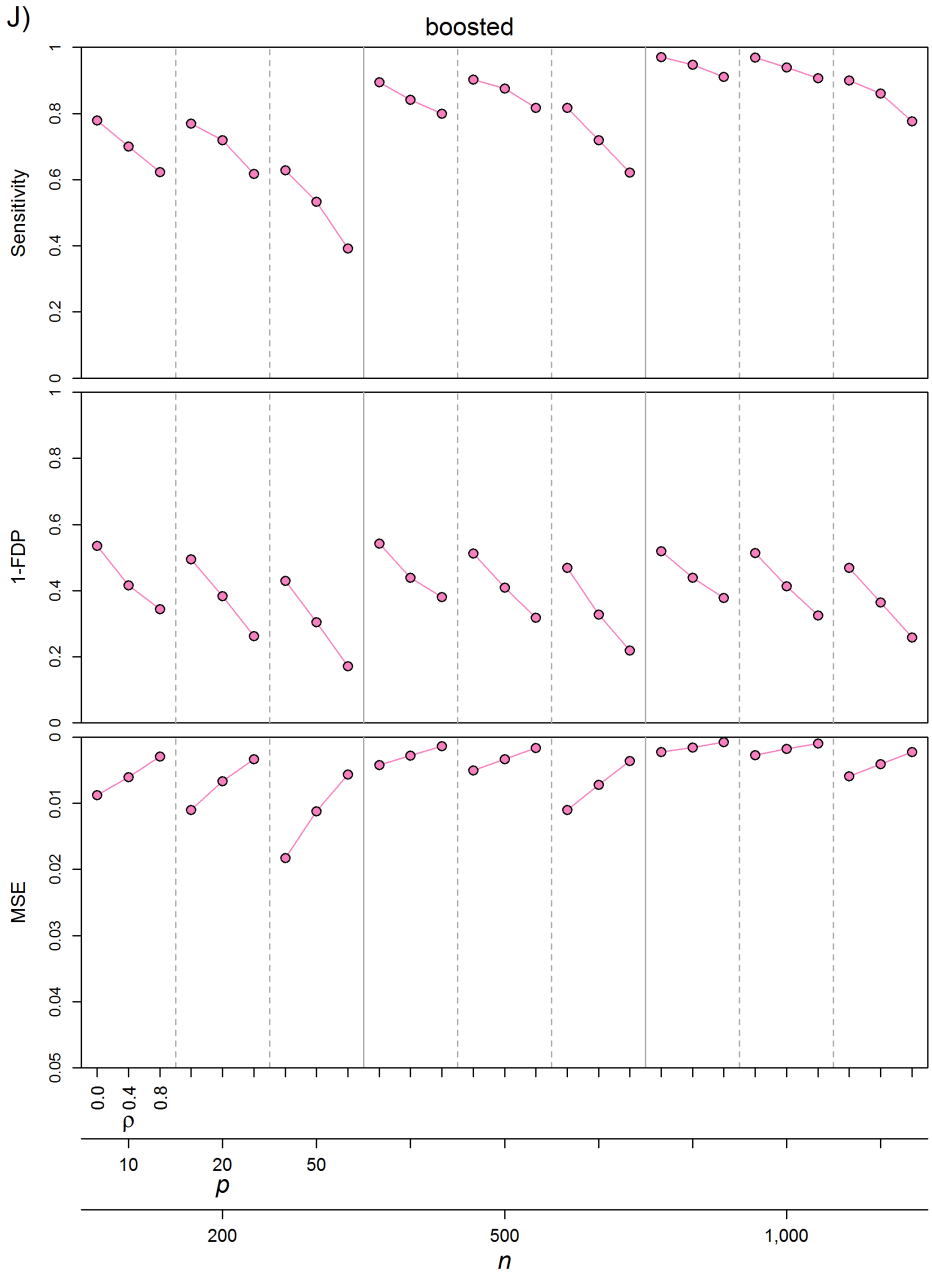

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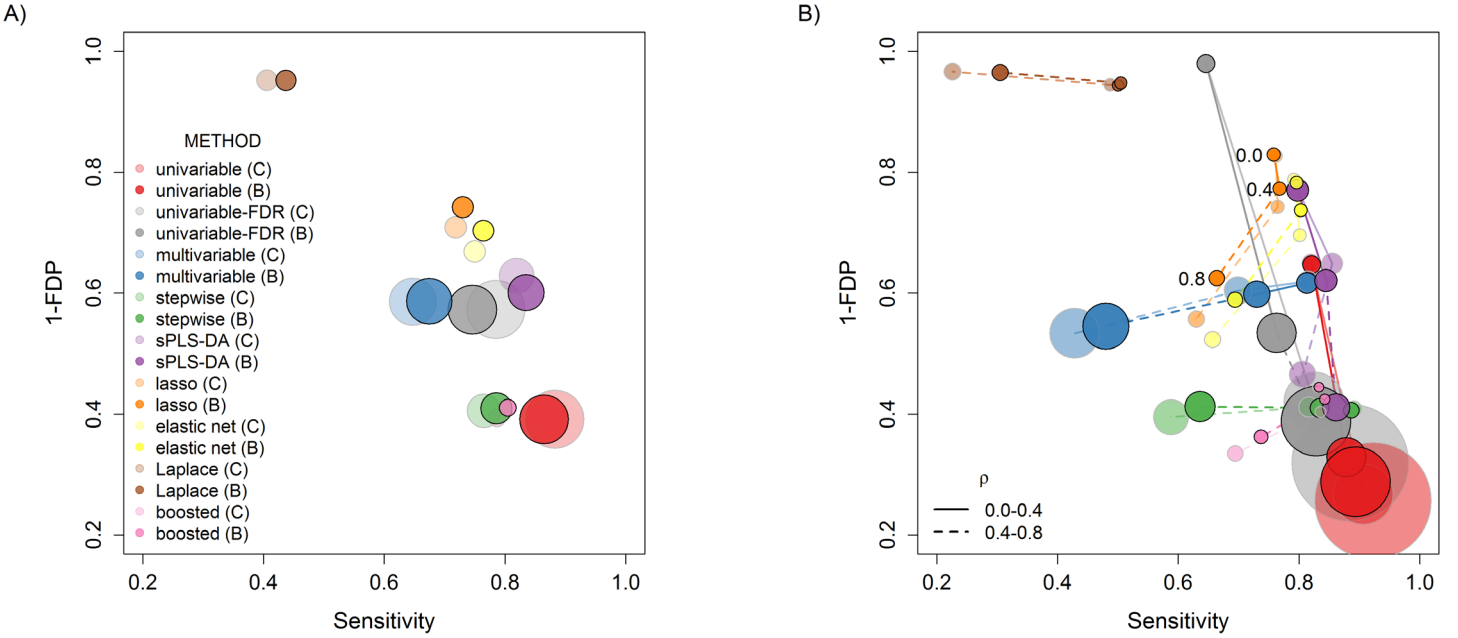


Figure S4. A comparison of performance for the simulations scenarios with blocked correlations (saturated colours, denoted B) versus constant correlations (lighter shaded colours, denoted C; note that these are the primary results presented in Figure 1). Results are presented for **(A)** all simulations, and **(B)** stratified by correlation levels.

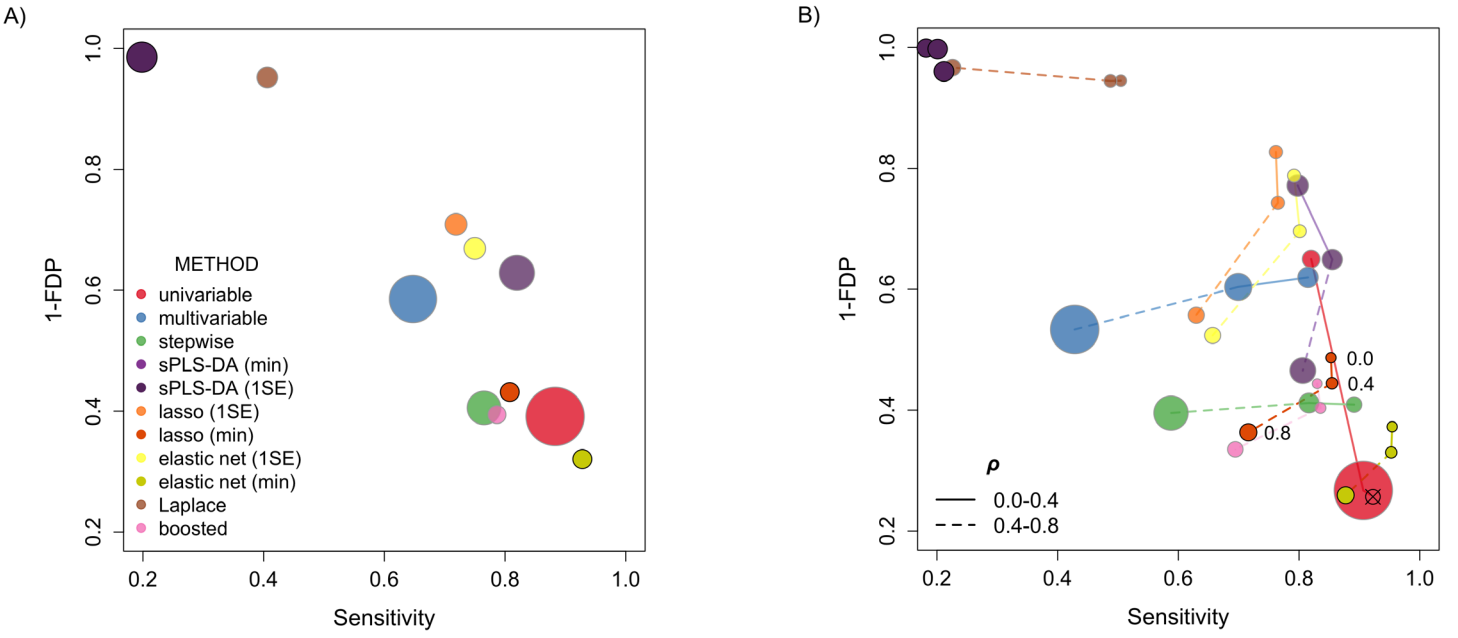
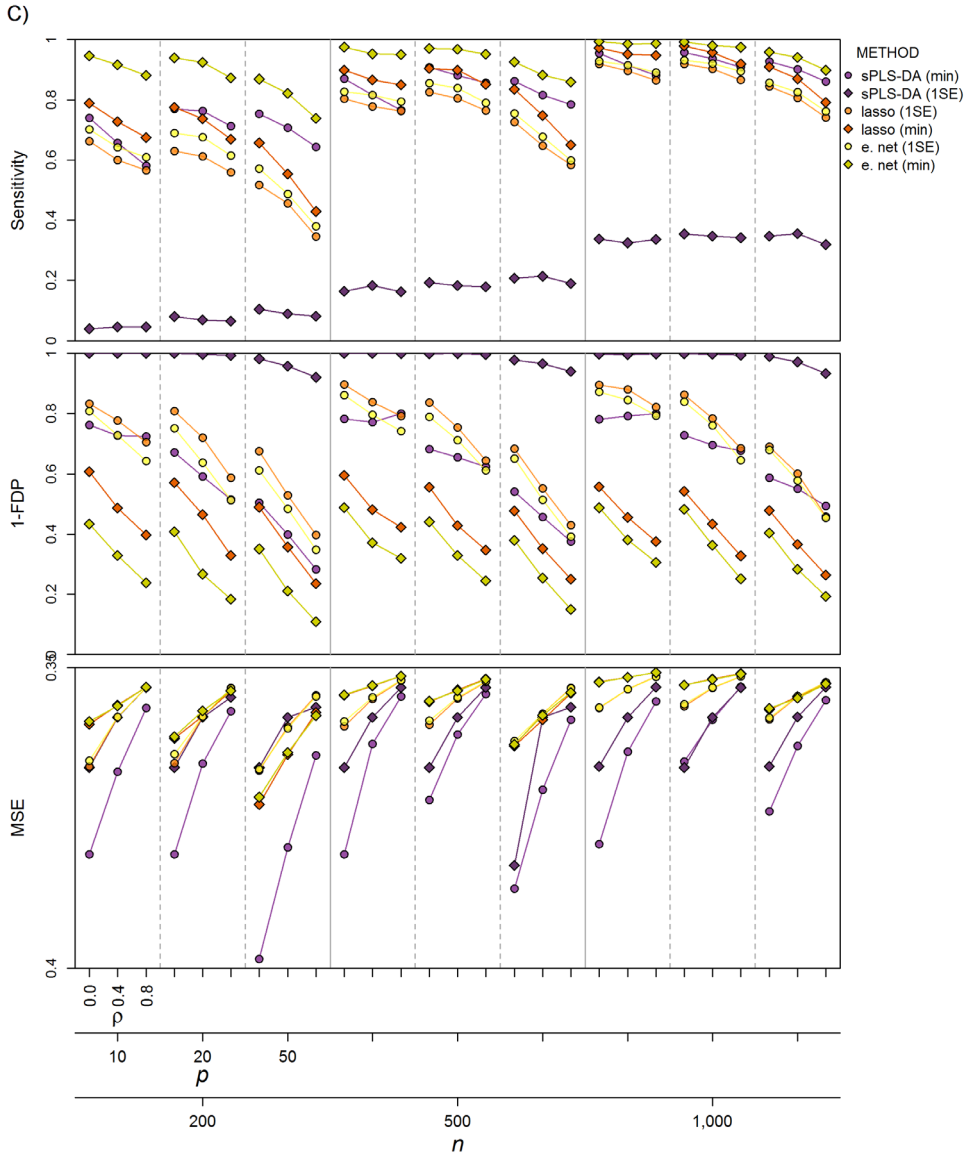


Figure S5. A comparison of performance using different cross-validation optimisation criteria: the primary analysis based on minimum misclassification error for sPLS-DA, and minimum + 1 standard error (SE) in cross-validation deviance for lasso and elastic net (grey circles with lighter shaded colours), versus the secondary analysis with alternative optimisation, minimum + 1 SE for sPLS-DA, and minimum for lasso and elastic net (black circles with darker saturated colours). These results are presented for **(A)** all simulations, **(B)** stratified by correlation levels, and **(C)** stratified by the three *a priori* known simulation scenario parameters (primary results, circles; these sensitivity analyses, diamonds). Note that to improve clarity, univariable-FDR results are not presented.



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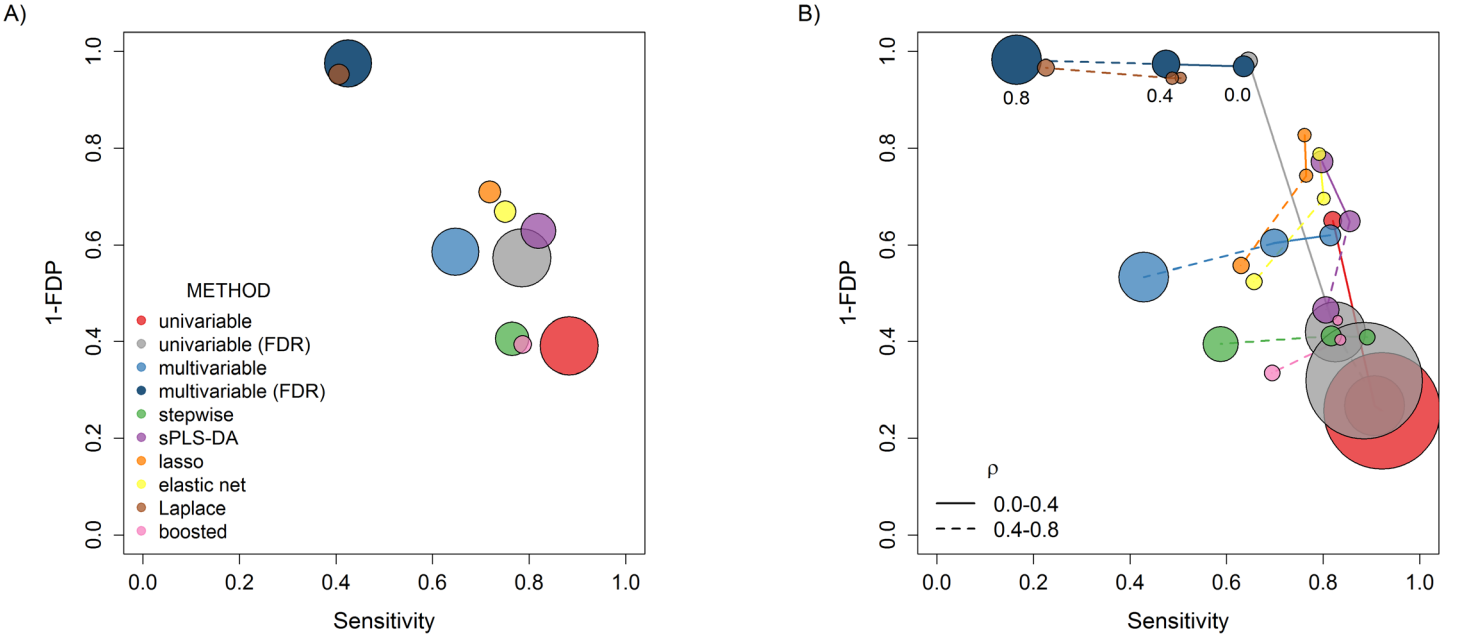

Figure S6. A comparison of the performance of multivariable regression with adjustment for multiple comparisons [false discovery rate (FDR) < 0.05]. Results are presented for **(A)** all simulations, **(B)** stratified by correlation levels, and **(C)** by the three *a priori* known simulation scenario parameters. Note that the axes in plots A and B extend from 0.0 to 1.0, and are not truncated at 0.2 as in most other bubble plots presented. Also, the median MSE values are identical for univariable/multivariable with FDR-correction and without, and are only visible for the former in plot (C).

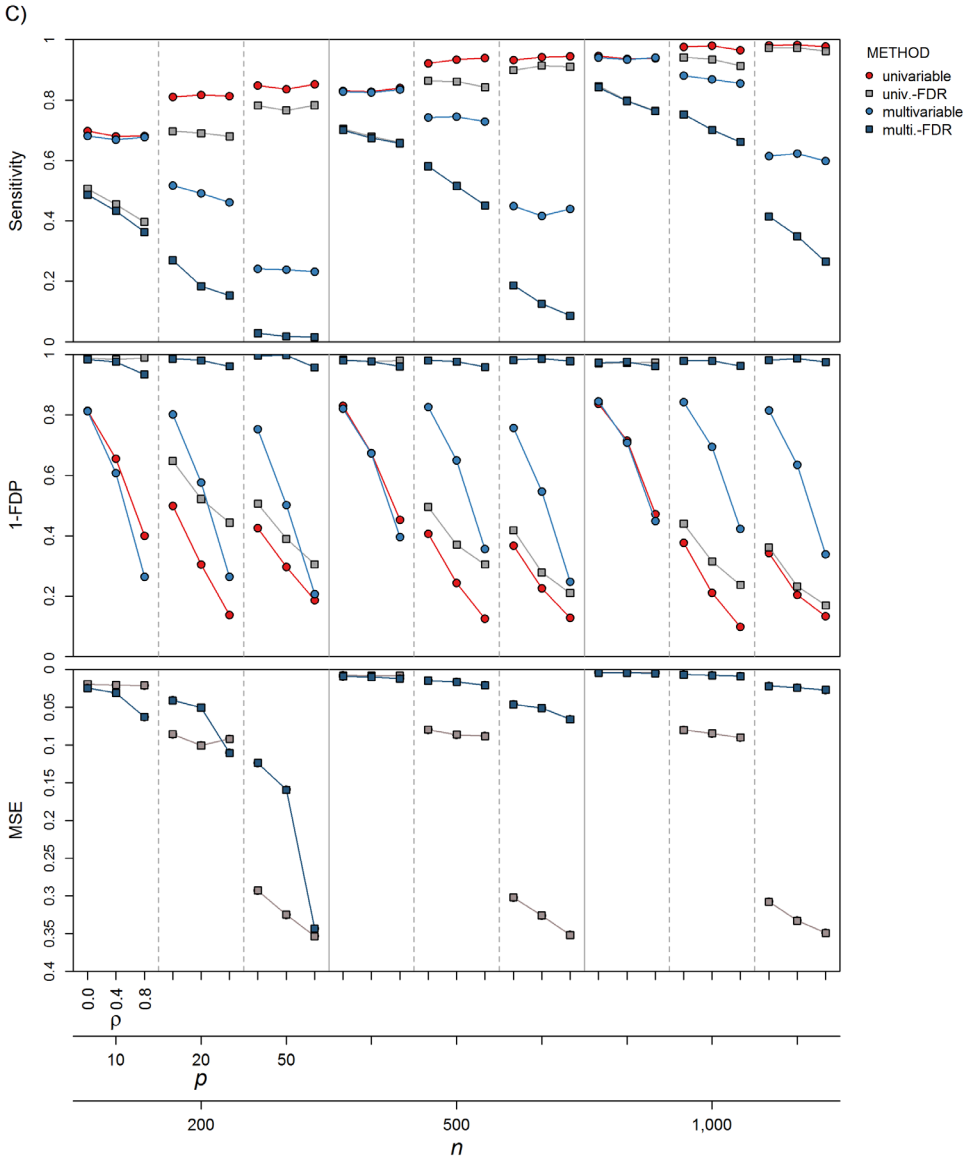

Figure S6. *Continued*

Chapter 7

General discussion

Numerous chemical exposures have been demonstrated to have detrimental effects on various aspects of the health of the fetus, child, and adult, both in the short-term and long-term. The evidence base for the safety of most environmental chemicals is sparse. One of the major challenges in environmental epidemiology, for both hazard identification and exposure– or dose–response assessment, is that human chemical exposure profiles are complex, while efficient statistical models to extricate and characterise exposure–outcome associations that are adjusted for confounding by co-occurring exposures are underdeveloped. In this dissertation, chemicals that are omnipresent in daily life were studied; their exposure patterns and their associations with prevalent health outcomes or biomarkers of adverse outcomes. The interplay between chemicals, gut microbiota, and health outcomes was investigated as well. Several underexploited statistical modelling approaches to assess the multi-pollutant burden were applied, and the performance of these variable selection models was compared. In this chapter, the main findings of this dissertation are briefly summarised, followed by a discussion of the methodological challenges encountered, lessons learned, implications for human health risk assessment, and suggestions for future research priorities.

Main findings in context

Exposure profiles of the study populations

In **Chapter 2** we characterised the levels and determinants of brominated flame retardant exposures in adult men of reproductive age from three countries. The men were sampled in the time period before production restrictions came into force. Median serum levels of the summed PBDE congeners (BDE-47, 99, 100, 153, and 154), the main constituents of the commercial Penta-BDE mixture, were 3.5 and 7 times higher in the Greenlandic compared to the Polish and Ukrainian men, respectively. A polybrominated biphenyl (BB-153) was also higher in Greenlandic men. In addition, in Chapters 3–4 we observed that concentrations of several other environmental chemicals, notably total mercury and PCB-153, were markedly higher in the Greenlandic study populations (Table 1).

A 2016 review reinforced our observation that there are large regional variations in levels, and congener profiles, of PBDEs and other flame retardants.[1] Around one third of US studies with blood biomarker data on tetra- to hepta-BDEs reported median concentrations >30 ng/g lipid weight, whereas median levels exceeded 10 ng/g for only a few studies from Europe and Asia.[1] Elevated concentrations in US populations are attributable to the high historical usage of PBDEs in the US, where they were predominantly added to polyurethane foam in furniture to comply with strict furniture flammability regulations in California.[2] Among biomonitoring surveys of PBDEs in non-US populations (in Europe, Asia, and Australia), levels in Greenlandic men are on the high end of the distribution whereas levels in Polish and Ukrainian men are on the low end of the distribution.[1]

Several factors may explain the high PBDE levels in the Greenlandic study population. Many persistent organic pollutants (POPs) undergo long-range transport to the Arctic

and accumulate, as evidenced by elevated levels of certain POPs in soils and biota. For the highly hydrophobic chemicals, cold temperatures enhance deposition and the cold condensation effect, slow degradation, and increase partitioning into organic matrices, thereby enhancing environmental accumulation and bioaccumulation in food-webs in polar areas.[3-5] Furthermore, Greenlandic Inuit consume higher trophic level foods, such as whale, seal, and polar bear, which are highly contaminated due to bioaccumulation and biomagnification.[6] Although there has been a transition to imported foods and more Westernised diets, locally-harvested foods account for around 20% of energy intake[7,8] and the amount consumed is a strong determinant of POP and mercury exposures.[9] Reviews by the Arctic Monitoring and Assessment Programme (AMAP) indicate that Arctic populations have elevated concentrations of many POPs. Within Arctic populations, Greenlanders have the highest observed POP concentrations with the exception of PBDEs; Inuit and other Aboriginal groups in Alaska and Northern Canada have higher levels of PBDEs, likely as a consequence of stricter US flammability regulations.[10,11] Body burdens of legacy POPs have decreased in Greenlandic populations in recent decades. Blood levels of PCB-153, DDE and HCB declined 49–60% between biomonitoring surveys in 1993–1995 and 2005–2009, with indications that this was primarily driven by decreased chemical concentrations in locally-harvested wildlife.[12]

The study described in Chapter 2 had only limited data on potential determinants of exposure. The general consensus is that diet is the primary source of exposure to PBDEs for European populations, and household dust is the primary source of exposure for the US population.[13,14] We found an imprecise positive association between summed-PBDEs and seafood intake. We also observed that some congeners were associated with age, although patterns were inconsistent. We would not expect to observe cross-sectional body burden-age trends (in adults) until 10–20 years post-ban for the PBDEs with longer elimination half-lives,[15,16] whereas earlier banned legacy POPs already exhibit age-dependent increases.[17,18] In other studies, higher concentrations of PBDEs have been associated with intake of fish and other seafood, recent purchase of a foam mattress or the use of foam instead of an innerspring mattress, wall-to-wall carpeting, the number of household electronics, and self-reported hand-to-mouth behaviours such as nail-biting.[19-21]

Population-specific chemical signatures. In **Chapters 2–5** we confirmed that chemical correlation structures are complex. Correlations within chemical classes are generally high, whereas correlations between chemical classes are generally moderate to low, although still large enough to warrant concerns about potential confounding and collinearity. As we demonstrate in the simulation study in Chapter 6 and discuss further herein, it is important to evaluate the correlation structure of the exposure matrix before choosing a statistical model, and to consider it when interpreting model output.

We also observed large differences in exposure contrast across chemicals in our study populations, as has been reported for other cohorts. For example, in the well-characterised

Table 1. Selected exposure biomarker concentrations from each chemical class examined in the study populations in this dissertation compared with other biomonitoring surveys.

	This dissertation								Related surveys		
	INUENDO (2002–2004)						HUMIS-NoMIC (2002–2009) Norway, women, n=993		US, NHANES[23] (2003–2004)	Arctic Canada	
	Greenland		Poland		Ukraine					(2007–2008)[10,b]	(2004)[24,c]
	Women n=513	Men n=199	Women n=180	Men n=197	Women n=557	Men n=206	Breast milk levels	Serum equivalent[a]	Adults n=1300–4525	Women n=485–491	Adults n=883
PCB-153 (ng/g)	107	223	11	17	27	45	32	26	24	47	190
DDE (ng/g)	302	568	348	516	653	1051	46	41	233	148	
HCB (ng/g)		59		12		135	11	174	15	32	
BDE-47 (ng/g)		1.96		0.63		0.27	1.05	0.72	18.0	8.8	5.7
PFOA (ng/mL)	1.84	4.60	2.51	4.86	0.96	1.33	0.04	NA	4.10		
PFOS (ng/mL)	20.1	47.4	7.8	17.7	5.0	7.3	0.11	NA	21.4		18.7
Hg (ng/mL)		8.66		1.01		0.84			1.00	5.0	
MECPP (ng/mL)	0.58	1.17	0.85	1.62	0.93	2.16					

Median or geometric mean values are presented. Serum or blood concentrations are reported, except for the Norwegian study population, for which breast milk concentrations are presented, along with: [a] The estimated serum concentration based on the mean reported serum:milk ratio.[25]
[b] Values for women of reproductive age from the International Polar Year Inuit Health Survey. Participants were Inuit and lived in Nunavut, Nunatsiavut, or the Inuvialuit Settlement Region.
[c] A study of Inuit from Nunavik, the northernmost area of the province of Quebec in Eastern Canada.

INMA-Sabadell Spanish birth cohort, the relative standard deviations (standard deviation/mean) in exposure biomarkers ranged from 60–167% for PCBs, PFASs and PBDEs to upwards of 353% for DDE and a maximum of 531% for a phthalate metabolite (7OH-MMeOP).[22] These large differences in contrast between chemicals necessitated scaling in variable selection models (as elaborated below), as contrast in exposure is positively related to the statistical power to detect exposure–outcome associations.

Epidemiological findings

In **Chapter 3** we assessed 330 exposure–outcome relationships using sparse partial least squares (sPLS) regression, a hybrid dimension reduction and penalised variable selection modelling approach. We studied men who had recently fathered a pregnancy (INUENDO cohorts) and detected 10 associations, both positive and inverse, between serum levels of certain phthalates, metals, and organochlorines and markers of male reproductive function. These included robust associations between DiNP phthalate metabolites and decreasing serum testosterone levels, and between PCB-153 and a decreasing proportion of progressively motile sperm.

A large study published after our study which was based on cross-sectional NHANES data found that several phthalate metabolites were associated with reduced testosterone in children and adults; however, only suggestive associations were found for men 40–60 years of age (for metabolites of DEHP and dibutyl phthalate), but not for other age groups.[26] The evidence for associations between adult exposure to phthalates and altered reproductive hormone profiles is suggestive, but remains inconclusive.[27]

Exposure during *in utero* development may represent a more sensitive period of exposure for male reproductive health. Several studies have found associations between prenatal exposure to several phthalates (including metabolites of DEHP and DiNP) and reduced anogenital distance in boys,[28-30] a sensitive marker of development correlated with reduced semen quality.[31] A substantial number of studies in rats have documented anti-androgenic effects of prenatal phthalate exposure to low-molecular weight [e.g., diethyl phthalate (DEP) and dibutyl phthalate (DBP)] and high-molecular weight (including DEHP) phthalates. The anti-androgenic effects of phthalates appear to be species-specific, as studies of mice[32] and experimental studies of human fetal testis xenografts[33] showed insensitivity to phthalate exposure. However, DBP has been shown to induce germ cell effects in both rat and human fetal testis xenograft models, including a loss of germ cells and abnormal germ cell aggregation.[34]

A number of epidemiological studies support an association between PCB exposure during adult life and reduced sperm motility,[35] and our finding bolsters this observational evidence. There is evidence from *in vitro* studies that 33 out of 96 environmental chemicals, including low levels of DDT, directly activate the calcium ion channel CatSper on human sperm, evoking a motility response and acrosomal exocytosis; this competition desensitises sperm to activation by the hormones progesterone and prostaglandins, compromising sperm

function.[36,37]

PFASs were not associated with any markers of male reproductive function in our study. A recent review of 14 studies on PFAS exposures and semen characteristics and reproductive hormones concluded that findings were inconsistent overall; however, the authors noted that several studies identified associations with lower testosterone levels and increased abnormal sperm morphology.[38] This was not confirmed in our study, as these results were null. Sperm morphology has been found to be the most predictive semen characteristic with respect to delayed conception.[39,40]

Few studies have investigated whether exposure to environmental chemicals is directly related to couple fecundity as measured by time-to-pregnancy (TTP). Studies assessing PFAS and phthalate exposures in relation to TTP in the INUENDO cohorts were largely null, although there were suggestive associations with a DiNP metabolite, PFNA, PCB-153, and *p,p'*-DDE and prolonged TTP, and between a DEHP metabolite and shorter TTP.[41-43] A prospective study of 501 couples in the United States found that a greater number of the male partner's chemical exposures were associated with TTP than the female partner's exposures, and that several PCBs, phthalates, phenols, lead and *p,p'*-DDE were associated with longer TTP.[44]

In **Chapter 4** we evaluated associations between prenatal exposure to 16 chemicals, as measured by maternal serum levels during pregnancy, and birth weight of infants born at term in the three INUENDO cohorts using elastic net penalised regression. A DEHP metabolite (MEHHP), PFOA, and *p,p'*-DDE were associated with decreased term birth weight, and a DiNP metabolite (MOiNP) was associated with increased term birth weight.

Recent reviews of 10 epidemiological studies on DEHP exposure and birth weight showed that the majority of associations were null, although most studies had smaller sample sizes ($n \leq 404$) and imprecise estimates.[45,46] Reviews of 14 studies on PFAS exposures support our finding of an association between PFOA and decreased birth weight[47,48]; however, a Danish study with one of the larger sample sizes ($n = 1507$) failed to replicate this association.[49] The evidence base is more limited for the other PFASs, with some studies, although inconsistent, supporting an association between PFOS and reduced birth weight.[48] A large pooled-analysis of 12 European studies found that PCB-153 but not *p,p'*-DDE was associated with lower birth weight.[50,51] In our study, the single-exposure PCB-153 association attenuated to null on adjustment for co-exposures.

Emerging evidence indicates that some environmental chemicals disrupt placental development and function—via mechanisms including altered microRNA expression, oxidative stress, and impaired angiogenesis—and that this may mediate the effects of chemicals on adverse birth outcomes.[52-54] Ultrasound measurements of fetal anthropometry (e.g., head circumference, femoral length) provide a more direct measure of fetal growth than birth weight, capturing growth at different stages of pregnancy and growth velocity,[55] and such studies[56,57] may provide more etiologically insightful results. Future studies might also attempt

to disentangle the associations between perinatal chemical exposures and *a)* fetal growth restriction, which is related to compensatory catch-up growth in early-life and excess adiposity,[58] and *b)* increased risk of obesity (i.e., chemical 'obesogens').[59] Potential sex differences in susceptibility to environmental exposures also warrants greater attention, and will generally require larger sample sizes to detect sexually dimorphic associations.

In **Chapter 5** we assessed whether maternal breast milk levels of 26 environmental chemicals, reflecting prenatal and postnatal exposures, and early-life gut microbiota markers were prospectively associated with asthma or lower respiratory tract infection (LRTI) in the Norwegian HUMIS-NoMIC birth cohort. We also assessed whether chemical exposures were associated with gut microbiota markers. We used elastic net penalised regression models and mediation analysis. An organochlorine pesticide (β-HCH) was positively associated with registry-based asthma assessed at a median of 10 years of age and maternal-reported asthma at 2 years of age. Several PCBs were inversely associated and others positively associated with asthma, whereas associations for PFASs and PBDEs were close to null, and no exposures were associated with maternal-reported LRTI by 2 years of age. There is a moderate level of evidence that exposure to organochlorine pesticides is associated with asthma, respiratory infections, and immune system biomarkers.[60-62] There is also emerging evidence that PFASs may be immunotoxic,[63] including studies finding that PFASs are associated with reduced vaccine-induced antibody concentrations.[64,65] The evidence base is limited for other chemical classes, including PBDEs.

A small number of *in vivo* studies have demonstrated that gut microbiota can metabolise specific environmental chemicals, and reciprocally, that environmental chemicals can perturb microbiota profiles and metabolic activity, some through activation of aryl hydrocarbon receptor (AhR) signalling.[66,67] To our knowledge, the study in this dissertation is the first to use human data to investigate potential chemical–microbiota associations. Except for an association between PCBs and reduced diversity, other associations were generally imprecise. In our study, we found that microbial diversity and functional metabolites (short-chain fatty acids) assessed at three time points in the first two years of life exhibited both positive and inverse associations with asthma and LRTI. The variable sample sizes and limited overlap at different sampling time points hindered statistical inferences about sensitive windows of exposure. Our findings provide limited evidence to support previous findings that diversity and SCFAs may play a protective role in asthma and respiratory infections.[68-70] The early-life composition of the respiratory microbiome has been linked to an increased risk of LRTIs and asthma.[69,71] The composition of the microbiota of the gut and respiratory tract is largely independent, although some determinants of colonisation and dysbiosis are shared, such as mode of delivery and antibiotic use.[69] Given the potential to manipulate microbiota composition and to prevent dysbiosis through diet, prebiotics and probiotics, and other modifiable aspects of the exposome, the exposome–microbiome frontier is a promising avenue of research for health promotion.[70,72-74]

Modelling of multiple exposures: statistical methods

Implementation considerations and limitations

In **Chapters 3–5** we applied two families of variable selection methods to evaluate multiple-exposure–outcome associations. We chose to apply models with strongly grounded theory, well-established in statistics and other research fields, and which are readily applicable using standard software. These methods should nudge results closer to identifying the true causal structure, as confirmed in the simulation study (Chapter 6) and other similar simulation studies.[75,76]

Model optimisation. We found that model selection and statistical inference were aspects of sPLS-regression and penalised elastic net regression modelling that were somewhat incomplete or lacking in the out-of-the box implementations. Small effect sizes are often expected in environmental epidemiology, and it became apparent that with weak signal the default single cross-validation approach to model optimisation (selection of model tuning parameters that determine the model selection or subset of exposures selected) sometimes yielded highly variable selection results. This has also been shown by others (e.g., Lim and Yu[77]). Therefore, we implemented a repeated *K*-fold cross-validation scheme, successively partitioning data, to achieve a more stable selection based on a large number of cross-validation error curves. Model selection aims to balance parsimony and predictive power while preventing over-fitting. Variability in model selection is inherent to *K*-fold cross-validation (except for deterministic leave-one-out cross-validation) due to the random partitioning of data. The number of *K* folds (e.g., 5, 10, 20) is somewhat influential, as the variance of the CV errors generally decreases as the number of folds increases; however, the choice *K* is less influential as the number of repetitions increases, as the variance of CV errors rapidly decreases as the number of repetitions of CV increases.[78] Based on the results of the repeated CV, we selected the model (level of penalisation) that corresponded to the minimum mean cross-validation error curve, or the maximum penalisation within one standard error of the minimum (the so-called "1 SE rule").[79,80] Model selection based on a percentile of repeated CV error, 0.5[81] or 0.95,[82] has also been proposed; or based on the minimizing the Akaike or the Bayesian information criterion (which rely on parametric assumptions); or based on stability of selection upon subsampling of the data,[83] or a combination of selection stability and predictive performance.[84] For the practitioner, the optimal choice will remain ambiguous until further theoretical or simulation results are available as to the most optimal criterion for the data structure at hand, and dependent on whether the researcher prioritises an exploratory or confirmatory assessment.

We also modified the cross-validation procedure in an elastic net regression R package (*glmnet*, used in Chapters 4–6) to search within a grid of the two tuning parameters α and λ, rather than pre-specifying the α parameter (determining the balance between the ridge and lasso penalties) and only tuning the λ penalisation parameter.[85] Not unexpectedly, minimum

cross-validation error values followed a continuous diagonal within the grid of α and λ, but the α level influenced the 'aggressiveness' of penalisation and which variables were removed (penalised to zero) first as λ increased.

To test the global null hypothesis that none of the exposure variables were predictive, we included a null model in the modified repeated cross-validation step in the sPLS procedure (Chapters 3 and 6).

Statistical inference. Recently, methods to integrate hypothesis testing (so called 'post-selection inference' and selective inference) into adaptive methods such as penalised regression have been developed, and extended to account for multiplicity with sequential stopping rules to control the false discovery rate (FDR).[86-90] Penalised models, similar to forward stepwise regression, are adaptive in that the strongest predictor variables are sequentially included, and thus classical hypothesis tests which assume models are static produce invalid *p*-values that are too small (*p*-values are biased downward). We calculated *p*-values using the *covariance test*, based on how much covariance between the outcome and the included (non-zero) variables is attributed to the last-entered variable (complete null hypothesis) (Chapter 4).[91]

A more recently developed and similar approach for valid inference after model selection is the *spacing test* (incremental null hypothesis),[92] which produces exact *p*-values and confidence intervals (exact in finite samples, i.e., does not require n or p to be large), and is less restrictive than the covariance test (which assumes the underlying model is linear and that selected variables are not too highly correlated with non-selected noise variables). This is based on polyhedral constraints on y in adaptive model selection events, where for selection event $Xy \leq b$ for a matrix of variables X and vector b, since each additional variable along the penalisation path is conditional (on the previously selected set of variables and the sign of the selected variables, and that each subsequent step represents competition among remaining p variables), for $\eta^T y | \{Xy \leq b\}$, η^T is a truncated Gaussian distribution which can be used for inference using classical tools.[93] Even more recently, G'Sell et al.[90] and Li and Barber[94] have proposed sequential stopping rules to control the FDR, valid as long as the *p*-values are independent under the null (as in the 'saturated model *p*-values' of Fithian et al.[95]). The performance of these methods in scenarios with (highly) correlated data structures requires further validation and evaluation.

Data splitting or data carving is another approach to statistical inference in which a portion of the data (often half of the data) is used to build and test the model, and the other portion of the data is used for replication and inference. However, this approach leads to a loss of power and is also stochastic (varying with the random data split), and was not deemed a favourable approach in the studies in this dissertation considering the moderate sample sizes. Bayesian variable selection methods, such as the Bayesian lasso regression evaluated in the simulation study (Chapter 6), have priors that adapt to the number of exposure variables, accounting for multiplicity, although they may be undesirably conservative for many explor-

atory environmental epidemiology assessments.[89]

These crucial practical implementation issues, unfortunately, can lead to a 'black-box' problem, and may lead to criticisms of naïve or sinister data massaging or dredging, and may justifiably frustrate sceptical readers seeking more insight into the stability and reproducibility of the findings. Until variable selection methods have undergone more rigorous performance evaluations, particularly with respect to implementation in scenarios typical of environmental epidemiology data, and until these methods become more standardised with respect to presenting uncertainties and sensitivity analyses, a recommendation would be to continue to present more conventional single-exposure–outcome assessments (i.e., univariate regression with FDR control) as a frame of reference for the reader. If multiple-exposure and single-exposure modelling results are divergent, authors may interpret for the reader which results they deem most robust in light of the data structure, notably the correlation structure of the exposure data. Another tactic is to employ several statistical methods in one study,[96] although this pluralistic approach also requires careful interpretation for the reader based on performance characteristics of the methods, and may deter readers more interested in the etiological implications than statistical inference from engaging with the research.

Integrating other modelling tools. A challenge we encountered was integrating some of the more established epidemiological analysis tools, such as multiple imputation and evaluation of effect measure modification, into the variable selection modelling approaches. To deal with missing data, and achieve matrix completion, we used multiple imputation in Chapter 5. We fitted elastic net regression to each imputed dataset and averaged coefficients across multiply imputed datasets if the exposure was selected (non-zero in elastic net models) in the majority of models,[97] somewhat akin to selection stability. An alternative would be to include weights accounting for the proportion of missing data for each X matrix variable.[97] Applying one penalised regression model to stacked multiple imputed datasets, incorporating weights for the proportion of missing data for each variable, has also been proposed,[97,98] although in our preliminary analyses it appeared this approach had low statistical power (low sensitivity; data not presented). Applying a group lasso penalty to the estimated regression coefficient of each selected variable across imputed datasets has also been proposed,[99] and as has combining bootstrap imputation (single imputation on multiple bootstrap samples) with selection stability, which can also be used for analyses of high dimensional ($p > n$) datasets.[100] How to combine multiple imputation with some of the aforementioned variable selection statistical inference methods is not readily apparent.

Another challenge specific to microbiome analysis is the integration of some of the microbiota-analytical approaches into variable selection modelling, including methods to account for the zero-inflated count data (as rare species have been shown to be etiologically relevant) and the compositional nature of microbiota data (e.g., log-ratio transformations).[101,102]

Identifying sensitive windows of exposure for disease onset or progression, for in-

stance by modelling of prenatal and postnatal chemical exposures in early life,[103] is hampered by the high correlations between these different periods of exposure. In Chapter 5, breast milk levels of chemical biomarkers were used as a proxy for perinatal exposures. We did not attempt to disentangle associations for specific prenatal and postnatal exposure periods. In this Norwegian cohort (HUMIS-NoMIC), with a relatively long average duration of breastfeeding compared to other birth cohorts (a median of 13 months of any breastfeeding), we observed that correlations between measured breast milk concentrations and the estimated[104] postnatal child serum concentrations diverged with time, as expected: they exceeded $r_p > 0.75$ from 0 to 6 months of age and subsequently dropped to $r_p < 0.75$ for around half of the lipophilic chemicals from 12 to 24 months of age (range $r_p = 0.49–1.00$) (unpublished data). Time-varying confounding and measurement error, in addition to high intra-individual correlations, will likely limit the power to detect windows of susceptibility.

More broadly, future methodological developments might focus on combining penalised regression and other variable selection approaches with other epidemiological estimation tools, such as models for longitudinal modelling; models for multiple time-varying covariates (e.g., joint marginal structural models[105]); models for decomposing the effect of a mediator into the proportion due to mediation versus interaction (i.e., four-way decomposition[106]); causal-modelling approaches (e.g., Mendelian randomisation and non-genetic instrumental variable analysis, negative control studies, within-sibship studies[107]); and models for non-linear associations [e.g., smoothing splines; highly relevant due to the observed phenomena of sub-linear thresholds, supra-linear plateaus, and non-monotonic (inverted) U-shaped dose–response curves attributable to saturation of biological pathways, cytotoxicity, receptor competition, negative feedback loops, etc.[108]].

In Chapter 3, where we investigated associations between multiple exposures and multiple outcomes, we did not attempt to delineate associations between the multiple exposures and the moderately correlated outcomes. We did not pursue structural equation modelling (SEM), or PLS-SEM for this purpose, as these models require strong statistical assumptions about correct model specification between all covariates, which are often not met, especially with cross-sectional data, and can lead to more severe biases than traditional regression or mediation analyses; in particular, incorrect specification of directionality and unmeasured confounding can lead to severe biases.[109]

Data processing and scaling. A standard step in penalised regression modelling is to mean-centre and scale variables, often by 1 standard deviation (SD; i.e., unit variance scaling or *z*-scores). Scaling ensures that variables with different dispersion have the same prior probability of selection in variable selection models based on penalisation. Scaling by 2 SD (as performed in Chapters 4–5) was advocated by Gelman[110] to improve comparability between continuous and binary variables, as regression coefficients for both would be on a common scale (with SD = 0.5). This has also been applied by Braun et al.[111] to simultaneously assess

chemicals with a high detection rate as continuous variables and chemicals with a low detect rate as binary (detectable vs. non-detectable) variables in a Bayesian hierarchical variable selection model. However, in a simulation study, scaling by 2 SD exhibited greater differential selection for unbalanced binary variables versus continuous and balanced variables compared to 1 SD scaling, whereby unbalanced variables were less likely to be selected.[81] An alternative, scaling by the interquartile range (by subtracting the median and dividing by the interquartile range) would be less sensitive to outliers than scaling by 1 or 2 standard deviations and may improve transportability of results.

We log-transformed chemical exposure data, which was right-skewed. Log-transformation can reduce heteroscedasticity, reduce the impact of extreme values, and furthermore, it has a pseudo-scaling effect in that differences between large and small values are diminished.[112] In the sPLS-regression analysis (Chapter 3) we only log-transformed variables; in comparison, scaling subsequent to log-transformation yielded slightly different selection results for only one of the 22 sPLSR models, whereas scaling was more influential in selection in penalised regression models, as expected. Log transformation has the well-known consequence of converting additive relationships into multiplicative associations. These data processing steps have implications for selection performance and the shape of exposure–outcome associations, and deserve further attention.

Measurement error. The signal-to-noise ratio is another aspect of the data structure that affects the performance of variable selection methods (not evaluated in the simulation study, Chapter 6). It is well-documented that many non-persistent environmental chemicals exhibit large within-subject temporal variability,[113,114] and substantial variability has also been observed for gut microbiota during early-life succession and in adulthood, although generally more moderate in magnitude barring interventions such as antibiotic-induced depletion.[115] Analyses of exposure–outcome associations based on only one biological sample for a chemical with an intraclass correlation coefficient of 0.2, such as some phthalates, led to a more than 70% attenuation bias in effect estimates in a simulation study.[116] Analytical variability can be relatively large for environmental chemicals as their concentrations are a 1000 times lower than those of many endogenous chemicals, food chemicals, and drugs.[117] Differences in the relative magnitude of measurement error lead to large differences in the *a priori* probability of detecting associations in multi-pollutant modelling.

Moreover, measurement errors are often positively correlated for chemical biomarkers, particularly within chemical classes, because of for instance similar pharmacokinetics, and this may be exacerbated by shared sample handling and analytical processes.[118] Non-differential correlated measurement error generally attenuates effect estimates toward the null, although Pollack et al.[119] demonstrated that in certain simulation scenarios, including scenarios with positively correlated biomarkers and negatively correlated measurement error, estimates were biased away from null; bias was negligible for truly null associations,

indicating that false positives were not induced by correlated measurement error.

Exposure–outcome estimates could be corrected post-selection using measurement error models [e.g., regression calibration and simulation extrapolation (SIMEX)]. These models have been little used, and have the caveat that they reduce precision in estimates (i.e., bias-variance trade-off), and can magnify confounding bias.[116,120] One possibility worth exploring would be to compare selection results for penalised models in which chemical exposure variables have been weighted by their noise (or the priors adapted in a Bayesian framework), perhaps by a hybrid of analytical variability and biological variability (e.g., laboratory coefficient of variation and the intra-class correlation coefficient from studies of repeated sampling).

Unmeasured confounding and model misspecification. An obvious limitation of the studies in this dissertation is that many exposures were not measured, some of which are likely correlated with the exposures we did consider. If an exposure is detected which is not causative but is highly correlated with a truly associated exposure, this can be informative with respect to revealing the causal structure.[86] Agier et al.[76] proposed alternative sensitivity and alternative FDP metrics which account for this partial agreement. However, exposure-wide association studies are susceptible to numerous sources of bias due to unmeasured confounding and model misspecification.[121] Assuming that some of the unmeasured exposures are correlated with the measured exposures and were causally associated with the health outcomes under study, our effect estimates would suffer from confounding (i.e., omitted-variable bias) (see Figure 1). Spurious associations can also be introduced in multiple-exposure modelling with unmeasured common causes (confounders) due to collider stratification bias.[122] Adjusting for a fixed set of potential confounders in multi-pollutant models implies that we are at best estimating direct effects, rather than the total effect of each exposure on the outcome.

In addition to bias and loss of precision due to model misspecification, it is likely that our models suffered to some degree of residual confounding due to unmeasured or mismeasured covariates (e.g., including education alone as a proxy of socioeconomic status). Pooling birth cohorts from different source populations, as in the INUENDO study (Chapters 2–4) and as applied in the European ENRIECO (Environmental Health Risks in European Birth Cohorts) and US ECHO (Environmental influences on Child Health Outcomes) consortia, can greatly enhance the study sample size and statistical power. However, as Rothman et al.[124] argue, representative sampling (e.g., of European populations) can in fact detract from drawing causal inferences due to threats to internal validity. We observed moderately strong country effects and heterogeneity for some of the associations in the analyses based on the INUENDO cohorts. This reinforces the importance of controlling for confounders in variable selection modelling in a pooled analysis. We performed confounder-adjustment either by pre-standardising exposure and outcome variables in sPLS-regression or by forcing unpenalised entry in elastic net regression. To limit potential residual confounding (or ecological)

bias, some studies perform a meta-analysis instead of pooled analysis, which generally results in a loss in precision.

A limitation of penalised regression and generally other data-driven variable selection methods is that they are unable to discriminate between confounders and other potential bias-inducing variables, namely colliders, intermediate, and instrumental variables. In our models, we conditioned on the variables we considered confounders based on background knowledge. However, as is essentially always the case, we were uncertain about the correct model specification. That we often expect the magnitude of associations between the causal exposure(s) of interest and the outcome to be quite small in environmental epidemiology—smaller than covariate–exposure and covariate–outcome associations—further complicates model interpretation. Attempting to characterise the magnitude of bias and loss of precision due to model misspecification is exceedingly daunting considering the complexity and number of unknowns for the chemical exposome.

In most scenarios, bias from omitting a (co-exposure) confounder is greater than bias induced by adjustment for an irrelevant variable or an indirect collider.[125] Notably, omitting a covariate can lead to both confounding bias and bias due to non-collapsibility

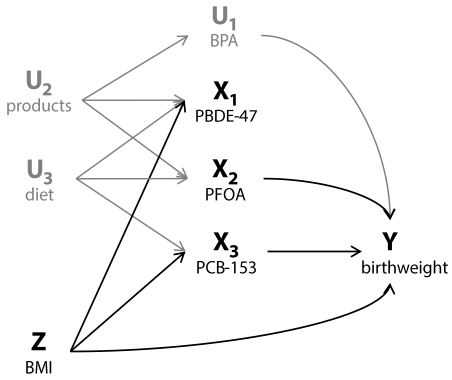

Figure 1. Directed acyclic graph of a theoretical set of relationships between multiple exposures (X_i), an outcome (Y), a measured confounding covariate (Z), and unmeasured covariates (U_i) to illustrate the potential ramifications of model misspecification in multi-pollutant modelling. X_i–Y represent the causal effects of interest; we are interested in estimating the effects of X_{1-3} on Y. X_i are often biomarker proxies for doses at the target tissues. The unmeasured covariates (U_2 and U_3) represent shared sources of the exposures, and U_1 an unmeasured exposure, correlated with X_{1-2}. A lack of an arrow indicates that the association is assumed absent. To obtain adjusted direct effect estimates for X_{1-3}–Y associations, we use a multi-pollutant variable selection method (e.g., elastic net penalised regression). Failure to adjust for U_1 (an unmeasured exposure of causal interest) introduces confounding bias of the X_i–Y associations. Adjustment for X_1, which is not causally associated with Y (for example, upon selection in a penalised regression model), introduces bias to X_{2-3}–Y paths (due to collider stratification, e.g., $X_2 \leftarrow U_3 \rightarrow [X_1] \leftarrow U_2 \rightarrow U_1 \rightarrow Y$). Adjustment for Z will remove bias from the X_3–Y path but will amplify the bias in the X_2–Y association ($X_2 \leftarrow U_3 \rightarrow [X_3] \leftarrow Z \rightarrow Y$). Thus multi-pollutant modelling will result in a trade-off between confounding bias reduction and bias amplification (and gains and losses in precision), determined partly by the size of specific associations and covariances between covariates, and collapsibility (whether adjustment alters the effect estimate, which differs for effect measures from linear and non-linear models).[122,123] This simplistic example ignores potential mediators, the potential time-varying nature of covariates, and measurement error, which further complicate statistical inference.

(when the unadjusted marginal effect estimate is not equivalent to the weighted-average of the covariate-stratified conditional effect estimates). Logistic regression models and other non-linear models are more susceptible to non-collapsibility, especially when the outcome is common (a reason why the change-in-estimate criterion for confounder selection is fallible for non-collapsible effect estimates).[126]

Generally, when unmeasured confounders are positively correlated with measured (co-exposure) confounders (e.g., a recently manufactured chemical which accumulates in lipid-rich foods like some legacy chemical exposures), increasing confounding bias with increasing confounder correlations will be offset by improved indirect partial adjustment; in the unlikely scenario where unmeasured confounders are completely uncorrelated with the measured co-exposure confounders (e.g., a chemical group with a completely novel exposure route), this unmeasured confounding can amplify bias considerably.[127,128] The direction and magnitude of the cumulative bias due to multiple unmeasured or mismeasured confounders is a complex function of the confounder measurement errors, prevalences or variances, covariance, and the size and direction of the confounder–exposure/outcome associations.[127,128]

Adjusting for a covariate only associated with the exposure (or an instrumental variable, only associated with the outcome through the exposure) will generally increase both bias and variance of effect estimates, whereas adjusting for a (non-confounding) determinant of the outcome will increase precision in linear regression but lead to a loss of precision in logistic regression.[129] When the strength of the association between the covariate and the exposure is considerably stronger than the strength of the association between the covariate and the outcome (a near instrumental variable), inclusion of the covariate will usually result in greater confounding bias reduction than bias amplification in a single-covariate scenario;[130] however, with multiple potential (co-exposure) confounders, bias amplification can accumulate at a greater rate than confounding bias reduction.[131] Thus, in our multi-pollutant models, we are more concerned about the potential bias arising from missing (co-exposure) confounders which are more strongly associated with the outcome than those which are more strongly associated with the measured exposures.

Alternative approaches

In **Chapter 6** the performance of several statistical modelling approaches for case-control studies was compared, with respect to selection accuracy and bias of effect estimates, across an extensive set of low-dimensional data scenarios in a simulation study. First, we confirmed that traditional univariable and multivariable logistic regression modelling approaches suffered from multicollinearity—bias and numerical instability, and poor sensitivity and elevated false positive findings—especially in the scenarios with correlated exposure data. Second, we observed that variable selection approaches were not a panacea, but that overall, penalised regression was attractive in that it achieved balanced sensitivity:FDP selection and minimally biased effect estimates. sPLS-DA performed nearly as well; however, disadvantages of this

method are that selection was quite sensitive to the model optimisation strategy, and coefficients for components are dimensionless, limiting their generalisability.

A plethora of statistical methods are available for evaluating associations with multiple-exposures, and many are still undergoing development. Hierarchical Bayesian modelling[132] (with e.g., a mixture of priors on exposures, such as a Dirichlet process prior, coupled with a variable selection prior) and Bayesian model averaging have been advocated as suitable methods[75,133,134] and have been applied in several environmental epidemiological studies.[96,135,136] Selection can be sensitive to the choice of prior distributions and hyper-parameters, potentially rendering them less accessible; using informative priors which assign a higher probability to likely confounders will improve their utility.

One attractive method is Bayesian kernel regression machine learning (BKMR), an exposure–response surface estimation method recently adapted by Bobb et al.[137] for evaluating the health effects of mixtures of pollutants. Deviations from additivity can be deduced from isoboles, or contours of the multidimensional exposure–response surfaces.[138] A hierarchical version of BKMR allows for pre-specifying groups of exposures, and uses a spike-and-slab prior to perform selection within groups. BKMR generates plots which help the analyst explore not only effect estimates for multiple-exposures, but also interactions, non-linearity, and the overall cumulative effect estimate. The method is currently limited to linear regression models, does not allow for data-driven grouping, requires the analyst to make statistical inference decisions about what level of posterior probability is noteworthy, and it would be worthwhile to test how sensitive the method's performance is to the tuning parameters. BKMR appears to be designed for analyses of a low to moderate number of exposures, and its applicability for 10s to 100s of exposures requires further validation. Nevertheless, it appears to be a user-friendly tool to explore low dimensional multi-pollutant–outcome investigations. Applying BKMR to the data on chemicals and birth weight from Chapter 4 yielded largely the same selection, and the model showed a clear overall negative association between all the chemicals and birth weight.

Interaction and cumulative effects. We focused primarily on estimating independent exposure–outcome associations, and examined interactions in secondary analyses. The statistical power to evaluate interactions is intractable for the analysis of all-way interactions for the typical cohort sizes with measured environmental chemical data, and power may be limited to detect more than a handful of two-way interactions. Nonetheless, in a simulation study[139] based on a matrix of 237 environmental exposures and linear regression modelling, for a low number of exposure–outcome associations and scenarios with up to two two-way interactions, a form of penalised regression (based on the group-lasso[140]) and an iterative model search algorithm (deletion/substitution/addition[141]) were shown to outperform boosted regression trees, a method previously applied and promoted as a strategy to identify interactions.[142] The performance of tree-based methods is expected to be relatively better in logistic models.

It may be desirable to focus more on cumulative effects in some settings.[143] Instead of focusing on estimating the independent exposures, another approach is to identify and estimate the effects of profiles or mixtures. One modelling strategy is weighted quantile sum regression, which estimates a body burden index, and quantifies which subset of exposures contributes most to the weighted sum of standardised exposures.[144] This assumes additive effects and may be most worthwhile when components within the mixture act via common biological pathways, or when the component represents an identifiable group of exposures that can be targeted for exposure reduction strategies. Statistical models will always be limited in their power to delineate the independent effects of very highly correlated exposures. Toxicological studies that experiment with different combinations of exposure mixtures might be the only solution if obtaining separate effect estimates (for a feasibly small set of exposures) is deemed necessary.

Implications

The variable selection methods we applied in this dissertation, and more broadly multi-pollutant models which account for co-occurring exposures, improve hazard identification and exposure–outcome estimation, and ultimately offer higher quality evidence for risk assessments. As others have noted,[76,143] the implications of incorrectly identifying an exposure–outcome association that is a false positive, yet highly correlated with a true positive, could still lead to effective policies that reduce human exposure given that highly correlated exposures likely share an exposure source and/or route.

Recent developments that are improving risk assessment of the chemical space include a public database on production and usage of >43 000 chemicals, which can be used for high throughput exposure modelling to predict human exposures,[145] and high throughput bioactivity assays and computational screening,[146] both enabling more informed prioritisation for risk assessment; and efforts to more systematically evaluate and integrate evidence from toxicology and epidemiology, and report on the weight of the evidence, in environmental health and specifically chemical risk assessment.[147] Also promising are efforts to replace hazardous or phase-out chemicals with fundamentally different structures, rather than structurally similar chemicals, and green chemistry initiatives (e.g., development of halogen-free firefighting foam).

Conclusions

In this dissertation, we applied state-of-the-art statistical methods to identify associations between chemicals and several health outcomes. Given the complex nature of environmental chemical exposures, epidemiologists and data analysts may need to increasingly engage with statistical methods for multi-pollutant modelling. In this chapter, various sources of bias and imprecision are discussed. The challenges specific to chemical exposome assessment, and the frequently small magnitude of effect estimates, may render epidemiological studies of

environmental chemical risks more susceptible to bias and complicated inference than for instance some clinical or occupational research scenarios with stronger main effects and weaker associations with confounders. Importantly, further research is necessary to refine strategies for evaluating the health risks of real-world exposure mixtures. Advancing multi-pollutant modelling will not negate the importance of subject matter knowledge necessary for etiologic inference.[148] We identified several classes of common environmental chemicals that were associated with adverse reproductive health outcomes and respiratory health outcomes. Many specific exposure–outcome associations in this dissertation require replication before robust inferences can be drawn about effects, and more broadly, more systematic assessments of our multi-pollutant burden are imperative to protect human health.

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Appendices

Summary

Samenvatting

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Publication list

Acknowledgements

Curriculum vitae

Summary

Chemicals are integral to many processes in modern society. Although they contribute to well-being and prosperity, some have been shown to be harmful to human health and the vast majority have not undergone adequate safety testing. Synthetic chemicals and naturally occurring metals are released due to human activities, and are distributed worldwide in the environment and in food webs. Many are also embedded in ordinary building materials and products. Humans are invariably exposed to low levels of these pollutants (i.e., environmental chemicals), predominantly through diet, and indoor dust and air. The aim of environmental epidemiology is to identify environmental exposures that causally affect human health and the degree to which they do so. Epidemiological results can inform risk assessments and strategies to prevent or reduce exposure to toxic agents, and thereby protect public health. Environmental epidemiological studies, and more broadly chemical risk assessments, have traditionally investigated a single exposure in isolation, on a chemical-by-chemical basis. Because many sets of environmental chemicals share a similar emission history or common exposure sources, exposures are often highly correlated within populations and within periods of time (i.e., age-period cohorts). Assessments of single exposure–outcome associations lead to inflated false positive discoveries, where an association may be misattributed to a correlated but non-causally related co-exposure, and to less accurate effect estimation due to confounding by co-exposures (**Chapter 1**). Assessments of associations between multiple exposures and an outcome using multivariable regression may lead to estimation problems arising from collinearity, namely unstable and imprecise health effect estimates, particularly as study samples are frequently modest in size.

A new generation of variable selection models has been proffered for modelling of collinear data, and to better identify and quantify individual exposure–outcome associations compared to traditional single-exposure regression models. Variable selection models were applied in this dissertation to investigate multiple common environmental chemical exposures and their potential effects on aspects of fetal, child, and reproductive health. These case studies encompassed a variety of study designs and data structures. The usefulness of several variable selection methods for multi-pollutant modelling was also systematically evaluated.

Case studies

Characterising exposure is an essential component of environmental epidemiological research and, ultimately, risk management. We describe the serum levels of brominated flame retardants—tri-to hexa- polybrominated diphenyl ether (PBDE) congeners and the most abundant brominated biphenyl (BB-153)—in 299 men of reproductive age from Greenland, Poland, and Ukraine (**Chapter 2**). The study populations were sampled in 2002 to 2004, prior to the phase out of PBDE commercial formulations in Europe and the United States. This study represents the first moderately sized biomonitoring surveys in these European countries and

the territory of Greenland. The men from Greenland had substantially higher serum levels of ∑PBDEs and BB-153 than the men from Poland or Ukraine, and lower concentrations than observed in other studies of US populations. We did not identify determinants that explained an appreciable amount of variance in serum levels, beyond geographic location, although data was available for only a limited set of potential determinants and the study power was modest. The study populations exhibited unique congener profiles. These population-specific profiles were also observed for the full set of chemicals assessed in the epidemiological studies in this dissertation. The existence of these profiles, and their correlated structures, are indicative of the need for multi-pollutant approaches. Methods such as penalised regression (e.g., elastic net) and dimension reduction (e.g., partial least squares (PLS) regression) modelling can better handle highly correlated exposures, and can be used to identify single exposures or clusters of exposures that drive risk.

There is strong evidence from experimental and occupational studies that exposure to certain chemicals contributes to the occurrence of male reproductive disorders, including diminished fecundity, i.e., the biological capacity to reproduce; however, the risks associated with low-level environmental exposures are poorly characterised or unknown for most widely used chemicals. In a cross-sectional study among 602 men from Greenland, Poland, and Ukraine, who had recently fathered a pregnancy, we evaluated associations between four chemical classes and markers of male reproductive function (**Chapter 3**). The study participants were recruited during antenatal care visits. The investigated exposures were metabolites of di(2-ethylhexyl) and diisononyl phthalates (DEHP, DiNP), perfluoroalkyl acids, metals, and organochlorine pesticides, and the investigated health outcomes were reproductive hormone levels, semen quality parameters, markers of sperm DNA damage and apoptosis, markers of epididymal and accessory sex gland function, and the ratio of Y- to X-chromosome–bearing sperm. We used sparse PLS regression modelling to analyse, simultaneously, the 15 exposure biomarkers in relation to each of the 22 outcomes. We identified 10 associations out of the 330 exposure–outcome associations assessed. Eight associations were indicative of deleterious effects, including robust associations between DiNP and decreasing testosterone, and PCB-153 and a decreasing proportion of progressively motile sperm. Two associations were indicative of potentially beneficial effects.

Exposure to environmental stressors before and during pregnancy can lead to a suboptimal intrauterine environment and impaired fetal development. Reduced birth weight, a marker of fetal growth, is associated with increased susceptibility to chronic diseases later in life. We used elastic net penalised regression to evaluate associations between prenatal exposure to three classes of environmental chemicals and birth weight in 1250 singleton infants born at term in study populations from Greenland, Poland, and Ukraine (**Chapter 4**). We identified associations within each of the three chemical classes, of which four were most robust; a DEHP metabolite (MEHHP), PFOA, and *p,p*'-DDE were associated with lower birth weight, and a DiNP metabolite (MOiNP) was associated with higher birth weight. The mag-

nitude of the effect estimates, scaled to the observed variability in exposure levels, was on par with those for established predictors of birth weight, such as multiparity, and approximately half that observed for cotinine levels, a biomarker of smoking.

Exposure in early-life to certain environmental chemicals can compromise the developing immune system, and may subsequently lead to an increased risk of respiratory and allergic disorders. Suggestive evidence of an increased prevalence of asthma in children treated with antibiotics in infancy, or born via Cesarean delivery as opposed to vaginal delivery, has led to the hypothesis that perturbations in commensal gut microbiota composition and function may also play an etiologic role. Gut microbiota and metabolites such as short-chain fatty acids (SCFAs) influence immune system maturation, and stimulate immune responses. We assessed associations between early-life exposures to four classes of environmental chemicals and asthma at 2 years of age and later in childhood, and the occurrence of a lower respiratory tract infection (LRTI) by 2 years of age in a Norwegian birth cohort of 993 mother–child pairs. We also evaluated the independent and potential mediating effects of microbial diversity and SCFAs measured at multiple time points in the first 2 years of life (**Chapter 5**). This study is one of the first epidemiological studies to evaluate the interplay between chemical exposures and gut microbiota in relation to health outcomes. An organochlorine pesticide was associated with a higher risk of registry-based asthma at around 10 years of age and maternal-reported asthma at 2 years of age, and several PCBs were associated with both higher and lower risks. Associations with a maternal-reported LRTI by 2 years of age were less precise. There was limited evidence that early-life chemical exposures perturbed microbial diversity or production of SCFAs, except for an association between ∑PCBs and decreased diversity at 2 years of age. Associations between microbial diversity and SCFAs at multiple ages and the outcomes, asthma and LRTI, were generally inconsistent and imprecise. There was no clear evidence that microbial diversity or metabolites mediated chemical–respiratory outcome associations.

Systematic comparison of variable selection methods

To complement the observations from the case studies described above, we conducted a simulation study to compare the performance of variable selection methods with more traditional regression-based approaches for evaluating the health effects of mixtures of exposures in case-control studies (**Chapter 6**). The performance of sparse PLS discriminant analysis, frequentist and Bayesian penalisation, boosting, and univariable, multivariable, and stepwise logistic regression was tested in 270 different simulation scenarios, representing data structures typical of environmental and occupational epidemiological studies. This study reinforced well-established theory, perhaps underappreciated in practice, that in the presence of correlated variables (ρ = 0.4 or 0.8), univariable regression yields a high proportion of false positive associations even with correction for multiple testing, whereas multivariable regression yields low sensitivity, and both methods yield an unfavourably high mean squared

error—a combination of bias and variance of effect estimates across simulations. This poor performance was exacerbated in scenarios with smaller sample sizes and relatively smaller effect sizes. Elastic net and lasso penalised logistic regression outperformed the other methods with respect to selection accuracy—also when sensitivity was weighted as twice or half as important as a low false discovery proportion—and the mean squared error values were competitively low.

In conclusion, application of modern variable selection methods in a varied set of epidemiological studies in this dissertation demonstrates how variable selection models can be used for more accurate hazard identification and characterisation. Nevertheless, there are theoretical limitations and practical challenges to multi-pollutant (chemical mixture) modelling approaches, underscoring the importance of continued methodological advancements in this emerging and rapidly evolving facet of environmental health science (**Chapter 7**). Furthermore, this dissertation supports that several environmental chemicals have an adverse impact on various dimensions of reproductive health. There was suggestive evidence for both positive and negative associations between chemical exposures and the risk of asthma. There was limited evidence that gut microbiota and short-chain fatty acid metabolites were perturbed by chemical exposures, or were associated with respiratory morbidity. The small to modest magnitude of effect estimates observed in these studies does not preclude an important population health burden given the ubiquity of the exposures and high prevalence of health conditions. Further research is warranted to bolster the evidence base for specific exposure–outcome associations, to integrate the internal and external exposome in epidemiological studies and elucidate underlying causal processes, and to more effectively assess the health risks of the complete chemical landscape.

Samenvatting

Chemische stoffen vormen een integraal onderdeel van veel processen in de moderne samenleving. Hoewel ze bijdragen aan welzijn en welvaart, blijken sommige stoffen schadelijk voor de menselijke gezondheid. Bovendien is het merendeel van de chemische stoffen niet getest op veiligheid. Synthetische chemische stoffen en natuurlijk voorkomende metalen kunnen vrijkomen als gevolg van menselijke activiteiten en worden hierdoor wereldwijd verspreid in het milieu en in voedselketens. Vele chemische stoffen zijn ook ingebed in bouwmaterialen en andere producten. Mensen worden voortdurend blootgesteld aan lage concentraties van deze verontreinigende stoffen (de zogenaamde milieuchemicaliën), via de voeding, door stof binnenshuis en via de lucht. Het doel van de milieuepidemiologie is het identificeren van milieublootstellingen die de gezondheid van de mens beinvloeden en het karakteriseren van de mate waarin zij dat doen. De resultaten van epidemiologische studies kunnen risicobeoordelingen en strategieën om de blootstelling aan toxische stoffen te voorkomen of te verminderen informeren en daarmee bijdragen aan de bescherming van de volksgezondheid. Traditioneel zijn milieuepidemiologische studies en risicobeoordelingen van chemische stoffen gebaseerd op één of een handvol chemische stoffen. Omdat veel chemische stoffen een vergelijkbare emissiegeschiedenis of een gemeenschappelijke blootstellingsbron delen, blijken blootstellingen vaak sterk gecorreleerd binnen een bepaalde populatie of binnen een bepaalde tijdsperiode (en dus ook binnen cohorten van leeftijdsgroepen). Hierdoor leidt een voor elke chemische stof afzonderlijke evaluatie van mogelijke gezondheids-associaties tot een vergrote kans op vals positieve bevindingen, waarbij een gevonden associatie ten onrechte wordt toegekend aan een gecorreleerde blootstelling die niet zelf causaal gerelateerd is aan de gezondheidsuitkomst. Bovendien kunnen correlaties er voor zorgen dat schattingen van de sterkte van een effect veel minder nauwkeurig worden (**hoofdstuk 1**). Evaluaties van associaties tussen meerdere blootstellingen en een uitkomst met behulp van multivariabele regressie kunnen, als gevolg van collineariteit, leiden tot onstabiele en onnauwkeurige schattingen, zeker omdat het aantal observaties in milieuepidemiologische studies vaak bescheiden is.

Er is een nieuwe generatie variabele-selectie modellen beschikbaar voor het modelleren van collineaire data. In vergelijking met traditionele enkelvoudige regressiemodellen zijn deze modellen beter in staat om individuele associaties met gezondheidsuitkomsten te identificeren en te kwantificeren. In dit proefschrift werden een aantal variabele-selectie modellen toegepast om de effecten van veelvuldig voorkomende chemische milieublootstellingen op de voortplanting en foetale en kindergezondheid te onderzoeken. De case studies omvatten een verscheidenheid aan studieontwerpen en datastructuren. De toepasbaarheid van de verschillende variabele-selectie methoden werd ook systematisch geëvalueerd.

Case studies

Karakterisering van blootstelling is een essentieel onderdeel van milieuepidemiologisch onderzoek en uiteindelijk risicomanagement. In hoofdstuk 2 wordt het serumgehalte van gebromeerde vlamvertragers, tri-tot hexa-polybromineerde difenylether (PBDE) congeners en het meest voorkomende gebromeerde bifenyl (BB-153), beschreven bij 299 mannen in de vruchtbare leeftijd afkomstig uit Groenland, Polen en Oekraïne (**hoofdstuk 2**). De studiepopulaties werden in de periode 2002 tot 2004 bemonsterd, voorafgaand aan het uitfaseren van PBDE bevattende commerciële mengsels in Europa en de VS. Dit is de eerste redelijk grote biomonitoring studie in deze Europese landen en Groenland. Mannen uit Groenland hadden aanzienlijk hogere serumniveaus aan ΣPBDEs en BB-153 dan mannen uit Polen of Oekraïne en lagere concentraties dan waargenomen in studies onder Amerikaanse populaties. Met uitzondering van geografische locatie hebben we geen determinanten kunnen achterhalen waarmee de variantie in serumniveaus verklaard zou kunnen worden. Gegevens waren echter alleen beschikbaar voor een beperkt aantal potentiële determinanten en de grootte van de studie was beperkt. De studiepopulaties vertoonden unieke congener-profielen. Deze populatie-specifieke profielen werden ook gezien voor andere chemicaliën die werden beoordeeld als onderdeel van de epidemiologische studies in dit proefschrift. Het bestaan van deze profielen, en de gecorreleerde structuren, illustreren de behoefte aan benaderingen die rekening houden met de aanwezigheid van meerdere verontreinigende stoffen. Methoden zoals gepenaliseerde regressie (bijvoorbeeld *Elastic Net*) en dimensiereductie (bijvoorbeeld *Partial Least Squares* regressie) kunnen beter overweg met hoog gecorreleerde blootstellingen en kunnen worden gebruikt om individuele blootstellingen, of clusters van blootstellingen, te identificeren die een effect op de gezondheid hebben.

Resultaten van experimenteel onderzoek en onderzoek naar beroepsmatige blootstellingen leveren sterke aanwijzingen dat blootstelling aan bepaalde chemicaliën bijdraagt aan mannelijke vruchtbaarheidsstoornissen, zoals een verminderde fecunditeit (de biologische capaciteit om te reproduceren). De risico's die verband houden met lage milieublootstellingen zijn voor de meest gebruikte chemicaliën echter slecht gekarakteriseerd of zelfs geheel onbekend. In een dwarsdoorsnede studie onder 602 mannen uit Groenland, Polen en Oekraïne die onlangs vader waren geworden, hebben we associaties geëvalueerd tussen stoffen in vier chemische klassen en indicatoren van de mannelijke voortplantingsfunctie (**hoofdstuk 3**). De deelnemers aan de studie werden geworven tijdens een bezoek aan de antenatale zorg. De onderzochte blootstellingen waren metabolieten van di (2-ethylhexyl) en diisononylftalaten (DEHP, DiNP), perfluoralkylzuren, metalen en organochloorpesticiden. Onderzochte gezondheidsparameters waren de hoeveelheid reproductie- hormoon, spermakwaliteit, beschadigingen aan het DNA of apoptose van sperma, indicatoren van de epididymale en bijbehorende geslachtsklierfunctie, en de verhouding tussen Y- en X-chromosoom dragend sperma. We hebben *Sparse Partial Least Squares* regressie modellen gebruikt om 15 blootstellings-indicatoren gelijktijdig te analyseren in relatie tot elk van de 22 uitkomsten. We

hebben 10 associaties geïdentificeerd uit het totaal van 330 mogelijke blootstelling-uitkomst associaties die werden beoordeeld. Acht associaties waren indicatief voor schadelijke effecten, waaronder robuuste associaties tussen DiNP en verlaagd testosteron, en tussen PCB-153 en een afnemende proportie van progressief motiel sperma. Twee associaties waren indicatief voor mogelijke gunstige effecten.

Blootstelling aan milieustressoren voor en tijdens de zwangerschap kan leiden tot een suboptimaal baarmoeder-milieu en verminderde foetale ontwikkeling. Verminderd geboortegewicht, een indicator van foetale groei, is geassocieerd met een verhoogde kans op chronische ziekten later in het leven. Met behulp van *Elastic Net* gepenaliseerde regressie hebben we associaties tussen prenatale blootstelling aan drie klassen milieuchemicalien en geboortegewicht bestudeerd bij 1250 eenling-zuigelingen, geboren na een normale zwangerschapsduur in studiepopulaties afkomstig uit Groenland, Polen en Oekraïne (**hoofdstuk 4**). We hebben associaties geïdentificeerd binnen elk van de drie chemische klassen, waarvan vier de meest robuust waren; een DEHP metaboliet (MEHHP), PFOA en *p,p'*-DDE waren geassocieerd met lager geboortegewicht en een DiNP metaboliet (MOiNP) was geassocieerd met hoger geboortegewicht. De omvang van de effectschattingen, geschaald naar de waargenomen variabiliteit in de blootstellingsniveaus, waren vergelijkbaar met die van reeds bekende voorspellers van geboortegewicht, zoals multipariteit, en ongeveer de helft van schattingen van het effect van cotinine niveaus, een kwantitatieve indicator voor het rookgedrag.

Blootstelling aan bepaalde milieuchemicaliën tijdens de kinderjaren kan de ontwikkeling van het immuunsysteem in gevaar brengen, en als gevolg daarvan leiden tot een verhoogd risico op ademhalings- en allergische stoornissen. Aanwijzingen voor een verhoogd voorkomen van astma bij kinderen die behandeld zijn met antibiotica, of die geboren zijn via een keizersnede in plaats van een vaginale geboorte, hebben geleid tot de hypothese dat verstoringen in de samenstelling en functie van de darmflora ook een rol kunnen spelen bij het ontstaan van ademhalings- en allergische stoornissen. De darmflora en metabolieten zoals korte-keten vetzuren (KKVZ) beïnvloeden de ontwikkeling van het immuunsysteem en stimuleren de immuunrespons. We hebben associaties geevalueerd tussen de vroegtijdige blootstelling aan vier klassen milieuchemicaliën en het voorkomen van astma bij kinderen van 2 jaar en ouder en een lagere luchtweginfectieop een leeftijd van 2 jaar in een Noors geboortecohort van 993 moeder-kind paren. We hebben ook de onafhankelijke en potentiële mediatie effecten geëvalueerd van microbiële diversiteit en KKVZ, gemeten op meerdere tijdspunten gedurende de eerste 2 levensjaren (**hoofdstuk 5**). Deze studie is één van de eerste epidemiologische studies die de wisselwerking tussen chemische blootstellingen en darmmicrobiota in relatie tot gezondheidsresultaten heeft geevalueerd. Een organische gechloreerde pesticide was geassocieerd met een hoger risico op asthma op leeftijd 2 en 10, en verschillende PCB's waren geassocieerd met hogere en lagere risico's. Associaties met een door de moeder gerapporteerde lagere luchtweginfectie bij het kind konden niet nauwkeurig worden vastgesteld. Er was beperkt bewijs dat chemische blootstelling gedurende de kinderjaren de

microbiële diversiteit of de productie van KKVZ kan verstoren. Wel vonden we een associatie tussen ΣPCB's en verminderde microbiële diversiteit bij leeftijd 2 jaar. Associaties tussen microbiële diversiteit en KKVZ voor meerdere leeftijden en de uitkomsten astma en lagere luchtweginfectie waren over het algemeen niet consistent en onnauwkeurig. Er waren geen duidelijke aanwijzingen dat de associaties tussen chemische blootstellingen en luchtweggezondheidsuitkomsten gemedieerd werden door microbiële diversiteit of metabolieten.

Systematische vergelijking van variabel selectiemethoden

Om de waarnemingen uit de bovenstaande case studies aan te vullen, hebben we een simulatiestudie uitgevoerd om de prestatie van variabele-selectie methoden te vergelijken met meer traditionele benaderingen in het kader van de evaluatie van de gezondheidseffecten van blootstellingsmengsels in case-control studies (**hoofdstuk 6**). De prestaties van *Sparse PLS discriminant analysis*, klassieke en Bayesiaanse penalisatie, *boosting* en univariabele, multivariabele en stapsgewijze logistische regressie werden getest in 270 verschillende simulatiescenario's, die gegevensstructuren vertegenwoordigen die typisch zijn voor milieu- en arbeids-epidemiologische studies. Deze studie bevestigt de verwachting op basis van theorie, die mogelijk in de praktijk wordt onderschat, dat in de aanwezigheid van gecorreleerde variabelen (ρ = 0,4 of 0,8) een univariabele regressie een hoog percentage vals positieve associaties oplevert, zelfs wanneer gecorrigeerd wordt voor kanskapitalisatie, terwijl multivariabele regressie een lage sensitiviteit heeft. Beide methoden hebben een hoge kwadratisch gemiddelde fout—een combinatie van vertekening en variantie van effectschattingen over de simulaties. Deze slechte prestatie werd verergerd in scenario's met kleinere steekproefgroottes en kleinere effecten. Gepenaliseerde methoden zoals *Elastic Net* en *Lasso* presteerden beter dan de andere methoden op het gebied van selectie-nauwkeurigheid, ook wanneer de sensitiviteit gewogen werd als tweemaal of half zo belangrijk als een lage proportie fout positieve bevindingen, en de kwadratisch gemiddelde fout was competitief laag.

Ter conclusie, de toepassing van moderne variabele-selectie methoden in een gevarieerde reeks epidemiologische studies in dit proefschrift laat zien hoe variabele-selectie modellen kunnen worden gebruikt om te komen tot een nauwkeurigere identificatie en karakterisering van gezondheids-gevaren als gevolg van blootstelling aan chemische stoffen. Niettemin zijn er theoretische beperkingen en praktische uitdagingen in het modelleren van de effecten van meervoudige blootstellingen (mengsels van chemische stoffen), die het belang van voortgezette methodologische vooruitgang onderstrepen in dit opkomende en snel evoluerende onderdeel van de milieuhygiëne (**hoofdstuk 7**). Bovendien ondersteunt dit proefschrift dat verschillende milieuchemicaliën een nadeligeinvloed hebben op verschillende dimensies van reproductieve gezondheid. Er was suggestief bewijs voor zowel positieve als negatieve associaties tussen chemische blootstellingen en het risico op astma. Er was beperkt bewijs dat darmflora en korte-keten vetzuurmetabolieten door chemische blootstellingen werden verstoord, of geassocieerd zijn met luchtwegaandoeningen. De kleine tot bescheiden

omvang van de effectschattingen die in deze studies werden waargenomen sluit niet uit dat er toch een belangrijke gezondheidsbelasting voor de bevolking is, gezien de alomtegenwoordigheid van de blootstellingen en de hoge prevalentie van de bestudeerde aandoeningen. Verder onderzoek is nodig om het bewijs te versterken voor de associaties tussen specifieke blootstellingen en gezondheidsuitkomsten, om het interne en externe exposoom te integreren in epidemiologische studies en de onderliggende causale processen te verhelderen en zo de gezondheidsrisico's van het volledige chemische landschap systematisch in kaart te brengen.

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Curriculum vitae

Virissa C. Lenters was born March 29, 1985, in Toronto, Canada. In 2006 she earned a Bachelor of Science (Honours) degree with Distinction in Environmental Life Sciences from Queen's University, Canada. In 2008 she completed a Master of Science in Epidemiology at Utrecht University, the Netherlands. The research for her Master's degree was performed at the Division of Environmental Epidemiology, Institute for Risk Assessment Sciences (IRAS), Utrecht University and the Department of Epidemiology, Julius Center for Health Sciences and Primary Care, University Medical Centre Utrecht. She studied air pollution and the risk of cardiovascular disease under the supervision of Dr. G. Hoek and Dr. C.S.P.M. Uiterwaal. After completing her Master's degree, she continued as a junior researcher at IRAS working on risk assessment of asbestos exposure and lung cancer. In 2009 she also began working on the research described in this dissertation, carried out as part of the European Union funded project Climate Change, Environmental Contaminants and Reproductive Health (CLEAR), under the supervision of Dr. R.C.H. Vermeulen, Dr. L. Portengen, Prof. D.J.J. Heederik, and Prof. A.H. Piersma. Starting in 2014, she coordinated the follow-up of a Dutch general population cohort and worked on an international study of the possible health effects of electromagnetic fields from mobile phones. In 2016 she joined Dr. M. Eggesbø's research group at the Norwegian Institute of Public Health in Oslo, Norway, to study the interplay between environmental chemical exposures and gut microbiota, and their relationship to child health.