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Association of persistent organic pollutants with sensorimotor neuropathy in participants with and without diabetes or prediabetes: results from the population-based KORA FF4 study

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Abstract:	ABSTRACT BACKGROUND. Exposure to persistent organic pollutants (POPs) has been associated with an increased type 2 diabetes (T2D) risk. It remains unclear whether POPs may also increase the risk of diabetes complications including neuropathy. We aimed to investigate the association of low-dose exposure to POPs with distal sensorimotor polyneuropathy (DSPN). METHODS. Our study was part of the second follow-up (FF4, 2013-2014, N = 2,279) of the population-based KORA S4 study (Augsburg, Germany). The study sample consisted of 200 participants, including four groups of 50 persons each with known T2D, prediabetes, newly diagnosed diabetes, and normal glucose tolerance (NGT) based on an oral glucose tolerance test. We analyzed the association of six most abundant serum concentrations of POPs with DSPN by multivariable logistic regression adjusted for age, sex, glycaemic status, body mass index, physical activity, smoking and alcohol consumption. We assessed effect modification by age, sex, glycaemic status and obesity. RESULTS. For all pollutants, the main models indicated no significant association of having DSPN. Two-pollutant models supported these findings, except for a lower odds ratio for the combination of polychlorinated biphenyl (PCB) 138 and beta-hexachlorocyclohexane (β-HCH) (OR: 0.59; 95% CI: 0.35 - 0.99). No effect modification was found by age, sex, glycaemic status and obesity. CONCLUSION. Low-dose exposure to POPs indicated no association with the odds of having DSPN in T2D, prediabetes and NGT.
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Submission of Manuscript:
"Association of persistent organic pollutants with sensorimotor neuropathy in participants with and without diabetes or prediabetes: results from the population-based KORA FF4 study"

Dear Editor,

We are pleased to submit the manuscript "Associations of persistent organic pollutants with sensorimotor neuropathy in participants with and without diabetes or prediabetes: results from the population-based KORA FF4 study" for consideration as Research Paper to Environment International.

Persistent organic pollutants (POPs) have been associated with several health endpoints like diabetes and diabetes related complications (Evangelou et al. Environ Int. 2016 May;91:60-8; Jaacks et al. Environ Int. 2015 Mar;76:57-70; Zonhg et al. Environ Int. 2018 May;114:334-342). Thereby, lipophilic chemicals like POPs are hypothesized to be related to neuropathies (Zeliger 2013. Interdiscip Toxicol. 2013 Sep;6(3):103-10) while, the risk of sensorimotor neuropathy is higher for people with diabetes (Pop-Busui et al. Diabetes Care. 2017 Jan;40(1)) and prediabetes (Herder et al. 2019. Trends Endocrinol Metab. 2019 May;30(5):286-298).

Our previous study, conducted in the same study region, found an increased chance of diabetes in association with two most abundant POPs (Wolf et al. Environ Int. 2019 Aug;129:221-228). The association between POPs and neuropathy has, however, only been investigated in only one epidemiological study so far (Lee et al. Diabetes. 2008 Nov;57(11):3108-11) which showed a higher prevalence of peripheral neuropathy among participants with type 2 diabetes compared to participants with impaired fasting glucose/prediabetes.

With this epidemiological work that is based on the well-characterized population-based KORA FF4 study, conducted in the south of Germany between 2013 and 2014, we aimed to fill this gap by examining the association between low-dose exposure to POPs and distal sensorimotor neuropathy. We observed no significant association between low-dose background levels of six most abundant POPs and the odds of having distal sensorimotor neuropathy. Effect modification analyses showed no significant results.
Despite the insignificances, this work adds necessary suggestive findings, as the evidence concerning POPs and neuropathy is scarce.

The manuscript and material have not been published and it is not being considered for publication elsewhere. All authors have read the manuscript,

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approved its submission and there is no conflict of interest. The KORA studies were approved by the by the Ethics Committee of the Bavarian Medical Association.

Yours sincerely,

Maximilian Schwarz (corresponding author)

Association of persistent organic pollutants with sensorimotor neuropathy in participants with and without diabetes or prediabetes: results from the population-based KORA FF4 study

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Supplementary material:1 document including detailed description of exposure measurement, 6 tables and 3 figures

HIGHLIGHTS

- We analyzed associations between low-dose exposure to persistent organic pollutants and distal sensorimotor polyneuropathy
- The results showed no significant associations between POPs and DSPN
- The findings were supported by a two-pollutant model
- We did not observe any effect modification by age, sex, diabetes or obesity

ABSTRACT

BACKGROUND. Exposure to persistent organic pollutants (POPs) has been associated with an increased type 2 diabetes (T2D) risk. It remains unclear whether POPs may also increase the risk of diabetes complications including neuropathy. We aimed to investigate the association of low-dose exposure to POPs with distal sensorimotor polyneuropathy (DSPN).

METHODS. Our study was part of the second follow-up (FF4, 2013-2014, N = 2,279) of the population-based KORA S4 study (Augsburg, Germany). The study sample consisted of 200 participants, including four groups of 50 persons each with known T2D, prediabetes, newly diagnosed diabetes, and normal glucose tolerance (NGT) based on an oral glucose tolerance test. We analyzed the association of six most abundant serum concentrations of POPs with DSPN by multivariable logistic regression adjusted for age, sex, glycaemic status, body mass index, physical activity, smoking and alcohol consumption. We assessed effect modification by age, sex, glycaemic status and obesity.

RESULTS. For all pollutants, the main models indicated no significant association of having DSPN. Two-pollutant models supported these findings, except for a lower odds ratio for the combination of polychlorinated biphenyl (PCB) 138 and beta-hexachlorocyclohexane (β-HCH) (OR: 0.59; 95% CI: 0.35 - 0.99). No effect modification was found by age, sex, glycaemic status and obesity.

CONCLUSION. Low-dose exposure to POPs indicated no association with the odds of having DSPN in T2D, prediabetes and NGT.

KEYWORDS. persistent organic pollutant, polyneuropathy, peripheral polyneuropathy, diabetes, KORA

1. INTRODUCTION

Persistent organic pollutants (POPs) are environmental contaminants including different sub-groups such as organochlorine (OC) pesticides like dichlorodiphenyltrichloroethane (DDT) or hexachlorobenzene (HCB), and industrial chemicals or by-products like polychlorinated biphenyls (PCB) (1). Although most POPs were banned decades ago, humans are still exposed mainly via dietary intake of contaminated food like agricultural products or seafood as well as breast milk (2, 3). POPs are mostly resistant to any kind of chemical or biological degradation and can accumulate in adipose tissue of organisms (4), where they form a persistent source of chronic internal exposure as they are slowly released into the circulation (1, 5). In addition, POP exposure is linked with adverse health effects like hormone-dependent cancer sites, impacts on the reproductive system, infectious diseases, metabolic disorders and obesity (6).

Peripheral polyneuropathy is a common long-term complication of people with diabetes and is associated with increased morbidity, mortality, and lower quality of life (7). The prevalence of distal sensorimotor polyneuropathy (DSPN) largely varied in previous studies due to differences in population, examination procedures, types of diabetes and the definition of polyneuropathy, ranging between 8 to 75% (8). For a population-based setting, the median prevalence of DSPN among people with type 2 diabetes (T2D) has been reported to be about 30% (8). In addition, there is emerging evidence that DSPN may be already present in people having prediabetes or the metabolic syndrome (9, 10). Individual factors that have been associated with DPSN are hyperglycaemia, height, body weight, general and abdominal obesity, blood pressure, smoking and lipid levels (9, 11).

Evidence is increasing that POPs are associated with an elevated risk of T2D, insulin resistance and metabolic syndrome, as they may act as endocrine disruptors and adversely affect β-cell

function (4, 12-16). However, evidence concerning the relationship between POPs and neuropathy is scarce. So far, only one study investigated the association between low-dose exposure to POPs and the risk of DSPN. In this study using a population-based survey from the United States (US), the prevalence of peripheral neuropathy was higher among participants with T2D, compared to participants with impaired fasting glucose/prediabetes (17). Additionally, peripheral neuropathy was strongly associated with OC pesticides (17). POPs are considered to act as neurological toxins by affecting dopamine and thyroid signaling, intracellular calcium dynamics, as well as oxidative stress (18). Evidence is conflicting from studies investigating accidental/occupational exposure reporting either no association (19) or an increased risk (20, 21) of having peripheral neuropathy or neurological abnormalities. A review on long-term health outcomes after a chemical plant explosion in Italy in 1976 reported subclinical reduced nerve conduction velocity (NCV) and nerve fiber damage by a neurological screening (22). However, no anomalies were found in later studies with regard to abnormal electrophysiological measurement, NCV, working memory, dexterity and mobility (assessed by walking speed, reach down test, coin flipping test) (22).

The aim of this cross-sectional study was to investigate the association between low dose of non-accidental exposure to six most abundant POPs and DSPN with a specific interest in the glycaemic status of the participants. We therefore selected a sub-sample of a German population-based cohort study including participants with known or newly diagnosed T2D, with metabolic abnormalities indicating prediabetes and people with normal glucose tolerance.

2. RESEARCH DESIGN AND METHODS

2.1 Study Design and Participants

The study used data from the Cooperative Health Research in the Region of Augsburg (KORA) FF4 study. KORA FF4 is the second follow-up examination (2013 to 2014) of the population-

based KORA S4 study (1999 to 2001) in the region of Augsburg, Germany, and two adjacent counties. From 6,417 eligible, randomly selected, individuals aged 25 to 74, 4,261 participated in KORA S4 and of these, 2,279 participants took part in KORA FF4. More detailed information concerning study design, sampling method and data collection have been described elsewhere (23, 24). For a sub-group of 767 KORA FF4 participants, who already had neurological measurements within the first follow-up examination KORA F4 (2006-2008) as well as participants with known diabetes, we carried out neurological measurements to assess DSPN. We selected 200 participants out of the 742 participants with a complete dataset after the follow-up examination was conducted. Participants were selected based on their glycaemic status determined at the follow-up examination using an interview and oral glucose tolerance tests (OGTT): 50 participants with normal glucose tolerance, 50 with prediabetes (impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT)), 50 with newly diagnosed diabetes and 50 with known T2D (Supplementary Figure 1). Characteristics of the study population, including age, sex, glycaemic status, anthropometry, socioeconomic status, medication intake, disease history and lifestyle factors were collected through self-administrated questionnaires and interviews by trained staff at the study center Augsburg. The KORA studies were approved by the Ethics Committee of the Bavarian Medical Association and all study participants gave their written informed consent.

2.2 Assessment of DSPN and Glycaemic Status

The assessment of DSPN was performed using the examination part of the Michigan Neuropathy Screening Instrument (MNSI) (25) as described in more detail elsewhere (26). The examination included appearance of feet, foot ulceration, ankle reflexes as well as vibration perception threshold. For assessing the vibration perception threshold, a C 64 Hz Rydel-Seiffer tuning fork was used and age-dependent lower limits of normal vibration threshold were taken into account

(using the formula for the fifth percentile according to Martina et al. (27) = 5.75 – 0.026 x age). Furthermore, the neuropathic assessment was extended by a bilateral examination of touch/pressure sensation using a Twin-Tip 10-g monofilament (Neuropen) (26, 28). The total MNSI scores range from zero to a maximum of 10 points, where zero is equal to normal findings within all assessment dimensions. The presence of DSPN was defined as MNSI scores > 3 as previously suggested (26, 29).

For patients without known diabetes, we also carried out a 75 g OGTT as described before (23, 30). We defined newly diagnosed diabetes as well as IGT, IFG and normal glucose tolerance according to the guidelines of the American Diabetes Association (ADA) (31). Known T2D was defined based on validated self-reported physician diagnosis or use of glucose-lowering medication (23) or diagnosed during previous KORA follow-up examinations. Prediabetes was defined by either isolated IFG, isolated IGT or a combination of IFG/IGT.

2.3 Assessment of Anthropometry

Weight, waist- and hip circumference were measured by trained staff according to a standard protocol (23). Overweight and obesity were defined as a body mass index (BMI) equal or above 25 and 30 kg/m^2, respectively. Abdominal obesity was defined as waist circumference above gender specific cutoff values according to the World Health Organization (WHO) (men: >102 cm; women: >88 cm; according to the WHO for substantially increased risk of metabolic complications) (32). As alternative measurement for abdominal obesity, we calculated waist-to-hip ratio (WHR) by dividing waist circumference by hip circumference and used recommended WHO cutoffs as indicators for obesity (men: ≥ 0.90 cm; women: ≥ 0.85 cm) (32). Medical interviews were used to assess baseline information on leisure time physical activity, smoking,

alcohol consumption, medication use and medical history, sociodemographic variables and parental diabetes (30).

2.4 Measurements of Blood Parameters and Persistent Organic Pollutants

Blood sampling was performed according to standardized protocols in a fasting condition and without stasis. The blood samples were centrifuged and cooled to 4 to 8°C immediately after extraction and then shipped in refrigerant packaging to the laboratory of Augsburg Central Hospital within 2-4 h. The samples were stored at -80°C until further analysis. We used gas chromatographic (GC) high-resolution mass spectrometry (MS) to analyze wet concentrations of six POPs following the Molecular Exposomics (MEX) Laboratory in-house method SOPD_BS1E, further described in the supplement. The following POPs were solid-phase extracted: hexachlorobenzene (HCB), beta-hexachlorocyclohexane (β-HCH), 4,4'-dichlorodiphenyldichloroethylene (4,4'-DDE) as well as polychlorinated biphenyl (PCB) 138, PCB-153 and PCB-180. In addition, we calculated the sum of total PCB exposure separately into one variable. POP concentrations below the limit of detection (LOD) were set to ½ LOD for each POP, ranging from two observations for PCB-180 to 39 observations for HCB. We a priori decided to use wet-weight concentrations of POPs (µg/l) as recommended by Lee et al. (1), because lipid-adjustment of POP concentrations could lead to an underestimation of the true association, whereas not considering lipid-levels could lead to an overestimation (1).

High-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, total cholesterol and triglyceride levels were determined by using assays either on a Dimension Vista 1500 instrument (Siemens Healthcare Diagnostics Inc., Newark, DE, USA) or on a Cobas c701/702 instrument (Roche Diagnostics GmbH, Mannheim, Germany). Since the measurement system changed from Siemens to Roche during the study period, we calibrated the measurements to enable

comparability. Concerning the calibration process and formulas, more detailed information has been given elsewhere (33). Glycated haemoglobin (HbA1c) was analyzed in whole blood primer using the VARIANT II TURBO HbA1c Kit – 2.0 system (Bio-Rad Laboratories Inc., Hercules, CA, USA).

2.5 Statistical Analysis

For descriptive purposes, we investigated the full sample but also stratified by prediabetes/glycaemic status. Baseline descriptive statistics were displayed with mean ± standard deviation (SD) for continuous variables and with absolute numbers (N) and percentages for categorical variables. Exposure variables were described, due to their skewed distribution, by their median value as well as the first (Q1) and third (Q3) quartile. To assess differences between each group of glycaemic status we performed *t*-tests or Wilcoxon-Mann-Whitney-U-tests for continuous variables and Pearson's Chi^2 tests for comparing categorical variables. We tested for differences separately for the three groups with prediabetes or diabetes compared to the group with normal glucose tolerance. Additionally, we calculated Spearman correlation coefficients for the POP concentrations, whereby values above 0.7 were regarded as high correlations. We also performed a Jonckheere-Terpstra-Test for trend between groups of different glycaemic status.

We carried out multivariable logistic regression using generalized linear models (GLM) to assess the association between exposure to POPs and the odds of having DSPN. All effect estimates were presented as odds ratios (OR) for an interquartile range (IQR) increase in POP concentration with associated 95% confidence intervals (CI). Based on the literature, we formed three sets of covariate adjustment. The minimum model consisted only of age and sex. The main model extended the minimum model by the variables BMI (kg/m^2), average alcohol consumption (g/day), leisure time physical activity (low: almost no or no physical activity; medium: regularly/unregularly approx. 1

h/week; high: regularly, > 2 h/week), smoking status (current, ex-, never-smoker) and glycaemic status. Additionally, we included HbA1c, total cholesterol and triglycerides to form an extended confounder model. The models were fitted for each POP separately. To study interdependencies of pollutant effects, we also performed two-pollutant models by adding a second pollutant into the models, if the Spearman correlation coefficient was < 0.7.

We examined effect modification by including an interaction term between the POP exposure and potential effect modifiers into our main model. We tested interaction by sex (male vs. female), age (< 75 years vs. ≥ 75 years), glycaemic status (no diabetes vs. prediabetes vs. incident diabetes vs. known T2D) and obesity (BMI < 30 kg/m^2 vs. ≥ 30 kg/m^2). We investigated two different definitions for abdominal obesity based on (i) waist circumference according to the WHO (waist circumference ≤ 102 cm (m), 88 cm (f) vs. > 102 cm (m), 88 cm (f)) and (ii) WHR according to WHO cutoffs (WHR < 0.90 cm (m), 0.85 cm (f) vs. ≥ 0.90 cm (m), 0.85 cm (f)).

2.6 Sensitivity Analyses

We performed several sensitivity analyses to test the robustness of our findings: (I) We log-transformed the values of POP exposure. (II) Instead of wet-weight concentrations, we used lipid-standardized POP concentrations (serum POP concentration divided by total serum lipids) according to the Phillips formula for total serum lipids (34). In the extended confounder model, we left out total cholesterol and triglyceride levels, for which we had already adjusted during the lipid-standardization procedure. (III) We used different cutoff values (MNSI > 2 / MNSI > 4) for defining the prevalence of DSPN. (IV) We used a clinical-driven definition of DSPN based on a bilateral impairment of foot-pressure sensation and/or impairment of food-vibration perception as described before (35). (V) We used quantile regression to test whether there are different exposure effects across the range of different MNSI Scores. (VI) Finally, we investigated dose-response

functions to examine deviations from linearity. We therefore added a smooth term with three degrees of freedom for each POP into a generalized additive model (GAM), fitted with the covariates of the main model.

Test results with two-sided p values < 0.05 were considered as statistically significant. All statistical analyses were carried out using RStudio version 1.2.1335 with R version 3.6.0 (The R Foundation for Statistical Computing, Vienna, Austria).

3. RESULTS

3.1 Study Population Characteristics

After exclusion of missing observations for exposure (N = 1) and outcome (N = 9), the study population consisted of 190 participants. Characteristics of the study population and serum POP concentrations, stratified by prediabetes/glycaemic status, are presented in Table 1. The overall mean MNSI score was 2.8 ± 1.6, the average age was 75.4 ± 4.8 years and there were more male participants (N = 109; 57.4%). For the descriptive analysis, we used NGT as reference group. In comparison with NGT, people with prediabetes, newly diagnosed diabetes and known T2D were more likely men, had higher levels of Triglycerides and HbA1c and had larger waist circumference and WHR. People with known T2D had also higher values for MNSI scores, DSPN prevalence (MNSI > 3) and BMI. People with newly diagnosed diabetes had higher BMI and lower physical activity. Median POP concentrations did not differ between the four glycaemic groups except for 4,4'-DDE, which showed higher concentrations for people with newly diagnosed diabetes. With deterioration of the glycaemic status, we observed higher values for MNSI score, DSPN (MNSI > 2), DSPN (MNSI > 3), BMI, physical activity, triglycerides, HbA1c, waist circumference, WHR and 4,4'-DDE levels. The Spearman correlation coefficients showed weak to moderate correlations

between the pollutants except for PCBs, which are highly correlated with other PCBs (Supplementary Table 1).

3.2 POPs and DSPN

The results of the multivariable logistic regression are presented in Table 2. In the minimally adjusted models, the effect estimates indicated no significant association but slightly decreased odds except for β-HCH and 4,4'-DDE. No significant associations were found after adjusting for further variables, but all seven main models pointed to decreased odds for DSPN. Similar were seen for the extended confounder model. In addition, we observed no pattern of the effect estimates, with more stringent confounder adjustment. Furthermore, the inclusion of a second pollutant did not affect the effect estimates (Figure 1), but PCB-138 turned to be significantly related to DSPN (OR: 0.59; 95% CI: 0.35 - 0.99) when additionally adjusted for β-HCH.

3.3 Effect modification

The results of the effect modifier analyses can be found in Figure 2. Overall, effect modification analysis showed no significant results when including an interaction term in the main model. Female participants (N = 81) indicated slightly lower effect estimates for all PCB exposures and higher estimates for HCB, β-HCH and 4,4'-DDE. Participants older than 75 years (N = 99), as well as obese participants (N = 75) showed lower effect estimates for all POP exposures, except for PCB-180 in older and HCB in obese participants. No consistent pattern was observed for any modifying effect by glycaemic status. Also, abdominal obese participants, defined by cut-off values for waist circumference (N = 127), generally indicated no effect modification, though for PCB-138 and PCB-153, effect estimates were lower for obese people. Finally, considering WHR

as cut-off for abdominal obesity, obese participants (N = 161) indicated slightly higher ORs, but CIs for non-obese participants were very wide.

3.4 Sensitivity Analysis

Using log-transformed POP concentrations showed a similar pattern compared to the main analysis (Supplementary Table 2). The same applies to the analysis for lipid-standardized POP concentrations (Supplementary Table 3). When defining DSPN with a cut-off of either MNSI > 2 or MNSI > 4 or using a clinical-driven definition of DSPN, the estimates were generally higher for the main models, but the association remained mostly inverse without any statistical significance (Supplementary Table 4, 5, 6). In addition, quantile regression indicated no major deviations from the main model. Since the distribution of our outcome (MNSI score) was skewed with about 28% of the participants reporting a MNSI score of 2.0, we could only investigate quartiles (Supplementary Figure 2). The inspection of the exposure-response curve by including a smooth term for POP concentrations indicated no major deviation from linearity (Supplementary Figure 3).

4. DISCUSSION

In this cross-sectional study, we did not observe an association between low-dose exposure to POPs and the odds of having DSPN in various glycaemic states. We did not observe any effect modification by age, sex, (abdominal) obesity or prediabetes/diabetes.

A link between POPs and neuropathy has been suggested (17). This study, including 246 participants from a US survey, also investigated the relationship of low-dose background exposure of POPs with the risk of polyneuropathy. The authors reported positive associations between POPs and the prevalence of peripheral neuropathy with an increased odds ratio for OC pesticides (17).

However, our findings did not confirm the reported positive associations. Interestingly, most effect estimates in our study pointed to a slightly inverse association, although not statistically significant and are therefore more in line with results from Grice et al. where the OC pesticide HCB was significantly protective for T2D (36).

Nevertheless, the effects and biological mechanisms of how low-dose POP exposure may lead directly to the onset of DSPN are not yet understood. Generally, discrepancies among studies emerge by different outcome assessments, distribution and measurement of exposure, statistical analysis and the study population. POPs are considered to act as general neurotoxins affecting neurological development, cognitive or motor deficits and different inter- and intracellular processes (e.g. oxidative stress) (18, 37, 38). Metabolic abnormalities including T2D are closely linked to neuropathy. Three major pathogenic pathways have been suggested for this relationship, although the associations are currently not yet understood: systemic inflammation, impaired function of the mitochondrion and endoplasmic reticulum, and oxidative stress (39-42). Briefly, these three mechanisms may affect various pathways and can induce cell damage or apoptotic processes in cells like neurons (39, 41). However, the trilateral interplay between POPs, diabetes and DSPN remain unclear. On the other hand, the associations between POPs and diabetes as well as between diabetes and DSPN are better known. POPs may act as diabetogenic agents affecting β-cell function and interacting with the endocrine system. Moreover, it is suggested that there are overlapping pathways, which are responsible for the onset of diabetes as well as for DSPN.

Our previous longitudinal study, including 396 participants of two German population-based cohorts, reported an increased risk of T2D for the POPs PCB-138, PCB-153 and the sum of PCBs 138, 153 and 180 (43). Interestingly, within the stratified analysis by research center, the CARLA (CARLA - Cardiovascular Disease, Living and Aging in Halle, Germany) cohort, located in

Eastern Germany, was mainly responsible for the significant results whereas the results for participants from the KORA cohort indicated no association (43). The participants in the study of Wolf et al. (43) as well as those in our study were selected from the same region and are part of the same study cohort. Nevertheless, both study samples are highly selected, and comparisons are difficult. Possibly, the KORA region itself and the people among this cohort are less exposed to POPs compared to people in other regions of Germany.

Older age is considered to be a strong and independent risk factor for developing DSPN (8). However, we did not see a modifying effect by age in our analyses. Likewise, Lee et al. reported similar results for younger or older participants (cut-off: younger: < 65 years; older: ≥ 65 years). Though, the participants in our study are about 12 years older compared to those of Lee et al. (mean age: 75.4 years vs. 63.6 years), our results pointed to decreased effect estimates of POPs on DSPN in older people. A possible explanation might be that both outcome and exposure are related to age. Survivor bias, a special form of the selection bias, might have influenced our associations as high risk patients might have dropped out (due to loss to follow-up, withdrawal or deaths). As a result, our cohort consisted of older and lower risk patients compared to the KORA baseline or the previous follow-up, respectively.

In addition to chronic low-dose exposure, previous studies also investigated cases of accidental or occupational POP exposure, which also reported contradictory results. For example, a European study of 156 workers of a pesticide manufacturing plant reported more signs of clinical sensory neuropathy among participants with chloracne (21). A study among US military veterans found higher odds of possible or probable peripheral neuropathy and increased odds of diagnosed peripheral neuropathy in participants exposed to an herbicide (20). Contrarily, no associations were found between occupational exposure to tetrachlorodibenzo-p-dioxin and peripheral

neuropathy among 281 workers of two US manufacturing plants (19). A recent review evaluated general health effects after accidental dioxin exposure at different time points. Reduced nerve conduction velocity and damage to nerve fibers were present shortly after exposure but disappeared in a follow-up screening (22). It is important to note that due to different methodological approaches, especially with regard source of exposure, comparability between our study and the studies mentioned above is limited. As another example, Lee et al. defined peripheral neuropathy through assessing foot sensation by having one or more insensate sites. Their main analysis used five groups of exposure whereas single concentrations of each POP were not investigated. Additionally, a comparison of exposure levels was not possible, as absolute values of POP exposure were not provided. The measured exposure values of previous studies focusing on accidental or occupational exposure are supposed to exceed the background levels that are found in the general population.

A controversially discussed methodological issue is the interplay of blood lipids and POP concentrations. Since POPs are lipophilic compounds, high levels of serum lipids are related to higher levels of serum POP concentrations and accounting for individual lipid levels seems reasonable. As Lee et al. mentioned, true associations may lie between lipid-adjusted and wet-weight exposure levels as under- or overestimation can occur depending on the used method (1). We a priori decided to use wet-weight concentrations and conducted sensitivity analyses additionally accounting for total cholesterol and triglycerides. In a recent European paper concerning methodological issues of POP concentrations and pancreatic cancer risk among 1.533 participants of a nested case-control study, Gasull et al. reported a wide range of unadjusted and lipid-adjusted POPs exposure between eight European countries. For example, with a median concentration of 4.40 µg/l in HCB, Spanish people were the most exposed. In comparison,

participants from the United Kingdom had HCB median values of 0.24 µg/l (44). In our study, the median values for both lipid-adjusted and wet-weight concentrations were generally low, e.g. with a concentration of 0.09 µg/l in β-HCH compared to median β-HCH levels of 0.35 µg/l in the study from Gasull et al. Compared to a stratified analysis by region, the β-HCH levels were even lower than the 25th percentile among the German participants of that study (0.09 µg/l vs. 0.26 µg/l). This indicates that the study region has an influence on POP exposure. Additionally, compared to the US National Health and Nutrition Examination Survey (NHANES) cohort, our lipid-adjusted 95th percentile of β-HCH (51.73 ng/g) was in between the 95th percentiles from NHANES 1999-2000 (68.9 ng/g) and NHANES 2001-2002 (43.3 ng/g), indicating similar exposure levels (45).

There are different statistical approaches dealing with POP exposure. Contrary to our analysis, Lee et al. used cumulative measures of POPs ranking the individual values of POPs and summing up the ranks afterwards. The resulting five categories of POPs were then categorized into tertiles (17). Since we were interested in the single effects, we a priori decided to model our data separately for each pollutant, although this does not reflect the reality in which POPs are a mixture of multiple congeners (4).

To our knowledge, this is the first European study addressing the association between low-dose background exposure of POPs and the odds of DSPN. The strengths of this study include that we used data from a well-characterized population-based cohort, including valid information of low-dose background exposure to POPs. Additionally, we used a validated assessment tool for DSPN. Moreover, the classification of metabolic status was assessed by an OGTT. This study also has several limitations. First, POP concentrations could only be measured for 200 people with 50 participants among each metabolic subgroup. The resulting statistical power is limited and therefore, some potential associations might have been not discovered. Second, the study

subsample was selected and therefore, does not represent the general population. Third, the observational study design does not allow any conclusions on causal relationships. Fourthly, we used the MNSI Score to define DSPN, but we did not check for any other impaired functions related to neuropathy. Finally, and probably most important, by design, no extrapolation to higher exposure levels were applied and inaccessible POPs can interfere with the results.

5. CONCLUSION

This cross-sectional study did not suggest an association between low-dose background exposure to six most abundant POPs and the odds of having DSPN.

Future research should consider larger sample sizes and measurements of more pollutants simultaneously. Prospective studies with several time points are needed to gain better insights into the complex interplay. Additionally, experimental studies would be favorable to clarify biological mechanisms and pathways of POPs on DSPN.

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DECLARATION OF INTEREST

The authors report no conflicts of interest.

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TABLES

Table 1: Basic characteristics and POP concentrations among all participants and subgrouped by glycaemic status.

Variable	Total	Normal glucose tolerance (NGT)	Prediabetes (IFG, IGT)	Newly diagnosed diabetes	Known diabetes	p-value[a]
	Mean ± SD or N (%)					
N	190	46	49	46	49	
MNSI	2.8 ± 1.6	2.5 ± 1.5	2.6 ± 1.4	2.7 ± 1.5	**3.3 ± 1.7[1]**	**0.012**
DSPN (MNSI > 2)	106 (55.8)	21 (45.7)	25 (51)	29 (63)	31 (63.3)	**0.043**
DSPN (MNSI > 3)	64 (33.7)	11 (23.9)	15 (30.6)	15 (32.6)	**23 (46.9)[2]**	**0.028**
DSPN (MNSI > 4)	29 (15.3)	6 (13)	6 (12.2)	4 (8.7)	13 (26.5)	0.155
Age (years)	75.4 ± 4.8	75.3 ± 4.9	75.2 ± 5.2	75.7 ± 4.9	75.6 ± 4.6	0.29
Sex (male)	109 (57.4)	18 (39.1)	**31 (63.3)[2]**	**29 (63)[2]**	**31 (63.3)[2]**	0.97
BMI (kg/m^2)	29.0 ± 4.8	27.1 ± 5.0	28.2 ± 3.6	**30.2 ± 4.6[1]**	**30.5 ± 5.1[1]**	**< 0.001**
Alcohol consumption (g/day)	16.5 ± 21.7	13.3 ± 20.2	15.5 ± 17.1	18.7 ± 27.0	18.4 ± 21.8	0.155
Smoking status:						0.812
current	9 (4.7)	2 (4.3)	3 (6.1)	0 (0)	4 (8.2)	
ex	87 (45.8)	21 (45.7)	20 (40.8)	20 (43.5)	26 (53.1)	
never	94 (49.5)	23 (50)	26 (53.1)	26 (56.5)	19 (38.8)	
Physical activity:						**0.011**
high	43 (22.6)	11 (23.9)	13 (26.5)	**9 (19.6)[2]**	10 (20.4)	
medium	71 (37.4)	23 (50)	18 (36.7)	**13 (28.3)[2]**	17 (34.7)	
low	76 (40)	12 (26.1)	18 (36.7)	**24 (52.2)[2]**	22 (44.9)	
Cholesterol (mmol/l)	5.4 ± 1.1	5.5 ± 0.9	5.6 ± 1.2	5.6 ± 1.1	5.1 ± 1.1	0.932
Triglyceride (mmol/l)	1.5 ± 0.8	1.1 ± 0.4	**1.6 ± 0.9[3]**	**1.7 ± 0.7[3]**	**1.7 ± 0.9[3]**	**< 0.001**
HbA1c (mmol/mol)	41.2 ± 7.5	36.5 ± 3.6	**38.0 ± 3.5[3]**	**42.5 ± 7.0[3]**	**47.7 ± 8.7[3]**	**< 0.001**
Waist circumference (cm)	102.3 ± 12.8	94.8 ± 12.8	**100.8 ± 9.5[1]**	**105.9 ± 12.3[1]**	**107.7 ± 12.7[1]**	**< 0.001**
Waist-to-hip ratio	0.94 ± 0.08	0.89 ± 0.09	**0.94 ± 0.07[3]**	**0.96 ± 0.07[3]**	**0.98 ± 0.07[3]**	**< 0.001**

Continued on p. 25

Table 1 (continued).

POPs (µg/l)	Total	Normal glucose tolerance (NGT)	Prediabetes (IFG, IGT)	Newly diagnosed diabetes	Known diabetes	p-value[a]
	Median (Q1 – Q3)					
PCB-138	0.47 (0.29-0.63)	0.46 (0.23-0.65)	0.49 (0.33-0.59)	0.50 (0.35-0.68)	0.44 (0.27-0.59)	0.658
PCB-153	0.84 (0.59-1.07)	0.75 (0.49-1.07)	0.87 (0.65-1.21)	0.94 (0.65-1.10)	0.75 (0.47-1.00)	0.801
PCB-180	0.86 (0.64-1.16)	0.82 (0.69-1.11)	0.96 (0.70-1.14)	0.91 (0.61-1.21)	0.79 (0.56-1.13)	0.808
Sum of PCBs	2.16 (1.57-2.93)	2.10 (1.49-2.91)	2.37 (1.81-2.94)	2.23 (1.65-2.94)	2.06 (1.43-2.45)	0.764
HCB	0.30 (0.17-0.57)	0.30 (0.10-0.61)	0.29 (0.20-0.47)	0.39 (0.10-0.57)	0.29 (0.17-0.51)	0.674
β-HCH	0.10 (0.06-0.17)	0.12 (0.06-0.16)	0.08 (0.06-0.15)	0.10 (0.07-0.17)	0.12 (0.07-0.19)	0.094
4,4'-DDE	1.15 (0.61-2.48)	0.86 (0.52-2.12)	1.07 (0.50-2.13)	**1.25 (0.97-2.67)[3]**	1.18 (0.66-2.40)	**0.029**

Statistical tests were performed between group 'normal glucose tolerance' and every other group as well as a trend-test between all four groups. OGTT: oral glucose tolerance test; IFG: impaired fasting glucose; IGT: impaired glucose tolerance; N: total number; SD: standard deviation; MNSI: Michigan Neuropathy Screening Instrument; DSPN: distal sensorimotor polyneuropathy; BMI: body mass index; Physical activity: high: regularly, > 2h/week; medium: regularly/unregularly approx. 1 h/week; low: almost no or no physical activity; HbA1c: glycated haemoglobin; POPs: persistent organic pollutants; Q1, Q3: first and third quartile; PCB: polychlorinated biphenyl; HCB: hexachlorobenzene; β-HCH: beta-hexachlorocyclohexane; 4,4'-DDE: dichlorodiphenyldichloroethylene. Numbers printed in bold indicate significance.

[a]: Jonckheere-Terpstra-test for increasing trend between groups of different glycaemic status
[1]: p-value < 0.05 of t-test
[2]: p-value < 0.05 of Pearson's Chi-square test
[3]: p-value < 0.05 of Wilcoxon-test

Table 2: Association between POP concentrations per IQR increase and DSPN (MNSI > 3).

POPs (µg/l)	IQR	Minimum model[a] OR (95% CI)	Main model[b] OR (95% CI)	Extended model[c] OR (95% CI)
PCB-138	0.34	0.67 (0.43; 1.04)	0.65 (0.40; 1.07)	0.66 (0.39; 1.11)
PCB-153	0.49	0.70 (0.48; 1.02)	0.72 (0.47; 1.09)	0.70 (0.45; 1.10)
PCB-180	0.52	0.74 (0.52; 1.05)	0.81 (0.57; 1.16)	0.80 (0.54; 1.17)
Sum of PCBs	1.36	0.66 (0.43; 1.00)	0.70 (0.45; 1.10)	0.68 (0.42; 1.10)
HCB	0.40	0.93 (0.68; 1.29)	0.87 (0.59; 1.28)	0.88 (0.60; 1.28)
β-HCH	0.11	1.01 (0.95; 1.08)	0.95 (0.89; 1.03)	0.95 (0.88; 1.02)
4,4'-DDE	1.86	1.07 (0.88; 1.29)	0.90 (0.73; 1.11)	0.90 (0.73; 1.11)

POPs: persistent organic pollutants; IQR: interquartile range; DSPN: distal sensorimotor polyneuropathy; MNSI: Michigan Neuropathy Screening Instrument; PCB: polychlorinated biphenyl; HCB: hexachlorobenzene; β-HCH: beta-hexachlorocyclohexane; 4,4'-DDE: dichlorodiphenyldichloroethylene; OR: odds ratio; CI: confidence interval.

[a]: Multivariable logistic regression adjusted for age (years) and sex.
[b]: Minimum model additionally adjusted for body mass index (kg/m^2), alcohol consumption (g/day), smoking status (current, ex, never), physical activity (high, medium, low) and glycaemic status.
[c]: Main model additionally adjusted for HbA1c (mmol/mol), total cholesterol (mmol/l) and triglycerides (mmol/l).

FIGURES

Figure 1: Odds ratio and 95% confidence interval for the associations between POP and DSPN using single and two-pollutant models, adjusted for main model covariates. Single pollutant estimates are displayed with dots in each segment for the respective pollutant. The colors indicate the second pollutant in the models, the estimates are displayed as triangles. N = 190.

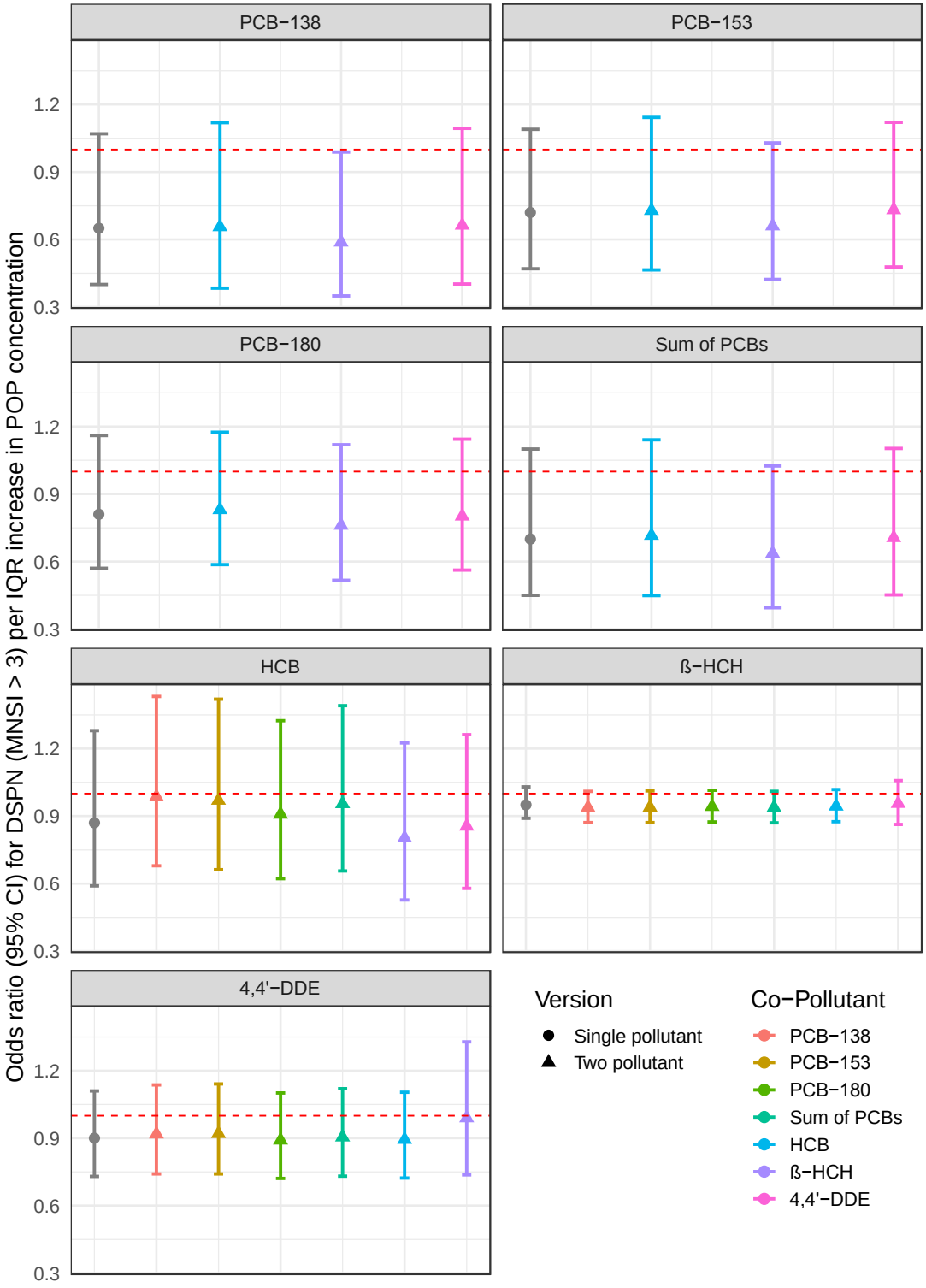
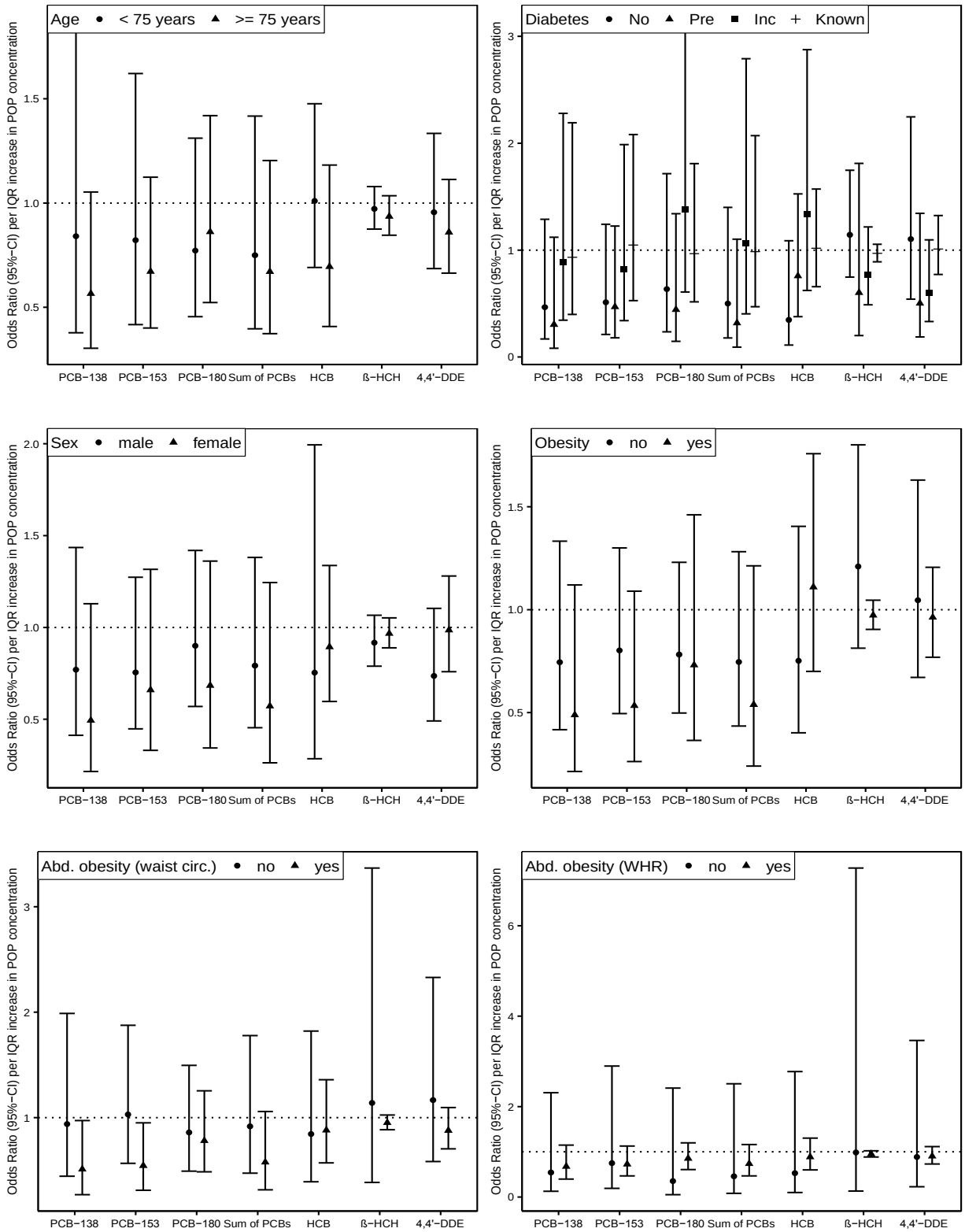


Figure 2: Effect modification by age (top left), glycaemic state (top right), sex (middle left), obesity [BMI < 30 vs. ≥ 30] (middle right), abdominal obesity, defined by waist circumference (bottom left) and waist-to-hip ratio (bottom right). All models were adjusted for main model covariates.



APPENDIX SUPPLEMENTARY DATA

Determination of POPs

Six POPs (hexachlorobenzene (HCB), beta-hexachlorocyclohexane (β-HCH), 4,4'-dichlorodiphenyldichloroethylene (4,4'-DDE) as well as polychlorinated biphenyl (PCB) 138, PCB-153 and PCB-180) were analyzed by gas chromatography (GC) high resolution mass spectrometry (MS). The isotope dilution technique was applied by using 13C-labeled analogues of the analytes as internal standards.

80 µL methanol were transferred to a 2 mL conical reaction tube and a labeled internal standard solution was added, as well as 250 µL acetonitrile and 250 µL toluene. After transferring 200 µL blood serum into this mixture everything was shaken thoroughly by use of a vortexer. The sample/solvent mixture was transferred to a glass column filled with (from bottom to top) 1 g silica gel, 1 g silica gel treated with 44% sulfuric acid, 1 g anhydrous sodium sulphate. The column was washed and activated before with 20 mL n-hexane/dichloromethane 1:1 (v/v). The elution of the analytes was carried out with 25 mL n-hexane/dichloromethane 1:1 (v/v). The eluate was reduced by a gentle stream of nitrogen and transferred to a 2 mL GC vial equipped with a small volume glass insert. The final volume was 20 µL.

The GC/MS conditions were as follows:

GC	
Type:	Agilent 5890 Series II
Column:	Stx-CLPesticides2, 30 m, 0.25 mm ID, 0.2 µm film thickness (Restek)
Temperature program:	60°C, 1.5 min, 12°C min-1, 140°C, 5°C min-1, 300°C, 10 min
Carrier gas:	Helium, head pressure: 16 psi
Injector:	Cooled injection system CIS 3 (Gerstel)

Temperature program:	120 °C, 12 °C s-1, 300 °C, 5 min
Temperature transfer line:	300 °C
Injection volume:	1 µl splitless
MS	
Type:	MAT 95S (Thermo)
Ionization mode:	EI+, 47 eV, 260°C
Scan mode:	Single ion monitoring
Resolution:	>8000

Quality Assessment/Quality Control for analysis of POPs

Every 20 serum samples a blank and internal control sample were analyzed. The results of the control samples were within ± 2 times the standard variation of previous analyses of that sample (N = 20). The analysis results were corrected for blank values, whereas the limit of detection was calculated as three times the standard deviation of the blank samples. The mean recoveries of the labeled internal standards were in the range of 64 % to 82 %.

Supplementary Table 1: Descriptive statistics of measured POP concentrations (in µg/l) and Spearman correlation coefficient.

	Mean ± SD	Range	IQR	Spearman correlation coefficient					
				PCB-138	PCB-153	PCB-180	Sum of PCBs	HCB	β-HCH
PCB-138	0.47 ± 0.25	0.07 - 1.34	0.34						
PCB-153	0.87 ± 0.43	0.18 - 2.75	0.49	**0.88**					
PCB-180	0.99 ± 0.64	0.07 - 6.59	0.52	**0.71**	**0.76**				
Sum of PCBs	2.34 ± 1.17	0.32 - 9.34	1.36	**0.91**	**0.94**	**0.91**			
HCB	0.44 ± 0.47	0.08 - 4.19	0.40	0.61	0.59	0.34	0.53		
β-HCH	0.21 ± 0.52	0.02 - 3.70	0.11	0.41	0.31	0.12	0.27	0.58	
4,4'-DDE	2.13 ± 3.00	0.16 - 23.52	1.86	0.52	0.53	0.16	0.40	0.41	0.54

POPs: persistent organic pollutants; SD: standard deviation; IQR: interquartile range; PCB: polychlorinated biphenyl; HCB: hexachlorobenzene; β-HCH: beta-hexachlorocyclohexane; 4,4'-DDE: dichlorodiphenyldichloroethylene. Numbers printed in bold indicate high correlation (> 0.70).

Supplementary Table 2: Association between log-transformed POP concentrations per IQR increase and DSPN (MNSI > 3).

log(POPs)	IQR	Minimum model[a] OR (95% CI)	Main model[b] OR (95% CI)	Extended model[c] OR (95% CI)
PCB-138	0.77	0.82 (0.57; 1.19)	0.81 (0.53; 1.23)	0.83 (0.53; 1.31)
PCB-153	0.60	0.79 (0.57; 1.10)	0.80 (0.55; 1.16)	0.79 (0.53; 1.18)
PCB-180	0.60	0.88 (0.64; 1.21)	1.01 (0.71; 1.45)	1.03 (0.70; 1.53)
Sum of PCBs	0.62	0.79 (0.55; 1.14)	0.85 (0.56; 1.29)	0.85 (0.54; 1.34)
HCB	1.20	0.98 (0.61; 1.58)	0.89 (0.52; 1.52)	0.88 (0.50; 1.54)
β-HCH	1.00	1.01 (0.72; 1.40)	0.70 (0.47; 1.03)	0.72 (0.48; 1.06)
4,4'-DDE	1.40	1.04 (0.68; 1.60)	0.74 (0.45; 1.19)	0.74 (0.45; 1.22)

POPs: persistent organic pollutants; IQR: interquartile range; DSPN: distal sensorimotor polyneuropathy; MNSI: Michigan Neuropathy Screening Instrument; PCB: polychlorinated biphenyl; HCB: hexachlorobenzene; β-HCH: beta-hexachlorocyclohexane; 4,4'-DDE: dichlorodiphenyldichloroethylene; OR: odds ratio; CI: confidence interval

[a]: Multivariable logistic regression adjusted for age (years) and sex.
[b]: Minimum model additionally adjusted for body mass index (kg/m^2), alcohol consumption (g/day), smoking status (current, ex, never), physical activity (high, medium, low) and glycaemic status.
[c]: Main model additionally adjusted for HbA1c (mmol/mol), total cholesterol (mmol/l) and triglycerides (mmol/l).

Supplementary Table 3: Association between lipid-standardized POP concentrations per IQR increase and DSPN (MNSI > 3).

POPs (ng/g)	IQR	Minimum model[a] OR (95% CI)	Main model[b] OR (95% CI)	Extended model[c] OR (95% CI)
PCB-138	48.26	0.67 (0.43; 1.04)	0.66 (0.41; 1.07)	0.68 (0.42; 1.12)
PCB-153	73.40	0.65 (0.44; 0.98)	0.69 (0.44; 1.07)	0.69 (0.44; 1.08)
PCB-180	67.11	0.74 (0.53; 1.04)	0.84 (0.61; 1.16)	0.84 (0.61; 1.16)
Sum of PCBs	187.12	0.64 (0.42; 0.98)	0.71 (0.46; 1.09)	0.72 (0.46; 1.11)
HCB	56.06	0.95 (0.71; 1.28)	0.89 (0.63; 1.26)	0.89 (0.63; 1.26)
β-HCH	15.58	1.01 (0.96; 1.07)	0.96 (0.90; 1.02)	0.96 (0.90; 1.02)
4,4'-DDE	253.50	1.06 (0.90; 1.25)	0.91 (0.76; 1.10)	0.92 (0.76; 1.10)

POPs: persistent organic pollutants; IQR: interquartile range; DSPN: distal sensorimotor polyneuropathy; MNSI: Michigan Neuropathy Screening Instrument; PCB: polychlorinated biphenyl; HCB: hexachlorobenzene; β-HCH: beta-hexachlorocyclohexane; 4,4'-DDE: dichlorodiphenyldichloroethylene; OR: odds ratio; CI: confidence interval

[a]: Multivariable logistic regression adjusted for age (years) and sex.
[b]: Minimum model additionally adjusted for body mass index (kg/m^2), alcohol consumption (g/day), smoking status (current, ex, never), physical activity (high, medium, low) and glycaemic status.
[c]: Main model additionally adjusted for HbA1c (mmol/mol).

Supplementary Table 4: Association between POP concentrations per IQR increase and DSPN (MNSI > 2).

POPs (µg/l)	IQR	Minimum model[a] OR (95% CI)	Main model[b] OR (95% CI)	Extended model[c] OR (95% CI)
PCB-138	0.34	1.04 (0.69; 1.57)	1.03 (0.66; 1.61)	1.19 (0.74; 1.89)
PCB-153	0.49	1.01 (0.72; 1.42)	1.04 (0.72; 1.50)	1.17 (0.79; 1.73)
PCB-180	0.52	0.87 (0.66; 1.13)	0.90 (0.69; 1.17)	0.94 (0.72; 1.22)
Sum of PCBs	1.36	0.91 (0.65; 1.28)	0.94 (0.65; 1.35)	1.03 (0.70; 1.52)
HCB	0.40	1.13 (0.84; 1.53)	1.07 (0.79; 1.45)	1.11 (0.81; 1.53)
β-HCH	0.11	0.99 (0.93; 1.06)	0.94 (0.87; 1.01)	0.93 (0.87; 1.00)
4,4'-DDE	1.86	1.12 (0.90; 1.38)	0.98 (0.78; 1.23)	0.98 (0.77; 1.25)

POPs: persistent organic pollutants; IQR: interquartile range; DSPN: distal sensorimotor polyneuropathy; MNSI: Michigan Neuropathy Screening Instrument; PCB: polychlorinated biphenyl; HCB: hexachlorobenzene; β-HCH: beta-hexachlorocyclohexane; 4,4'-DDE: dichlorodiphenyldichloroethylene; OR: odds ratio; CI: confidence interval

[a]: Multivariable logistic regression adjusted for age (years) and sex.
[b]: Minimum model additionally adjusted for body mass index (kg/m^2), alcohol consumption (g/day), smoking status (current, ex, never), physical activity (high, medium, low) and glycaemic status.
[c]: Main model additionally adjusted for HbA1c (mmol/mol), total cholesterol (mmol/l) and triglycerides (mmol/l).

Supplementary Table 5: Association between POP concentrations per IQR increase and DSPN (MNSI > 4).

POPs (µg/l)	IQR	Minimum model[a] OR (95% CI)	Main model[b] OR (95% CI)	Extended model[c] OR (95% CI)
PCB-138	0.34	0.63 (0.35; 1.16)	0.78 (0.39; 1.56)	0.95 (0.46; 1.96)
PCB-153	0.49	0.69 (0.42; 1.16)	0.90 (0.50; 1.62)	1.10 (0.58; 2.08)
PCB-180	0.52	0.77 (0.49; 1.23)	1.00 (0.66; 1.53)	1.08 (0.75; 1.57)
Sum of PCBs	1.36	0.67 (0.38; 1.17)	0.92 (0.50; 1.68)	1.10 (0.60; 2.01)
HCB	0.40	0.89 (0.56; 1.39)	0.84 (0.50; 1.44)	0.89 (0.53; 1.50)
β-HCH	0.11	1.02 (0.95; 1.10)	0.96 (0.88; 1.05)	0.96 (0.88; 1.05)
4,4'-DDE	1.86	1.11 (0.90; 1.37)	0.93 (0.73; 1.18)	0.94 (0.74; 1.21)

POPs: persistent organic pollutants; IQR: interquartile range; DSPN: distal sensorimotor polyneuropathy; MNSI: Michigan Neuropathy Screening Instrument; PCB: polychlorinated biphenyl; HCB: hexachlorobenzene; β-HCH: beta-hexachlorocyclohexane; 4,4'-DDE: dichlorodiphenyldichloroethylene; OR: odds ratio; CI: confidence interval

[a]: Multivariable logistic regression adjusted for age (years) and sex.
[b]: Minimum model additionally adjusted for body mass index (kg/m^2), alcohol consumption (g/day), smoking status (current, ex, never), physical activity (high, medium, low) and glycaemic status.
[c]: Main model additionally adjusted for HbA1c (mmol/mol), total cholesterol (mmol/l) and triglycerides (mmol/l).

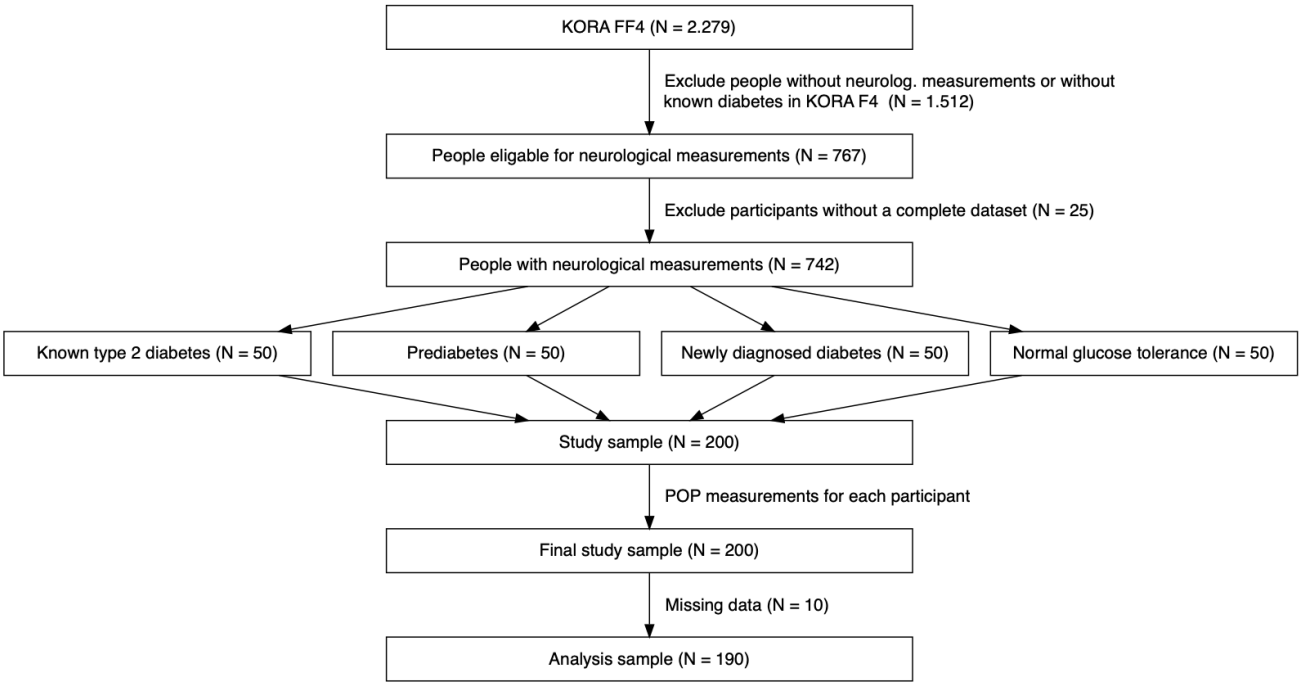
Supplementary Table 6: Association between POP concentrations per IQR increase and clinical DSPN.

POPs (µg/l)	IQR	Minimum model[a] OR (95% CI)	Main model[b] OR (95% CI)	Extended model[c] OR (95% CI)
PCB-138	0.34	1.00 (0.65; 1.52)	1.01 (0.63; 1.62)	1.09 (0.67; 1.80)
PCB-153	0.49	1.01 (0.71; 1.44)	1.06 (0.71; 1.57)	1.18 (0.77; 1.81)
PCB-180	0.52	0.78 (0.55; 1.09)	0.82 (0.58; 1.15)	0.88 (0.63; 1.23)
Sum of PCBs	1.36	0.87 (0.59; 1.27)	0.89 (0.59; 1.34)	0.98 (0.64; 1.50)
HCB	0.40	0.96 (0.70; 1.31)	0.85 (0.58; 1.24)	0.88 (0.60; 1.28)
β-HCH	0.11	1.03 (0.97; 1.10)	0.99 (0.92; 1.06)	0.99 (0.92; 1.06)
4,4'-DDE	1.86	**1.31 (1.05; 1.63)**	1.16 (0.91; 1.48)	1.15 (0.90; 1.46)

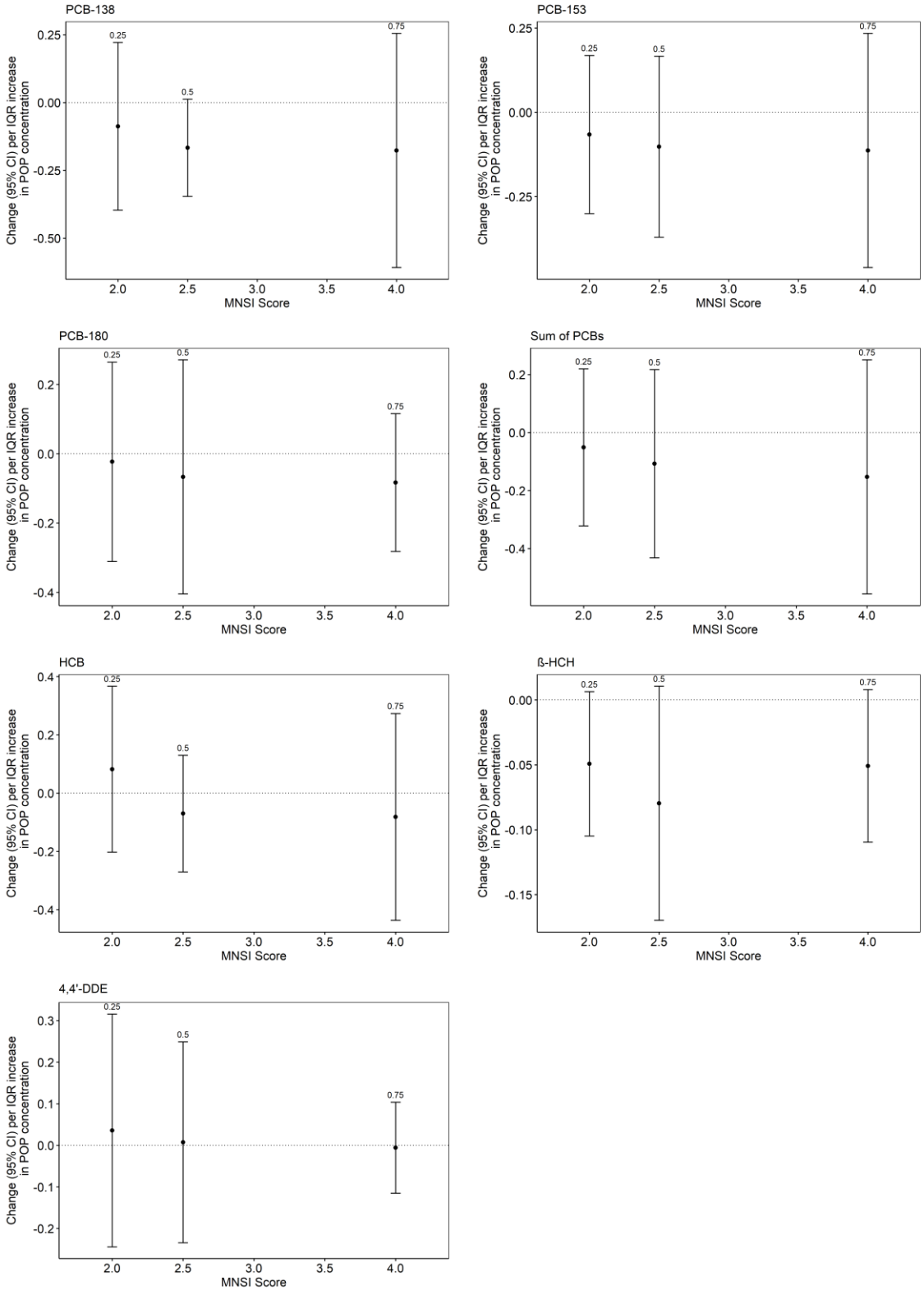
POPs: persistent organic pollutants; IQR: interquartile range; DSPN: distal sensorimotor polyneuropathy; MNSI: Michigan Neuropathy Screening Instrument; PCB: polychlorinated biphenyl; HCB: hexachlorobenzene; β-HCH: beta-hexachlorocyclohexane; 4,4'-DDE: dichlorodiphenyldichloroethylene; OR: odds ratio; CI: confidence interval

[a]: Multivariable logistic regression adjusted for age (years) and sex.
[b]: Minimum model additionally adjusted for body mass index (kg/m^2), alcohol consumption (g/day), smoking status (current, ex, never), physical activity (high, medium, low) and glycaemic status.
[c]: Main model additionally adjusted for HbA1c (mmol/mol), total cholesterol (mmol/l) and triglycerides (mmol/l).

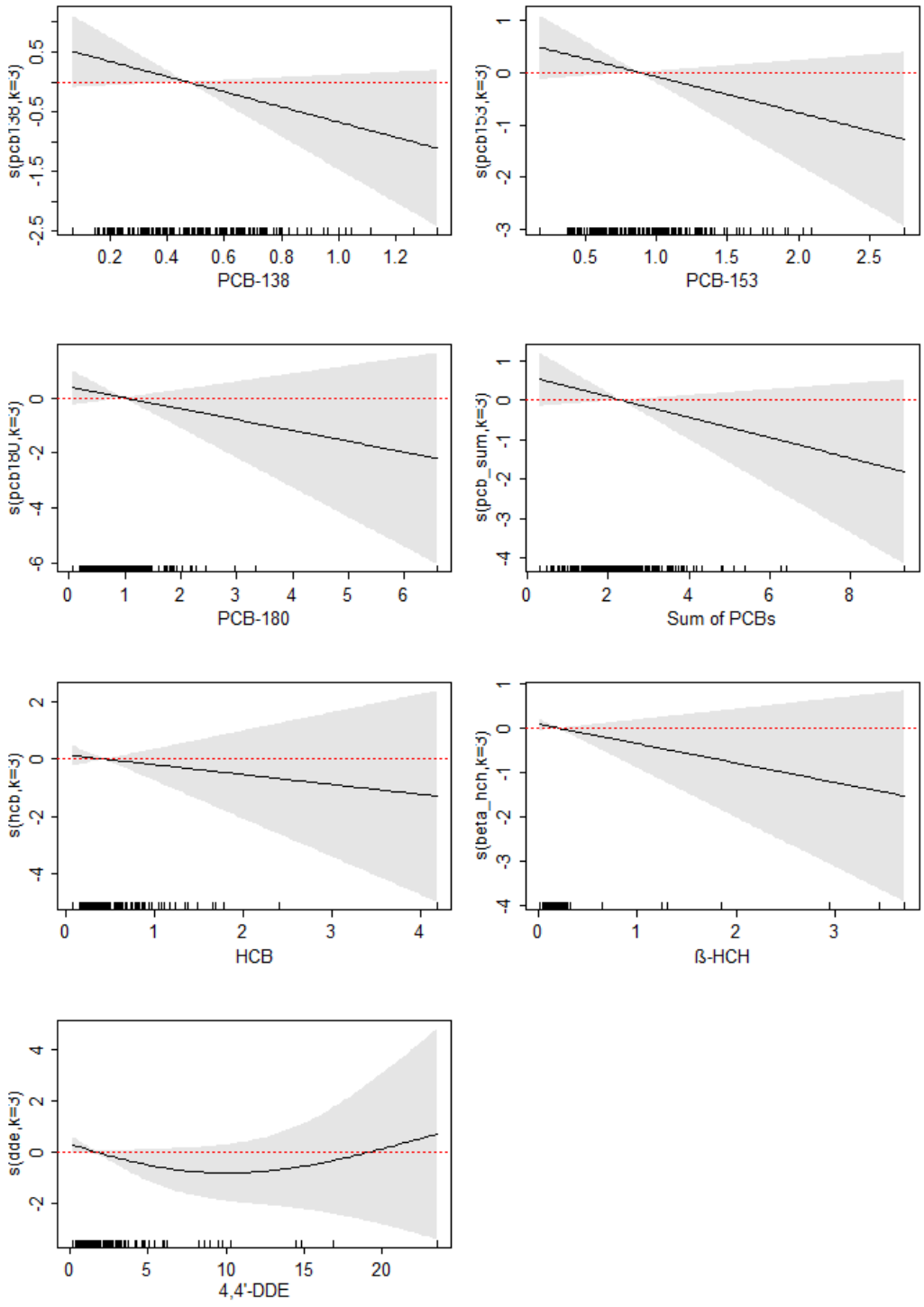
Supplementary Figure 1: Flowchart of the study population



Supplementary Figure 2: Quantile Regression per IQR increase in POP concentration using the 25th, 50th and 75th percentile. The x-axis shows absolute differences in MNSI Score for the 25th, 50th and 75th percentile. The y-axis represents changes in coefficients per IQR increase in POP concentration. All models were adjusted for main model covariates.



Supplementary Figure 3: Dose-response analysis. A smooth term with three degrees of freedom was used for each pollutant. All models were adjusted for main model covariates.



Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: