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## Environmental risk scores of persistent organic pollutants associate with higher ALS risk and shorter survival in a new Michigan case/control cohort

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### Abstract

**Background:** Amyotrophic lateral sclerosis (ALS) is a fatal, progressive neurodegenerative disease caused by combined genetic susceptibilities and environmental exposures. Identifying and validating these exposures are of paramount importance to modify disease risk. We previously reported that persistent organic pollutants (POPs) associate with ALS risk and survival and aimed to replicate these findings in a new cohort.

**Methods:** Participants with and without ALS recruited in Michigan provided plasma samples for POPs analysis by isotope dilution with triple quadrupole mass spectrometry. Odds ratios for risk

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#### Author Contributions

SAG and JB directly accessed and verified the underlying clinical data reported in the manuscript. SAB and JB directly accessed and verified the underlying persistent organic pollutant data reported in the manuscript. All authors had full access to all the data in the study and accept responsibility to submit for publication.

SAG: Conceptualization, Funding acquisition, Investigation, Writing – original draft

JB: Formal Analysis, Writing – review & editing

DGJ: Formal Analysis, Writing – review & editing

BM: Formal Analysis, Writing – review & editing

RJR: Literature search, Writing – review & editing

SAB: Conceptualization, Funding acquisition, Investigation, Writing – review & editing

ELF: Conceptualization, Funding acquisition, Investigation, Resources, Supervision, Writing – review & editing

#### Declaration of interests

SAG: Listed as an inventor on a patent, Issue number US10660895, held by University of Michigan titled “Methods for Treating Amyotrophic Lateral Sclerosis” that targets immune pathways for use in ALS therapeutics. Served on a DSMB and provided scientific advisory for a documentary about ALS.

JB: Nothing to declare

DGJ: Nothing to declare

BM: Nothing to declare

RJR: Nothing to declare

SAB: Nothing to declare

ELF: Listed as an inventor on a patent, Issue number US10660895, held by University of Michigan titled “Methods for Treating Amyotrophic Lateral Sclerosis” that targets immune pathways for use in ALS therapeutics.

models and hazard ratios for survival models were calculated for individual POPs. POP mixtures were represented by environmental risk scores (ERS), a summation of total exposures, to evaluate the association with risk (ERS<sup>risk</sup>) and survival (ERS<sup>survival</sup>).

**Results:** Samples from 164 ALS and 105 control participants were analyzed. Several individual POPs significantly associated with ALS, including 8 of 22 polychlorinated biphenyls (PCB) and 7 of 10 organochlorine pesticides (OCPs). ALS risk was most strongly represented by the mixture effects of OCPs alpha-hexachlorocyclohexane, hexachlorobenzene, trans-nonachlor and cis-nonachlor and an interquartile increase in ERS<sup>risk</sup> enhanced ALS risk 2.58 times ( $p<0.001$ ). ALS survival was represented by the combined mixture of all POPs and an interquartile increase in ERS<sup>survival</sup> enhanced ALS mortality rate 1.65 times ( $p=0.008$ ).

**Conclusions:** These data continue to support POPs as important factors for ALS risk and progression and replicate findings in a new cohort. Assessments of POPs in non-Michigan ALS cohorts are encouraged to better understand the global effect and the need for targeted disease risk reduction strategies.

### Keywords

amyotrophic lateral sclerosis; environmental exposures; organochlorine pesticides; persistent organic pollutants; environmental risk scores; ERS

## Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease chiefly characterized by progressive muscle weakness, although non-motor symptoms involving cognitive domains are also common.<sup>1,2</sup> Most individuals that develop ALS lack a known genetic cause, potentially implicating environmental exposures superimposed on monogenic and polygenic risk according to the gene-time-environment hypothesis.<sup>1</sup> Multiple exposure types are linked to ALS, including pesticides, metals, electromagnetic fields, and physical activity.<sup>3,4</sup> Identifying exposures and related mechanisms that increase ALS risk is critical to inform new therapeutic targets and prevention efforts.<sup>5</sup>

We previously reported that higher plasma persistent organic pollutant (POP) concentrations correlate with ALS risk and poorer survival.<sup>6,7</sup> We focused on three POP classes, including polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), and polybrominated diphenyl ethers (PBDEs). The goal of the current work was to validate previously published associations<sup>6,7</sup> using samples from new ALS participants and develop a set of environmental risk scores (ERSs), which summarized the combined effects of POPs exposures on both ALS risk and survival.

## Methods

### Participants

This study included participants with ALS and neurologically healthy controls, using previously reported methods.<sup>3,4,6,7</sup> Briefly, ALS patients meeting Gold Coast criteria were recruited during clinical visits to the University of Michigan Pranger ALS Clinic. All

ALS patients were eligible if they could provide informed consent in English. Controls were enrolled from across Michigan using electronic study postings on Facebook and the University of Michigan Health Research Platform and random address mailings. Controls were required to consent in English and were excluded if they had a personal or family history of a neurodegenerative disease in a first or second degree relative. Controls were also excluded if they had an active infection, cancer, or autoimmune disease. Recruitment of both cases and controls was impacted by restrictions resulting from the COVID-19 pandemic. All participants provided informed consent and the study was approved by our Institutional Review Board (University of Michigan IRBMED, HUM28826). Controls were compensated for participating. Following consent, participants provided a blood sample obtained via peripheral venipuncture. Plasma was separated and aliquoted into vials and remained frozen at  $-80^{\circ}\text{C}$  until processed for POP analysis. For all participants, sex and age are recorded and weight and height measurements are either reported by the participant or extracted from medical records to calculate body mass index (BMI;  $\text{kg}/\text{m}^2$ ). Previously, we found high correlation between self-reported and measured BMI.<sup>8</sup> For cases, variables relevant to ALS phenotyping and prognosis were abstracted from medical records [e.g., onset segment, revised ALS functional rating scale (ALSF<sub>RS</sub>-R), non-invasive ventilation (NIV) use, original/revised El Escorial Criteria]. Participants continued to receive clinical care at our center and therefore clinical status following sample collection could be recorded.

### Persistent Organic Pollutant Assays

Detailed methods are reported in the supplement. Briefly, plasma samples were analyzed using a modified method presented by the CDC, 2007<sup>9</sup> in the organic chemistry laboratory in the Exposure Assessment Core (EAC) at the University of Michigan School of Public Health. The EAC organic chemistry laboratory updated its methodology in 2019 and, compared to our prior report, utilizes an isotope dilution technique, simpler extraction and cleanup protocols, similar chromatography protocols, and triple quadrupole mass spectrometry (MS/MS) for detecting POPs. This updated method also combines OCP and PBDE gas chromatography GC/MS runs, with a separate run for PCBs. Quality assurance includes authentic standards (certified surrogate and calibration standard solutions), blanks, matrix spikes, duplicates, and analyses of standard reference materials.

### Statistical Analysis

**Pre-processing.**—POPs found below the limit of detection (LOD) in over 50% of samples or with likely identification or quantitation issues (e.g., peak overlap or contamination) were omitted from analysis. PCB-15, p,p'-DDT (dichlorodiphenyltrichloroethane), and endosulphan I were separately removed due to potential batch contamination issues. Sample concentrations not omitted but otherwise missing (not detected) were singly imputed using  $LOD/2$ . For some participants that had multiple longitudinal measurements taken over time, the earliest available measurement was used for this analysis. Additionally, for participants with replicates at the first measurement, concentrations were averaged.

**Descriptive Analyses and Missing Data.**—Descriptive statistics were tabulated for the study population stratified by case/control status and compared with t-tests and Chi-

squared tests, as appropriate. Descriptive statistics summarizing POPs exposures were also calculated for cases and controls and differences were evaluated using a Wilcoxon rank sum test with Benjamini-Hochberg correction to adjust for multiple testing. The correlation structure among POPs was assessed by Spearman rank correlation heatmap.

Multiple imputation using chained equations via the mice package in R<sup>10</sup> was used to impute missing adjustment covariates separately for cases and controls to facilitate imputing ALS-specific variables (e.g., onset segment) for cases. The imputation model for controls included sex, BMI, military service, smoking status, education, family history of ALS, all POPs, age at sample acquisition, and rate of change in BMI five years prior to study entry. The imputation model for cases included all variables in the control imputation model, in addition to bulbar versus non-bulbar onset, El Escorial criteria, ALSFRS-R at first visit, NIV use, and the cumulative hazard rate (Nelson-Aalen estimator) defined from diagnosis.<sup>11</sup> Twenty imputed datasets were generated using predictive mean matching for continuous variables (BMI and BMI rate of change over 5 years prior to study entry), polytomous logistic regression for categorical variables (education and smoking status), and logistic regression for binary variables (ALS family history and military service).

**Case/Control.**—For each POP, covariate adjusted logistic regression models assessed differences between interquartile range (IQR)-standardized, log-transformed POP measures and case/control status. Adjustment covariates for single POP models were age at sample acquisition, sex, education, BMI, and rate of BMI change over the five years prior to study. Rubin's rules pooled results across all 20 imputed datasets and Benjamini-Hochberg correction controlled the false discovery rate due to multiple testing.

To consider multiple POPs simultaneously, an ERS for ALS risk (ERS<sup>risk</sup>) was developed. Stacked adaptive elastic net,<sup>12</sup> used to understand the joint associations between POPs and case/control status and to select POPs linked to case/control status, determined the ERS weights. Outcome models were subsequently fit on each imputed dataset associating the resulting IQR-standardized ERS with case/control status and results were pooled using Rubin's rules.

A matched sensitivity analysis was also performed using the MatchIt package in R to better control for differences in sex distribution between cases and controls. Here, one-to-one case/control matching with replacement was exact on sex and age based on a caliper width of one year (i.e., matches must be within one year of one another). A geographic restriction was also placed on cases and controls to be within 160 kilometers of the study site. After obtaining matches, weighted logistic regression models with a quasibinomial link function were fit on each imputed dataset to assess the association between IQR-standardized ERS and case/control adjusted for education, BMI, and rate of change in BMI. Measures of uncertainty were obtained via bootstrap with 100 replicates for each imputed dataset and inference was pooled across imputed datasets using Rubin's rules.

**Survival.**—For each POP, covariate adjusted Cox proportional hazards models assessed differences between IQR-standardized, log-transformed POP exposure and survival from diagnosis. Adjustment covariates were diagnosis age, sex, BMI, rate of BMI change over

the five years prior to study entry, bulbar versus non-bulbar onset, original or revised El Escorial criteria, log-transformed time between symptom onset and diagnosis, NIV use, education, military service, smoking status, and family history of ALS. Survival time in the Cox proportional hazards models was defined from diagnosis, with the endpoint being either an observed death or the last visit date. Benjamini-Hochberg correction was applied to adjust for multiple testing. Rubin's rules were used to pool estimates across imputed datasets for all models. Next, a survival ERS (ERS<sup>survival</sup>), based on our prior publication<sup>7</sup> with updated imputation,<sup>12</sup> was constructed via stacked Cox ridge regression and the resulting IQR-standardized ERS was associated with survival post-diagnosis using Cox regression. The ERS<sup>survival</sup> model adjusted for age at diagnosis, sex, BMI, rate of BMI in over the five years prior to study entry, bulbar versus non-bulbar onset, original or revised El Escorial criteria, log-transformed time between symptom onset and diagnosis, NIV use, education, military service, smoking status, and family history of ALS.

## Results

### Participants and POPs Analysis

Samples from 164 ALS and 105 control participants were included (Table 1). Imputed values for cases were BMI (n=7), military service (n=2), education (n=4), ALSFRS-R (n=2), time from symptom onset to diagnosis (n=1). Imputed values for controls were BMI (n=12), military service (n=7), education (n=7). Cases were older, had higher proportion of males, and lower educational attainment versus controls. Otherwise, characteristics of cases did not differ from controls except for ALS-specific variables. The limits of detection and unadjusted POP concentration distributions are shown in Supplementary Table 1. Correlations of POP plasma measures were most pronounced among PCBs and PBDEs (Figure 1).

### ALS Risk

Results for single pollutant class models to case/control status are presented in Figure 2. Odds ratios (ORs) for PCBs were widespread, ranging from 0.95 for PCB-28/31 to 2.23 for PCB-209. PBDE ORs were more uniform, ranging from 1.23 for PBDE-99 to 1.37 for PBDE-153. OCP ORs ranged from 1.14 for pentachlorobenzene to 2.10 for cis-nonachlor. POPs with  $p < 0.05$  included PCB-157, PCB-167, PCB-194, PCB-202, PCB-206, PCB-208, PCB-209, alpha-hexachlorocyclohexane, trans-nonachlor, oxychlorane, cis- / trans- heptachlorepoxide, hexachlorobenzene, cis-nonachlor, and p,p'-dichlorodiphenyldichloroethylene (p,p'-DDE).

To test whether aggregated POPs associated with ALS status, mixture analyses were performed. The ERS<sup>risk</sup> (Supplemental Figure S1) was most strongly represented by the combination [determined by adaptive elastic net OR and standard deviation (SD)] of , alpha-hexachlorocyclohexane (OR = 1.167, SD = 1.00), hexachlorobenzene (OR = 1.14, SD = 1.14), trans-nonachlor (OR = 1.03, SD = 0.83), and cis-nonachlor (OR = 1.34, SD = 0.89). The combined effects of these POPs as a mixture associated with ALS using the ERS<sup>risk</sup> model: an increase from the 25<sup>th</sup> to 75<sup>th</sup> percentile of ERS<sup>risk</sup> based on the control

population corresponds to an OR = 2.58,  $p < 0.001$ . Therefore, POPs overall associated with ALS risk.

Given the imbalance of sex in the cohort, a matched analysis was performed, which matched 76 cases to 41 controls by replacement algorithm (Supplementary Table S2). The matched analysis for ALS risk showed that POPs significant ( $p < 0.05$ ) in the unmatched analysis had the same effects direction in the matched analysis, albeit with lower  $p$ -values due to a smaller sample size (Supplemental Figure S2). Further, the matched ERS<sup>risk</sup> OR = 2.08 (95%CI 0.62–6.93,  $p = 0.232$ ), again showed a positive mixture effect on ALS risk. Overall, results from the matched analysis were consistent with the unmatched models, supporting the findings from the unmatched analyses despite the imbalance in sex.

### ALS Survival

As per our previous work,<sup>7</sup> we hypothesized that each individual POP may play a small role on survival, but only the cumulative effect of all POPs expressed as an ERS<sup>survival</sup> would correlate with shorter survival. As anticipated, 24 of 36 POPs had a negative impact on ALS survival (hazard ratio (HR) > 1) (Figure 3). Of these, PBDE-47 and trans-nonachlor had a  $p < 0.05$ . Moreover, the ERS<sup>survival</sup> constructed from the cumulative effect of all POPs correlated, as expected, with shorter survival (ERS<sup>survival</sup> HR = 1.65,  $p = 0.001$ ) (Figure 3). Thus, the survival models recapitulate our earlier findings. Survival contour plots by continuous ERS<sup>survival</sup> are shown for the cohort (Supplementary Figure 3), highlighting that survival probability decreases as ERS<sup>survival</sup> increases. Survival plots by ERS quartile are also provided (Supplementary Figure 4) showing shorter survival of participants in Quartile 4 versus Quartile 1 (HR = 2.31, 95% CI 1.30–4.13,  $p = 0.005$ ), resulting in an additional 0.88 years of survival for participants in Quartile 1—the lowest exposure group.

### Discussion

We previously reported that higher plasma POPs concentrations are associated with greater ALS risk<sup>6</sup> and shorter survival.<sup>7</sup> Herein, we confirmed these results in an independent cohort, assaying POPs using updated MS/MS and GC/MS methods. Regarding ALS risk, associations were most significant for individual OCPs and with the cumulative ERS<sup>risk</sup>. Regarding survival, multiple POPs contributed incrementally to overall survival, as reflected by a multipollutant ERS<sup>survival</sup>, as we previously published.<sup>7</sup> Overall, this report recapitulates our prior studies<sup>6,7</sup> and strongly supports the concept that POPs may play an important role in ALS pathogenesis and progression in Michigan cohorts.

Of individual POPs, we identified that OCPs correlate the strongest to ALS risk and survival, aligned with multiple studies that identified pesticides as ALS risk factors. A recent meta-analysis found a pooled OR of 1.48 based on self-reported prior pesticide exposure and occupational history among 2,001 ALS participants across 24 largely retrospective case/control studies.<sup>13</sup> Thus, most reports relied on recall of prior exposures (e.g., direct report of pesticide exposure) or exposures inferred from various occupational settings (e.g., prior farming occupation), which is subject to recall bias and may lead to conflicting findings in the literature. Pesticide studies by geographic proximity face their own challenges, such as the difficulty in estimating exposures. A recent Italian study found



that proximity to agricultural land was not linked to ALS.<sup>14</sup> Conversely, an analysis of ALS cases in a commercial database linked to United States Geological Survey chemical application data found increased risk from closer proximity to herbicides glyphosate and 2,4-dichlorophenoxyacetic acid and insecticides carbaryl and chlorpyrifos.<sup>15</sup> In contrast to these retrospective studies, our previous<sup>6,7</sup> and current reports more accurately assessed exposures directly by quantitating pesticide levels in plasma, overcoming the limits of recall bias and proximity studies.

Our group previously reported higher OCPs in blood from ALS participants.<sup>6</sup> Of the OCPs, oxychlordane, cis-nonachlor, and p,p'-DDE are consistently elevated in ALS cases. These OCPs fall into the general category of insecticides and their metabolites, e.g., p,p'-DDE. Although the exact mechanisms of action of these compounds remain under study, they are all neurotoxicants and some target ion channels, such as voltage-gated sodium channels and GABA receptor-gated chloride channels.<sup>16</sup> For instance, elevated cis-chlordane decreases survival, alters Na<sup>+</sup>/K<sup>+</sup>-ATPase function, and impairs electrophysiology in human stem cell-derived motor neurons, and reduces motor neuron outgrowth in zebrafish embryos.<sup>16</sup> There is also a strong association between pesticide and herbicide exposure to Parkinson's disease.<sup>17</sup> Putative mechanisms include proteasome inhibition, altered ion channel function, and mitochondrial dysfunction and oxidative damage.<sup>17</sup> One toxic herbicide, paraquat, not identified in our study, induces nigrostriatal injury and oral administration results in a Parkinson's like syndrome in animals.<sup>17</sup> Similar mechanistic preclinical research is lacking for OCPs in ALS and constitutes a significant unmet need in the field.

We also examined PCBs and PBDEs. Although these compounds have largely been banned, their prior production, multitude of uses, environmental persistence, and bioaccumulation have led to ongoing exposures via air, water, and diet.<sup>18</sup> PCBs had diverse uses in hydraulic and heat-transfer fluids, lubricants, insulating media in transformers and capacitors, carbonless carbon paper, and additives in polymers, coatings, and adhesives.<sup>18</sup> There are 209 PCB congeners that vary in their pharmacokinetics and biological effects; however, exposures usually involve congener mixtures, complicating the specific species conferring risk.<sup>18</sup> In the current study, PCB ORs for ALS risk varied, aligned with our previous report,<sup>6</sup> which also showed a range of ORs by various PCBs that differ in chlorine position on the biphenyl backbone. The chlorination pattern of PCBs also influences developmental neurotoxicity, with reported changes on motor activity and cognition.<sup>19</sup> Mechanisms remain under study, but select PCBs bind to the aryl hydrocarbon receptor, altering gene expression linked to energy metabolism, lipid synthesis, xenobiotic metabolism, and immune function.<sup>20</sup> Aryl hydrocarbon receptor agonists can also induce expression and enhance the stability of TDP-43,<sup>21</sup> an RNA/DNA-binding protein that accumulates in neurons and is an ALS hallmark. Future studies are needed to reveal how select PCBs promote ALS risk, as these compounds remain a major environmental hazard.

Regarding PBDEs, the finding that PBDE-153 marginally enhanced ALS risk ( $p = 0.110$ ), aligned with our previous study.<sup>6</sup> PBDEs were specifically developed as flame retardants, and, despite a structural resemblance to PCBs, differ in exposure pathways, exposure timing, and use pattern.<sup>18</sup> Like PCBs, PBDE toxicity involves mixtures of multiple PBDE congeners. Potential mechanisms of PBDE-mediated neurotoxicity and neuronal apoptosis

include impaired  $\text{Ca}^{+2}$  homeostasis<sup>22</sup> and oxidative stress.<sup>23</sup> In mammary tissue, PBDEs can damage DNA and trigger cytokine production;<sup>24</sup> however, whether similar mechanisms occur in the nervous system remains unexplored. Overall, as for OCPs, insight into PCB- and PBDE-mediated pathological mechanisms in ALS are lacking, but constitute an important research avenue to advance understanding of disease pathogenesis.

As previously reported, the ERS provided the most robust measure of ALS survival.<sup>7</sup> Indeed, systematic reviews and meta-analyses report robust associations of a wide variety of neurological disorders with environmental risks, including Alzheimer's disease and dementia,<sup>25</sup> Parkinson's disease,<sup>26</sup> multiple sclerosis,<sup>27</sup> and autism.<sup>28</sup> Actual calculated ERSs based on lifestyle and reported exposures robustly associate with multiple cancers, especially when linked with genetic risk.<sup>29,30</sup> In addition to our own work,<sup>6,7</sup> the use of a quantitative ERS based on pollutant measurements from biofluids to predict the accuracy of disease risk and survival is a burgeoning application, including associating blood metals to metabolic syndrome,<sup>31</sup> urinary phthalate mixtures with preterm delivery,<sup>32</sup> urinary metals mixtures with heart rate,<sup>33</sup> and urinary metals with glucose homeostasis.<sup>34</sup> ERSs have been used to also capture other forms of exposure such as sex, history of infectious mononucleosis, and smoking status in the Genes and Environment in Multiple Sclerosis project,<sup>35</sup> which were linked to clinical and neuroimaging outcomes.<sup>36</sup>

Although the science of associating risk from POPs on ALS is in its infancy, POPs have an impact on planetary well-being and the Stockholm Convention on POPs was designed to address the health impacts of these chemicals.<sup>37</sup> Yet, POPs are detected throughout the globe in sediments, soil, ash, and the food supply and including areas where they were not used such as the Arctic and Antarctica.<sup>38</sup> Global monitoring of POPs from air samples indicates an overall decrease in concentrations,<sup>39</sup> although levels of some species, such as chlordane, heptachlor, and hexachlorobenzene, are on the rise.<sup>40</sup> POPs also accumulate in water bodies and climate change may impact exposure of wildlife as a result of ice melting and iceberg calving allowing trapped POPs to become reintroduced into the environment. In addition to POPs, there is an increasing focus of climate change and other exposures, such as air pollution on neurological diseases. Further, recent papers support the global interest in understanding exposures that impact ALS.<sup>3</sup>

The primary limitations of this study are the small sample size and that it was conducted at only a single center. In addition, this analysis represents a cross-sectional analysis and longitudinal studies are important future avenues for study. Controls were compensated for participation, which could lead to bias. Since we updated the method for detecting POPs, to reflect more current technology, the results here are not directly comparable to our prior publication.<sup>6</sup> Alternatively, the use of a new method may be considered a strength since it demonstrated POPs associations to ALS risk that occurred independent of method. The control population had a higher proportion of females versus the case population. However, the matched analysis found that the significant POPs had overall similar effects compared to the full study population. The controls were also younger than the cases, but again the matched analysis showed similar results. Additionally, POPs have very long clearance or elimination half-lives depending on factors such as the compound and dose. Elimination half-life estimates for PCBs range from about 6 months for lower chlorinated



congeners to potentially over 100 years for more persistent compounds,<sup>41</sup> although most estimates do not extend beyond about 20 years. Results for OCPs are generally similar with recent data showing half-lives ranging between about 6 and 17 years for several representative pesticides.<sup>42</sup> Thus, even after multiple half-lives have elapsed, POPs will still be detectable in blood. This analysis also does not consider the impact of participant-specific genetics on the perturbations on causal pathways, although this is an important future direction. Strengths of this study include the creation of ERSs that go beyond single pollutant exposures to quantify the effects of multipollutant exposures. Further, measuring exposure biomarkers to calculate a quantitative ERS avoided recall bias associated with questionnaires.

In conclusion, we demonstrated that multipollutant risk and survival ERSs are associated with higher ALS risk and shorter ALS survival, respectively. Among the POPs, several OCPs had high individual ORs for ALS risk and shorter survival. These results confirm our initial reports from a separate cohort of ALS cases and controls<sup>6,7</sup> and suggest that quantitative assessments of environmental pollutants from available biofluids could identify persons at future risk of ALS. Replication in other non-Michigan cohorts is beneficial to determine the national and global distribution of this risk. Beyond ALS, the development of multipollutant ERSs for other neurodegenerative diseases could have similar broad implications for detecting early disease risk and implementing prevention strategies.<sup>5</sup>

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Data sharing

Sharing of non-identifiable data will be considered at the reasonable request of a qualified investigator. Request should be made to the corresponding author and requires the submission of a proposal to be approved by a study committee and an institutionally signed data sharing agreement.

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**What is already known on this topic –**

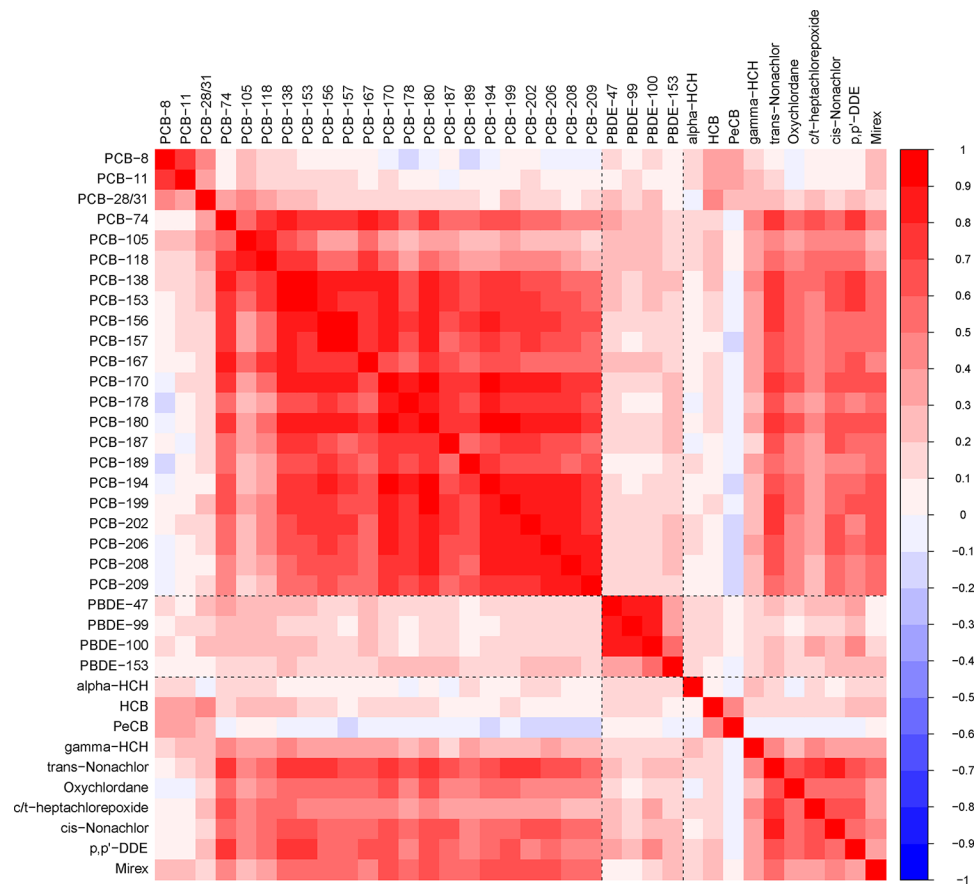
Environmental exposures are linked to amyotrophic lateral sclerosis (ALS) risk. A prior cohort demonstrated that persistent organic pollutants (POPs) associate with ALS risk and survival, but validation of these findings in a new cohort is needed.

**What this study adds –**

This new cohort again demonstrates that mixtures of POPs significantly associate with ALS risk and survival, providing validation of the originally published work.

**How this study might affect research, practice or policy –**

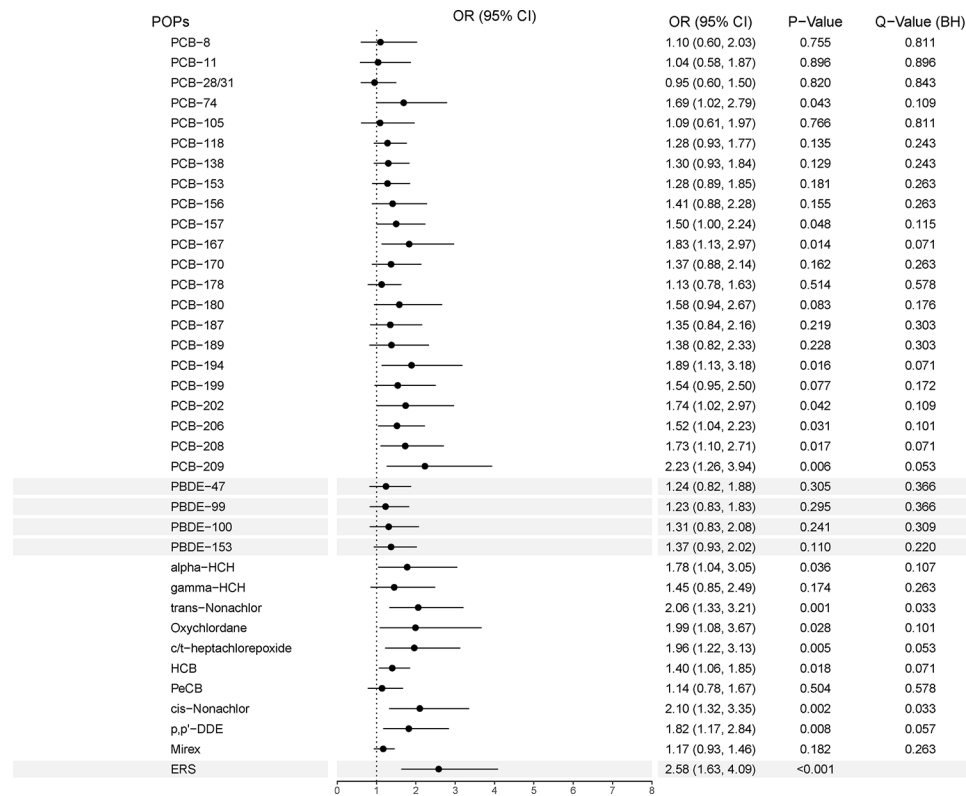
POPs are of a global concern and could play a role in the worldwide burden of ALS. These findings continue to support the need for more widespread assessments of POPs on ALS to help better define the global impact of POPs on ALS which could in turn be the basis of risk reduction efforts.



**Figure 1. POP correlations for cohort samples. Spearman correlations of POPs.**

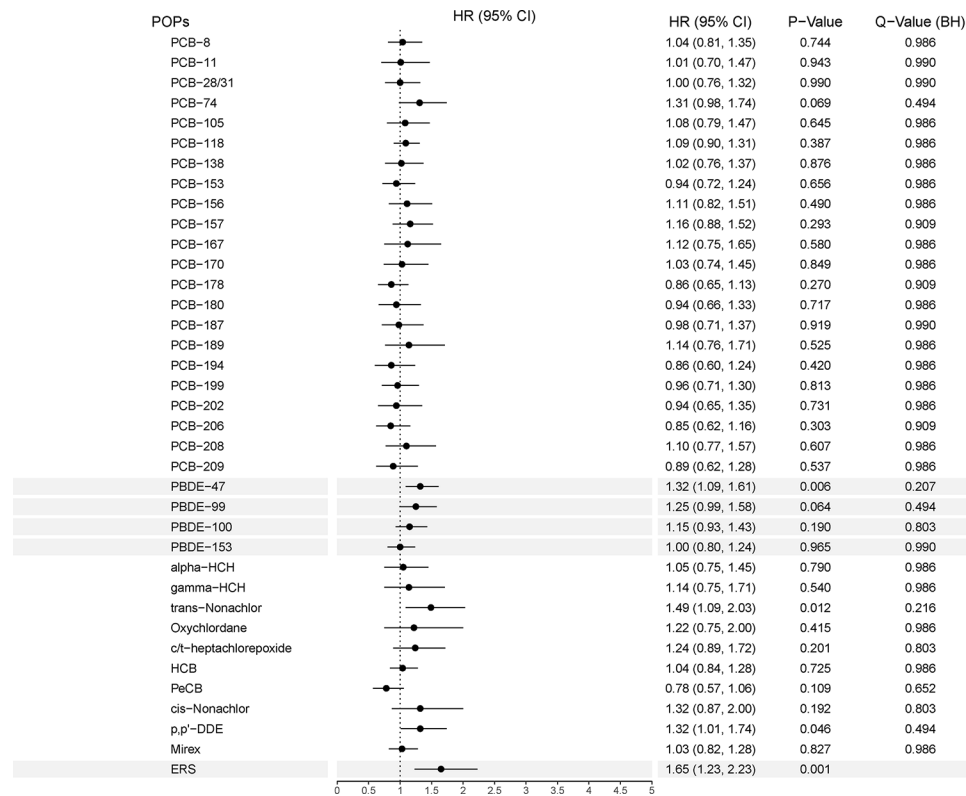
Correlations are strongest within the polychlorinated biphenyl and polybrominated diethyl ether groups. Red, positive correlation; Blue, negative correlation. Abbreviations: POP, persistent organic pollutant; PBDE, polybrominated diphenyl ether; PCB, polychlorinated biphenyl; HCH, hexachlorocyclohexane; HCB, hexachlorobenzene; PeCB, pentachlorobenzene; DDE, dichlorodiphenyldichloroethylene.





**Figure 2. Cohort adjusted single pollutant and mixture associations between POPs and case/control status.**

Single POP logistic regression models where the outcome is case/control status, the variables of interest are log-transformed, interquartile range-standardized POP concentrations, and the covariates are continuous age at sample acquisition, sex, education, continuous BMI, and continuous BMI slope. Abbreviations: BH, Benjamini-Hochberg; BMI, body mass index; CI, confidence interval; DDE, dichlorodiphenyldichloroethylene; ERS, environmental risk score; HCB, hexachlorobenzene; HCH, hexachlorocyclohexane; OCP, organochlorine pesticide; OR, odds ratio; PBDE, polybrominated diphenyl ether; PCB, polychlorinated biphenyl; PeCB, pentachlorobenzene; POP, persistent organic pollutant.



**Figure 3. Cohort survival analysis.**

One-at-a-time Cox proportional hazards model with the outcome of survival from diagnosis (in years), covariate of interest is log-transformed, interquartile range-standardized POPs, and adjustment covariates are log-transformed time between symptom onset and diagnosis, age at diagnosis, sex, onset segment, El Escorial criteria, BMI at survey consent, BMI slope over five years prior, education, military service, smoking status at first visit, family history of ALS, and NIV use. There are 163 cases for this model because one participant is missing a symptom onset date. Abbreviations: BH, Benjamini-Hochberg; BMI, body mass index; CI, confidence interval; DDE, dichlorodiphenyldichloroethylene; ERS, environmental risk score; HCB, hexachlorobenzene; HR, hazard ratio; OCP, organochlorine pesticide; PBDE, polybrominated diphenyl ether; PCB, polychlorinated biphenyl; PeCB, pentachlorobenzene; POP, persistent organic pollutant.

**Table 1.****ALS and Control Participant Demographics**

Covariate	Cases (N = 164)	Controls (N = 105)	P-Value
Age at sample collection (years)	65.3 (57.2–72.1)	62.4 (55.2–68.5)	0.016
Body Mass Index (kg/m <sup>2</sup> ) <sup>*</sup>	26.3 (23.2–30.1)	26.5 (23.4–29.7)	0.747
Sex			0.001
Female	82 (50.0)	74 (70.5)	
Male	82 (50.0)	31 (29.5)	
Military Service			0.414
No	148 (90.2)	93 (88.6)	
Yes	14 (8.5)	5 (4.8)	
Missing	2 (1.2)	7 (6.7)	
Education			<0.001
High School or Less	54 (32.9)	8 (7.6)	
Some Postsecondary	58 (35.4)	24 (22.9)	
Bachelor's Degree	29 (17.7)	36 (34.3)	
Graduate Degree	19 (11.6)	30 (28.6)	
Missing	4 (2.4)	7 (6.7)	
Race			NA
American Indian / Alaska Native	1 (0.6)	0 (0.0)	
Asian	3 (1.8)	3 (2.9)	
Black or African American	3 (1.8)	5 (4.8)	
White	157 (95.7)	97 (92.4)	
ALSFRS-R <sup>**</sup>	36 (31–41)		
Time Between Symptom Onset and Diagnosis (Years) <sup>***</sup>	1.02 (0.59–1.67)		
Time Between Diagnosis and Sample Acquisition (Years)	0.48 (0.26–0.94)		
Observed Death			
Yes	147 (89.6)		
No	17 (10.4)		
El Escorial Criteria			
Possible / Suspected	28 (17.1)		
Probable, Lab Supported	32 (19.5)		
Probable	47 (28.7)		
Definite	57 (34.8)		
Onset Segment			
Bulbar	55 (33.5)		
Cervical	49 (29.9)		
Lumbar	59 (36.0)		
Cannot be Determined	1 (0.6)		

Table of descriptive statistics for the study population. For continuous variables, median (25<sup>th</sup> – 75<sup>th</sup> percentile), and for categorical variables, N (%). P-values for continuous and categorical variables correspond to t-tests and chi-squared tests, respectively.

\* Body Mass Index is observed for 157 cases and 93 controls.

\*\* ALSFRS-R is observed for 162 cases.

\*\*\* Time between symptom onset and diagnosis is observed for 163 cases. ALSFRS-R, revised amyotrophic lateral sclerosis functional rating scale.

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