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Pop, heavy metal and the blues: secondary analysis of persistent organic pollutants (POP), heavy metals and depressive symptoms in the NHANES National Epidemiological Survey

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ABSTRACT

Objectives: Persistent environmental pollutants, including heavy metals and persistent organic pollutants (POPs), have a ubiquitous presence. Many of these pollutants affect neurobiological processes, either accidentally or by design. The aim of this study was to explore the associations between assayed measures of POPs and heavy metals and depressive symptoms. We hypothesised that higher levels of pollutants and metals would be associated with depressive symptoms.

Setting: National Health and Nutrition Examination Survey (NHANES).

Participants: A total of 15 140 eligible people were included across the three examined waves of NHANES.

Primary and secondary outcome measures: Depressive symptoms were assessed using the nine-item version of the Patient Health Questionnaire (PHQ-9), using a cut-off point of 9/10 as likely depression cases. Organic pollutants and heavy metals, including cadmium, lead and mercury, as well as polyfluorinated compounds (PFCs), pesticides, phenols and phthalates, were measured in blood or urine.

Results: Higher cadmium was positively associated with depression (adjusted Prevalence Ratios (PR) =1.48, 95% CI 1.16 to 1.90). Higher levels of mercury were negatively associated with depression (adjusted PR=0.62, 95% CI 0.50 to 0.78), and mercury was associated with increased fish consumption (n=5500, r=0.366, p<0.001). In addition, several PFCs (perfluorooctanoic acid, perfluorohexane sulfonic acid, perfluorodecanoic acid and perfluorononanoic acid) were negatively associated with the prevalence of depression.

Conclusions: Cadmium was associated with an increased likelihood of depression. Contrary to hypotheses, many of persistent environmental pollutants were not associated or negatively associated with depression. While the inverse association between mercury and depressive symptoms may be explained by a protective role for fish consumption, the negative associations with other pollutants remains unclear.

Strengths and limitations of this study

- Strengths of this study include the quality and size of the National Health and Nutrition Examination Survey (NHANES) database and the rigour of its measures.
- Owing to the vast number of potential persistent organic pollutants, we did not have the ability to assess all agents of relevance.
- Owing to the exploratory nature of this analysis and the absence of a singular a-priori target, the potential for type 1 errors should be noted.
- It is unclear if these data represent true neurobiological effects, or are proxy markers of residual confounding due to other operative variables.

This exploratory study suggests the need for further investigation of the role of various agents and classes of agents in the pathophysiology of depression.

BACKGROUND

Persistent environmental pollutants have a ubiquitous presence. Such environmental pollutants include the inorganic heavy metals and persistent organic pollutants (POPs). Easily transported by wind and water, these pollutants are found across the globe, often far from their original source.[1–4] While many POPs (eg, pesticides) were explicitly designed to have bioactive properties, the bioactive effects of many compounds were only discovered subsequent to their development and use.[5–7]

Many environmental pollutants are known to exert significant impacts on human health in a dose-dependent fashion. POPs, due to

their potential to bioaccumulate and biomagnify in human and animal tissues, appear to become progressively concentrated up the food chain.[8] POPs have documented adverse effects on a diversity of biological systems, and extensive exposure can cause illness and death. They can disrupt endocrine, reproductive and immune systems, are associated with risk for cancer and, germane to this report, are linked to neurobehavioural disorders. As an exemplar, in a recent study evaluating the neurotoxic effects of POPs, a Spanish birth cohort demonstrated that prenatal exposure to polychlorinated biphenyl-153 is associated with worse psychomotor development, supporting the importance of understanding how POPs interfere in brain development, in order to guide and develop environmental strategies to minimise the impact on brain health.[9]

Other POPs also exert effects on neuronal systems in preclinical models. Neurotoxic effects of hexachlorocyclohexane pesticides have been reported in animals after ingestion of a single dose, while prolonged exposure to low doses in rats resulted in significantly altered Skinner box behaviour (operant conditioning), and reduced nerve conduction velocity.[10 11] Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans, frequently referred to as 'dioxins', are associated with neurodevelopmental delays and subtle neurobehavioural effects, including neonatal hypotonia.[12] Bisphenol A (BPA), used in the lining of food containers, is implicated in neuroendocrine and developmental abnormalities.[13] Neurobehavioural effects following perinatal exposure are reported in animals (monkey, rat and mouse) and humans.[14] In rhesus monkey infants exposed in utero and during lactation, impaired learning ability and hyperactivity were reported.[12]

Some previous studies have investigated the relationship between exposure to environmental pollutants and neurobehavioural effects in humans. One study reported that solvent exposure was linked to an increased risk of anxiety and depressive disorders in adults.[15] In another cohort study of 1512 participants, prenatal and early childhood tetrachloroethylene contaminated drinking water exposure was associated with an increased risk of bipolar disorder, post-traumatic stress disorder and schizophrenia.[16] Exposure to organophosphate pesticides in farmers was linked to higher levels of somatisation, as well as higher blood levels of superoxide dismutase and lipid peroxidation and lowered total antioxidant capacity.[17] This may be germane to psychiatric health due to the demonstrated role of oxidative stress in a number of psychiatric disorders.[18 19] Data also have linked heavy metal exposure to mood disorders. Symptoms of manganese poisoning can include euphoria, apathy, hallucinations, flight of ideas, compulsivity agitation and verbosity; and symptoms of mercury poisoning can include irritability, poor concentration, memory deficiencies, anxiety and depression.[20 21] In two selected samples with a psychotic disorder or bipolar disorder, blood levels of cadmium and lead were found to be higher than in healthy control participants. Although very limited in scope, these data point to a possible impact of these pollutants in mental health.[22 23] Heavy metals such as cadmium, arsenic and lead are found in cigarettes, and this may be one of the multitudes of pathways whereby smoking may be detrimental to health.[24]

An understanding of which pollutants, if any, can affect the development of depressive symptoms is imperative given the widespread use of these pollutants and the substantial burden of disease imposed by unipolar depression.[25] The aim of this study, therefore, was to explore the associations between numerous assayed measures of POPs and heavy metals and depressive symptoms in the National Health and Nutrition Examination Survey (NHANES). We hypothesised positive relationships between POPs and heavy metals and depression.

METHODS
Participants
This study included adults who participated in one of the three latter waves (2005–2006, 2007–2008 and 2009–2010) of the NHANES, for which data is publicly available (http://www.cdc.gov/nchs/nhanes.htm). Of a sample of 18 318 individuals who participated in one of the three waves and were aged over 18 years, those who did not have a valid depression screen were excluded from the analyses, resulting in a sample of 15 760. The maximum sample size for analyses including missing data on pollutants was 15 140 participants. For total sample size according to class of pollutant see table 1.

Measurements
The outcome, depressive symptoms, was assessed using the nine-item depression module of the Patient Health Questionnaire (PHQ-9). The PHQ-9 is based on the Diagnostic and Statistical Manual of Mental Disorders Fourth Edition (DSM-IV) criteria for major depressive disorder and has been considered to be reliable and valid for use in the general population.[26] To indicate the presence of significant depressive symptoms we employed a cut-off point of 9/10.[27] This is largely recognised as the optimal trade-off between sensitivity and specificity.[28]

The exposures of interest included the following:

Heavy metals
Blood lead, cadmium and mercury were assessed in all three waves (n=16 691). Whole blood lead (Pb), cadmium (Cd) and mercury (Hg) concentrations were determined using inductively coupled plasma mass spectrometry.[29–31]

Polyfluorinated compounds
Levels of polyfluorinated compounds (PFCs) were measured in subsamples drawn from each of the three waves

Table 1 Sample sizes for different classes of pollutants measured in the three NHANES waves assessed

NHANES wave	Heavy metals (blood)	PFCs (blood)	Pesticides (urine)	Phthalates (urine)	Phenols (urine)	Organophosphate (urine)
2005–2006	4608	1513	1521	1521	1521	NA
2007–2008	5172	1689	1767	1767	1767	1753
2009–2010	5360	1726	1789	NA	1789	NA

NA, not applicable; NHANES, National Health and Nutrition Examination Survey.

(n=5442). The quantitative detection of perfluorooctane sulfonamide (PFOSA), 2-(N-methyl-perfluorooctanesulfonamido) acetic acid (Me-PFOSA-AcOH), 2-(N-ethyl-perfluorooctanesulfonamido) acetic acid (Et-PFOSA-AcOH), perfluorobutanesulfonate (PFBuS), perfluorohexanesulfonate (PFHxS), perfluorooctanesulfonate (PFOS), perfluoroheptanoate (PFHpA), perfluorooctanoate (PFOA), perfluorononanoate (PFNA), perfluorodecanoate (PFDeA), perfluoroundecanoate (PFUA), and perfluorododecanoate in serum was performed using solid phase extraction (SPE) coupled to high-performance liquid chromatography (HPLC)—turbo ion spray ionisation (TIS)—tandem mass spectrometry (MS/MS; online SPE-HPLC-TIS-MS/MS).[29–31]

Environmental pesticides and phenols

Urinary levels of pesticides and phenols were measured in subsamples drawn from each of the three waves (n=5588). The method employed SPE coupled online to HPLC and MS/MS for measuring phenols and pesticides. With the use of isotopically labelled internal standards, the sensitivity is sufficient for measuring urinary levels of phenols in non-occupationally exposed participants.[29–31]

Phthalates

Urinary phthalates were measured in subsamples drawn from the first two waves (n=3566). The test utilises HPLC—electrospray ionisation (ESI)—MS/MS (HPLC-ESI-MS/MS) for the quantitative detection of the following metabolites: monomethyl phthalate (mMP), monoethyl phthalate (mEP), monobutyl phthalate (mBP), mono-isobutyl phthalate (miBP), mono-(3-carboxypropyl) phthalate (mCPP), monocyclohexyl phthalate (mCHP), mono-(2-ethylhexyl) phthalate (mEHP), monooctyl phthalate (mOP), monobenzyl phthalate (mBzP), monoisononyl phthalate (mNP), mono-(2-ethyl-5-oxohexyl) phthalate (mEOHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (mEHHP), mono-(2-ethyl-5-carboxypentyl) phthalate (mECPP), monocarboxyoctyl phthalate (MCOP) and monocarboxynonyl phthalate (MCNP). Samples were then processed using enzymatic deconjugation of the glucuronidated phthalate monoesters followed by online SPE coupled with reversed HPLC-ESI-MS/MS.[29–31]

Organophosphates

Urinary organophosphates were measured in a subsample drawn from the 2007–2008 wave (n=2593). Urine samples were spiked with stable isotope analogues of the dialkyl phosphate (DAPs) metabolites and water was removed from the samples. The dried residue was dissolved in acetonitrile and diethyl ether, and the DAPs were chemically derivatised to their respective chloropropyl phosphate esters. The chloropropyl phosphate esters were concentrated, and analysed using gas chromatography—MS/MS (GC–MS/MS).[29–31]

Other exposures, such as demographic and socioeconomic characteristics, medical conditions, relevant biological measures and other lifestyle factors were available from the three mentioned waves of data collection. Income was documented and divided into four categories (less than US\$10 000/year, US\$10 000–US\$25 000, US\$25 000–US\$45 000 and over US\$45 000). The poverty index ratio (PIR) is based on the number of family members and annual family income, calculated using poverty thresholds provided by the US Census Bureau.[32] It was divided in quartiles as a further control variable. Country of birth and ethnicity was documented and grouped as USA versus other and Caucasian, Hispanic, African-American and other, respectively. Chronic medical conditions including cardiovascular and thyroid conditions, asthma, arthritis, liver disease, chronic obstructive pulmonary disease and cancer were self-reported. Smoking was assessed with a biomarker of smoke exposure, serum cotinine levels,[33] which allow for the additional control of secondhand smoke; it was also categorised in quartiles. Biological measurements comprised creatinine, cholesterol and glucose serum levels. Fish consumption was estimated at the 2005–2006 wave of data collection by Food Frequency Questionnaire.[34] We included this variable due to mercury accumulation in some fish species.

Statistical analysis

The three continuous waves of NHANES were combined according to the Centre for Disease Control and Prevention (CDC) instructions to achieve maximum statistical power.[29–31] Multivariate models were developed to identify factors associated with the presence of significant depressive symptoms, incorporating potential confounders including socioeconomic status, ethnicity, comorbid medical illnesses and relevant biological measurements. We conducted four different hierarchical models; unadjusted; adjusted for income, the PIR, country of birth, ethnicity, gender and age bands (categorised in 10-year bins; Model 1), the latter plus medical

conditions and smoking exposure (Model 2) and all plus biological measures (Model 3). Prevalence ratios (PR) for depression were calculated using binary Poisson regression with robust estimation of SEs.[35] Furthermore, Spearman rank correlation was used to test the association between mercury and total fish consumption levels. The distribution of measured pollutants were extremely skewed, therefore the data were categorised into quartiles, as in previous manuscripts based on NHANES data.[36–39]

RESULTS

The prevalence of likely depression, adjusted for the sample design was 7.1% (5.4% in the 2005–2006 wave, 8.1% in the 2007–2008 wave and 7.7% in the 2009–2010 wave). Characteristics for the whole group and those with and without depression are shown in table 2.

Table 3 shows associations between blood heavy metals and depression. Serum cadmium was associated with a higher prevalence of depression (adjusted (Adj) PR=1.48, 95% CI 1.16 to 1.90) after adjustment for confounders. There was no association between depression and lead. Mercury was associated with a lower prevalence of depression (Adj PR=0.62, 95% CI 0.50 to 0.78). Frequency of fish consumption was associated with total circulating mercury (n=5500, r=0.366, p<0.001).

Several PFCs (table 4) were associated with a lower prevalence of depression; perfluorooctanoic acid (Adj PR=0.63, 95% CI 0.44 to 0.89), perfluorohexane sulfonic acid (Adj PR=0.67, 95% CI 0.49 to 0.92), perfluorodecanoic acid (Adj PR=0.62, 95% CI 0.45 to 0.86) and perfluorononanoic acid (Adj PR=0.63, 95% CI 0.43 to 0.92). With the exception of the phenols, benzophenone-3 (Adj PR=0.55, 95% CI 0.34 to 0.88), triclosan (Adj PR=0.66, 95% CI 0.47 to 0.93) and ethyl

paraben (Adj PR=0.76, 95% CI 0.57 to 1.00), there were no associations between the phthalates, pesticides and organophosphates and depression (tables 4–6).

DISCUSSION

In this study, cadmium was associated with an increased likelihood of depression. However, contrary to our hypothesis, other heavy metals and POPs demonstrated either no association or negative associations with likely depression.

Heavy metals are very well-established neurotoxins, with extensive data on mercury, lead and other agents. They can interfere with major cellular systems at levels well below those causing classic signs of toxicity.[40 41] Cadmium induces neurotoxicity via multiple pathways, including interference with the blood–brain barrier, increases in oxidative stress, interference with zinc and calcium-dependent processes and metallothionein and induction of apoptosis. Intriguingly, many of these pathways are now known to be intimately involved in the pathophysiology of mood disorders.[42] Cadmium ions bind to mitochondria and can inhibit respiration and oxidative phosphorylation.[40] Furthermore, cadmium has been associated with synaptic malfunction through attenuation of Na(+)-dependent glutamate uptake and blockage of voltage-gated Ca(2+).[43] It is worth mentioning that ketamine, a N-methyl-D-aspartate receptor antagonist that, in turn, increases presynaptic glutamate release,[44] has emerged as a new drug for depression treatment.[44] Similarly, lead impacts many pathways known to be operative in the genesis of depression, including neurogenesis and apoptosis, oxidative stress and glutathione, glutamate, calcium and calmodulin and neurotransmitters such as acetylcholine.[18 19 45 46] In one study examining mental health in individuals exposed via

Table 2 Characteristics of the sample divided for the presence of significant depressive symptoms (PHQ9>9)

	No depressive symptoms	Depressive symptoms	Total sample
Female sex (%)	49.6	63.7	50.8
Age*	46 (31–63)	46 (33–58)	46 (31–63)
Non-Caucasian ethnicity (%)	51.8	57.3	52.2
Born outside USA (%)	24.9	18.1	24.3
Yearly family income over 45k (%)	44.9	23.5	43.1
Cardiovascular illness (%)	9.8	17.8	10.5
Chronic obstructive pulmonary disease (%)	6.3	17.4	7.3
Cancer (%)	9.3	11	9.5
Liver disease (%)	3	7.7	3.4
Arthritis (%)	26.3	43.2	27.8
Asthma (%)	12.9	23.3	13.8
Thyroid disease (%)	9	14.9	9.5
Family PIR*	2.27 (1.17–4.18)	1.23 (0.73–2.27)	1.82 (0.96–3.60)
Cholesterol levels*	192 (166–221)	193 (166–224)	191 (165–221)
Creatinine levels*	0.87 (0.72–1.01)	0.80 (0.70–0.97)	0.85 (0.72–1.00)
Glucose levels*	92 (84–103)	93 (85–106)	92 (84–103)

*Continuous measures shown as median (IQR).
PHQ-9, nine-item depression module of the Patient Health Questionnaire; PIR, poverty index ratio.

Table 3 Prevalence ratios for upper quartile (with first quartile used as reference) for multivariate associations between blood heavy metals and depression

	Unadjusted n=15 140	Model 1* n=14 016	Model 2† n=12 807	Model 3‡ n=12 746
Cadmium	2.40 (1.93 to 3.00)	1.89 (1.49 to 2.40)	1.49 (1.16 to 1.91)	1.48 (1.16 to 1.90)
Lead	0.95 (0.81 to 1.13)	0.98 (0.78 to 1.25)	0.79 (0.62 to 1.00)	0.81 (0.64 to 1.02)
Mercury	0.45 (0.36 to 0.56)	0.56 (0.45 to 0.70)	0.62 (0.50 to 0.78)	0.62 (0.50 to 0.78)

*Adjusted for demographics and socioeconomic status (age, sex, poverty, family income, ethnicity and country of birth).
†Adjusted for the above, cotinine levels and chronic medical illness.
‡Adjusted for the above and blood levels of cholesterol, glucose and creatinine.

mining to heavy metals, including cadmium, zinc, lead and copper, higher total scale scores on the Symptom Checklist 90 (SCL-90) were seen in the exposed compared with the control group, and ratings on somatisation, obsessive–compulsive, interpersonal sensitivity, depression, anxiety, hostility, paranoid ideation, psychoticism and other symptoms were higher in the exposed individuals.[47] In a clinical study, higher levels of cadmium and lead were found in individuals with depression, and higher levels of lead in those with schizophrenia.[48] Lower selenium is linked to depression risk.[49]

Contrary to expectations, mercury was inversely correlated with depression in our study. This finding requires replication and further exploration, as this relationship may be secondary to other factors associated with mercury consumption. It is thought that mercury consumption may be a proxy of seafood consumption, which may itself be the operative factor, or may be a proxy of factors such as socioeconomic status and omega 3 intake.[8 50] Thus, the protective effect of fish consumption, which has been shown to be associated with reduced depression risk, may outweigh any potential

negative contribution of mercury to depression prevalence.[51]

That several PFCs were associated with a lower risk of depression was an unexpected finding, given many of these compounds have shown significant neurodevelopmental effects.[52] Perfluorooctanoic acid belongs to the family of perfluorinated compounds, which are used extensively in industrial and consumer applications, including food packaging, clothing, fabrics and carpets. They are ubiquitous in the environment, being found in dust and human milk. Prenatal exposure is linked to developmental sequelae.[53] Perfluorooctanoic acid also alters brain proteins including CaMKII, GAP-43 and synaptophysin that have critical roles in neuronal growth and synaptogenesis.[54] Prenatal exposure results in neurobehavoural deficits in adult mice, including hyperactivity, altered spontaneous behaviour and impaired habituation, features found in many primary psychiatric disorders.[55] These effects were shown to worsen with age.

Moreover, perfluorooctane sulfonic acid perturbs the functioning of motor neurones by altering α-Tubulin and proliferating cell nuclear antigen.[56] It also induces

Table 4 Prevalence ratios for upper quartile (with first quartile used as reference) for multivariate associations between blood polyfluorinated compounds and depression

	Unadjusted n=5026	Model 1* n=4645	Model 2† n=4272	Model 3‡ n=4266
Perfluorooctanoic acid	0.49 (0.33 to 0.71)	0.66 (0.46 to 0.93)	0.61 (0.43 to 0.0.87)	0.63 (0.44 to 0.89)
Perfluorohexane sulfonic acid	0.49 (0.36 to 0.66)	0.66 (0.47 to 0.93)	0.66 (0.47 to 0.93)	0.67 (0.49 to 0.92)
2-(N-ethyl-PFOSA) acetate§	0.62 (0.31 to 1.26)	0.77 (0.39 to 1.50)	0.73 (0.38 to 1.41)	0.72 (0.38 to 1.35)
2-(N-methyl-PFOSA) acetate	0.84 (0.55 to 1.26)	0.94 (0.63 to 1.42)	0.79 (0.55 to 1.28)	0.82 (0.57 to 1.17)
Perfluorodecanoic acid	0.55 (0.40 to 0.75)	0.64 (0.48 to 0.86)	0.62 (0.45 to 0.85)	0.62 (0.45 to 0.86)
Perfluorobutane sulfonic acid¶	0.89 (0.27 to 2.86)	1.29 (0.45 to 3.70)	1.47 (0.51 to 4.19)	1.50 (0.52 to 4.29)
Perfluoroheptanoic acid§	0.89 (0.68 to 1.18)	0.62 (0.32 to 1.22)	0.52 (0.24 to 1.11)	0.53 (0.25 to 1.14)
Perfluorononanoic acid	0.61 (0.43 to 0.87)	0.64 (0.41 to 0.99)	0.62 (0.42 to 0.92)	0.63 (0.43 to 0.92)
Perfluorooctane sulfonamide¶	0.58 (0.37 to 0.90)	0.93 (0.58 to 1.50)	0.89 (0.55 to 1.43)	0.89 (0.54 to 1.45)
Perfluoroundecanoic acid§	0.60 (0.40 to 0.91)	0.68 (0.44 to 1.05)	0.73 (0.47 to 1.12)	0.72 (0.47 to 1.09)
Perfluorododecanoic acid§	0.95 (0.69 to 1.30)	0.80 (0.39 to 1.37)	0.80 (0.39 to 1.63)	0.83 (0.41 to 1.69)
Perfluorooctane sulfonic acid	0.52 (0.29 to 0.66)	0.60 (0.34 to 1.05)	0.65 (0.38 to 1.12)	0.68 (0.39 to 1.16)

*Adjusted for demographics and socioeconomic status (age, sex, poverty, family income, ethnicity and country of birth).
†Adjusted for the above, cotinine levels and chronic medical illness.
‡Adjusted for the above and blood levels of cholesterol, glucose and creatinine.
§Only three groups formed because of the lack of granularity in data.
¶Only two groups formed because of the lack of granularity in data.

Table 5 Prevalence ratios for upper quartile (with first quartile used as reference) for multivariate associations between urinary phthalates and phenols and depression

Phthalates	Unadjusted n=3342	Model 1* n=3117	Model 2† n=2747	Model 3‡ n=2710
Monocarboxynonyl Phthalate	0.91 (0.57 to 1.46)	1.04 (0.68 to 1.59)	0.96 (0.60 to 1.55)	0.93 (0.58 to 1.48)
Monocarboxyoctyl Phthalate	0.91 (0.56 to 1.48)	1.06 (0.69 to 1.63)	1.08 (0.68 to 1.71)	1.05 (0.66 to 1.66)
Mono-2-ethyl-5-carboxypentyl phthalate	0.84 (0.51 to 1.38)	0.89 (0.53 to 1.51)	0.76 (0.42 to 1.35)	0.76 (0.42 to 1.37)
Mono-N-butyl phthalate	1.65 (0.98 to 2.76)	1.35 (0.78 to 2.36)	1.17 (0.63 to 2.17)	1.14 (0.65 to 1.99)
Mono-(3-carboxypropyl) phthalate	1.34 (0.82 to 2.21)	1.45 (0.88 to 2.40)	1.20 (0.67 to 2.13)	1.18 (0.67 to 2.07)
Monoethyl phthalate	1.48 (0.94 to 2.34)	1.26 (0.76 to 2.08)	1.18 (0.72 to 1.96)	1.28 (0.77 to 2.11)
Mono-(2-ethyl-5-hydroxyhexyl) phthalate	0.94 (0.55 to 1.61)	0.96 (0.55 to 1.68)	0.86 (0.46 to 1.59)	0.79 (0.43 to 1.45)
Mono-(2-ethyl)-hexyl phthalate	0.91 (0.50 to 1.63)	0.93 (0.50 to 1.73)	0.94 (0.48 to 1.85)	0.96 (0.49 to 1,87)
Mono-N-methyl phthalate§	0.86 (0.57 to 1.30)	0.82 (0.56 to 1.20)	0.72 (0.52 to 0.98)	0.73 (0.54 to 1.01)
Mono-isononyl phthalate¶	1.27 (0.65 to 2.50)	1.45 (0.76 to 2.79)	1.50 (0.73 to 3.06)	1.38 (0.70 to 2.73)
Mono-(2-ethyl-5-oxohexyl) phthalate	0.93 (0.53 to 1.63)	0.99 (0.55 to 1.78)	0.89 (0.45 to 1.78)	0.84 (0.44 to 1.59)
Mono-benzyl phthalate	1.51 (0.85 to 2.69)	1.28 (0.66 to 2.46)	1.02 (0.48 to 2.14)	0.99 (0.52 to 1.88)
Mono-isobutyl phthalate	1.54 (0.88 to 2.69)	1.34 (0.77 to 2.33)	1.13 (0.58 to 2.18)	1.11 (0.60 to 2.06)
Phenols	**n=5160**	**n=4775**	**n=4202**	**n=4185**
Bisphenol A	1.44 (0.98 to 2.10)	1.22 (0.80 to 1.85)	1.00 (0.64 to 1.55)	0.94 (0.60 to 1.46)
Benzophenone-3	0.47 (0.33 to 0.67)	0.54 (0.37 to 0.80)	0.55 (0.34 to 0.88)	0.55 (0.34 to 0.88)
4-tert-octylphenol¶	1.47 (10.7 to 2.02)	1.35 (0.95 to 1.92)	1.35 (0.94 to 1.95)	1.34 (0.93 to 1.94)
Triclosan	0.52 (0.40 to 0.69)	0.63 (0.47 to 0.84)	0.65 (0.46 to 0.91)	0.66 (0.47 to 0.93)
Butyl paraben§	1.00 (0.72 to 1.41)	0.92 (0.66 to 1.28)	0.94 (0.66 to 1.34)	0.96 (0.67 to 1.37)
Ethyl paraben§	0.83 (0.62 to 1.09)	0.74 (0.56 to 0.99)	0.73 (0.55 to 0.97)	0.76 (0.57 to 1.00)
Methy paraben	1.04 (0.73 to 1.48)	0.75 (0.49 to 1.15)	0.77 (0.51 to 1.18)	0.76 (0.50 to 1.14)
Propyl paraben	1.13 (0.87 to 1.64)	0.91 (0.59 to 1.40)	1.03 (0.68 to 1.58)	1.02 (0.67 to 1.54)

*Adjusted for demographics and socioeconomic status (age, sex, poverty, family income, ethnicity and country of birth).
†Adjusted for the above, cotinine levels and chronic medical illness.
‡Adjusted for the above and blood levels of cholesterol, glucose and creatinine.
§Only three groups formed because of the lack of granularity in data.
¶Only two groups formed because of the lack of granularity in data.

brain oxidative stress by dysregulating cyclin-dependant kinase 5 and peroxiredoxin, and causes an inflammatory glial response.[57] It impairs calcium signalling,[58] and interferes with gene transcription related to neuroactive ligand-receptor interaction, the cell cycle, cell communication, long-term potentiation and depression, and peroxisome proliferator-activated receptor (PPAR) signalling.[59] Neurotoxicity has been shown even after a

Table 6 Prevalence ratios for upper quartile (with first quartile used as reference) for multivariate associations between urinary organophosphate pesticides and depression

Pesticides	Unadjusted n=5160	Model 1* n=4775	Model 2† n=4202	Model 3‡ n=4185
2,5-dichlorophenol	1.76 (1.29 to 2.39)	1.29 (0.90 to 1.85)	1.17 (0.78 to 1.74)	1.18 (0.80 to 1.73)
O-phenyl phenol§	1.38 (0.97 to 1.97)	1.27 (0.91 to 1.80)	1.21 (0.86 to 1.70)	1.12 (0.80 to 1.56)
2,4-dichlorophenol	1.35 (1.04 to 1.76)	1.10 (0.82 to 1.49)	1.04 (0.74 to 1.46)	1.04 (0.74 to 1.46)
2,4,5-trichlorophenol§	1.29 (0.92 to 1.80)	1.14 (0.77 to 1.68)	1.08 (0.72 to 1.63)	1.09 (0.72 to 1.66)
2,4,6-trichlorophenol	1.21 (0.82 to 1.77)	1.02 (0.70 to 1.47)	1.01 (0.67 to 1.52)	1.01 (0.67 to 1.52)
Organophosphates	**n=1785**	**n=1627**	**n=1472**	**n=1465**
Dimethylphosphate§	0.96 (0.53 to 1.74)	1.26 (0.73 to 2.19)	1.20 (0.78 to 1.85)	1.20 (0.77 to 1.86)
Diethylphosphate§	0.85 (0.51 to 1.42)	0.96 (0.58 to 1.57)	0.99 (0.55 to 1.79)	0.98 (0.55 to 1.75)
Dimethylthiophosphate	0.69 (0.48 to 0.99)	0.89 (0.51 to 1.55)	0.98 (0.52 to 1.83)	1.02 (0.52 to 1.99)
Diethylthiophosphate§	0.65 (0.41 to 1.01)	0.88 (0.58 to 1.34)	1.07 (0.73 to 1.55)	1.08 (0.76 to 1.55)
Dimethyldithiophosphate¶	0.81 (0.49 to 1.35)	1.01 (0.65 to 1.57)	1.02 (0.69 to 1.51)	1.01 (0.66 to 1.55)

*Adjusted for demographics and socioeconomic status (age, sex, poverty, family income, ethnicity and country of birth).
†Adjusted for the above, cotinine levels and chronic medical illness.
‡Adjusted for the above and blood levels of cholesterol, glucose and creatinine.
§Only three groups formed because of the lack of granularity in data.
¶Only two groups formed because of the lack of granularity in data.

single exposure.[60] Perfluorodecanoic acid stimulated the proliferation of glioblastoma cell growth, although the behavioural effects of this agent are unknown.[58]

Polyfluoroalkyl chemicals (PFCs), which are widely used in consumer products, are associated with diverse adverse health impacts. There is a report of human neurotoxic effects of perfluorooctane sulfonic acid and perfluorooctanoic acid exposure. Among 1400 pairs of pregnant women and their offspring who were randomly selected from the Danish National Birth Cohort, there was an association between delayed gross motor development in infancy and maternal perfluorooctane sulfonic acid levels, although levels were not associated with delays in other developmental milestones, nor attention.[61] In another epidemiological study, perfluorooctane sulfonic acid, perfluorooctanoic acid and perfluorohexane sulfonic acid but not perfluorononanoic acid, exposure was linked to attention deficit hyperactivity in adolescents.[62]

There are no published reports on perfluoroundecanoic acid, 2,5-dichlorophenol or O-phenyl phenol and the brain or neurotoxicicity. However, BPA is capable of altering neuronal function via multiple pathways, and developmental exposure to even low doses of BPA during development results in impairment of neurobehavioural development and results in enduring impacts on brain structure, function and behaviour in animal studies. Nanomolar doses of BPA can alter hippocampal long-term potentiation, resulting in significant effects on memory.[63] BPA exposure in animal models results in hyperactivity, as well as disturbed short-term and long-term memory with adult or prenatal exposure.[64 65] This can be ameliorated by administration of N-acetyl cysteine.[66] There are case reports of BPA exposure and neurobehavioural abnormalities in humans, particularly excitability, lethargy and stress abstinence, associated with dietary exposure to food heated in plastics.[67] In adolescent inner city children, phthalate exposure was linked to poorer social cognition, social communication and social awareness.[68] BPA exposure has been putatively linked to schizophrenia.[69] A relationship between increased hyperactivity and aggression in 2-year olds and prenatal BPA exposure has been suggested.[70] For these reasons, we had hypothesised a positive relationship between these agents and depression; however, these relationships were not observed and, indeed, the inverse was observed in many instances. It is not clear why this may be the case.

This study has many strengths but also some limitations. The strengths of this study include the quality and size of the NHANES database and the rigour of its measures. However, given the vast number of potential POPS, we did not have the ability to assess all agents of relevance. Moreover, given the exploratory nature of this analysis and the absence of a singular a-priori target, the potential for type 1 errors should be noted. It also remains to be clarified if these data represent true neurobiological effects, or are proxy markers of other

operative variables. Different pollutants have a tendency to cluster differently with important socioeconomic variables. Pesticides, for instance, are associated with a lower income.[71] PFCs, which come from domestic utensils, are associated with the higher end of the income spectrum. This was nicely demonstrated in our analysis. Although we attempted to control for such effects, we cannot exclude that residual confounding is ultimately responsible for the associations we found between PFCs and depression.[72]

CONCLUSION

In this large, population-based study of adults, cadmium levels were positively associated with depression; however, other investigated POPs and heavy metals were either not associated or inversely associated with depression. This was in contrast to our hypotheses and the explanation for these negative findings remains uncertain. Nevertheless, this study provides tantalising clues about agents and classes of agents requiring closer analysis.

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