Title

Prenatal and childhood exposure to organophosphate pesticides and functional brain imaging in young adults

Authors

Sagiv, Sharon K

Baker, Joseph M

Rauch, Stephen

et al.

Publication Date

2023-11-01

DOI

10.1016/j.envres.2023.117756

Environmental Research 242 (2024) 117756

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Prenatal and childhood exposure to organophosphate pesticides and functional brain imaging in young adults

Sharon K. Sagiv [a,*], Joseph M. Baker [b], Stephen Rauch [a], Yuanyuan Gao [b], Robert B. Gunier [a], Ana M. Mora [a], Katherine Kogut [a], Asa Bradman [a,c], Brenda Eskenazi [a,1], Allan L. Reiss [b,d,e,1]

[a] Center for Environmental Research and Community Health (CERCH), School of Public Health, University of California at Berkeley, Berkeley, CA, USA
[b] Center for Interdisciplinary Brain Sciences Research, Department of Psychiatry and Behavioral Sciences, School of Medicine, Stanford University, Stanford, CA, USA
[c] Department of Public Health, University of California, Merced, CA, USA
[d] Department of Radiology, School of Medicine, Stanford University, Stanford, CA, USA
[e] Department of Pediatrics, School of Medicine, Stanford University, Stanford, CA, USA

ARTICLE INFO

Keywords:
Organophosphates
Dialkyl phosphates
Prenatal
Neurodevelopment
Neuroimaging
fNIRS

ABSTRACT

Background: Early life exposure to organophosphate (OP) pesticides has been linked with poorer neurodevelopment from infancy to adolescence. In our Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) birth cohort, we previously reported that residential proximity to OP use during pregnancy was associated with altered cortical activation using functional near infrared spectroscopy (fNIRS) in a small subset (n = 95) of participants at age 16 years.

Methods: We administered fNIRS to 291 CHAMACOS young adults at the 18-year visit. Using covariate-adjusted regression models, we estimated associations of prenatal and childhood urinary dialkylphosphates (DAPs), non-specific OP metabolites, with cortical activation in the frontal, temporal, and parietal regions of the brain during tasks of executive function and semantic language.

Results: There were some suggestive associations for prenatal DAPs with altered activation patterns in both the inferior frontal and inferior parietal lobes of the left hemisphere during a task of cognitive flexibility (β per ten-fold increase in DAPs = 3.37; 95% CI: −0.02, 6.77 and β = 3.43; 95% CI: 0.64, 6.22, respectively) and the inferior and superior frontal pole/dorsolateral prefrontal cortex of the right hemisphere during the letter retrieval working memory task (β = −3.10; 95% CI: −6.43, 0.22 and β = −3.67; 95% CI: −7.94, 0.59, respectively). We did not observe alterations in cortical activation with prenatal DAPs during a semantic language task or with childhood DAPs during any task.

Discussion: We observed associations of prenatal OP concentrations with mild alterations in cortical activation during tasks of executive function. Associations with childhood exposure were null. This is reasonably consistent with studies of prenatal OPs and neuropsychological measures of attention and executive function found in CHAMACOS and other birth cohorts.

1. Introduction

Early life exposure to organophosphate (OP) pesticides has been linked with poorer neurodevelopment in children, which is concerning given the widespread use of these pesticides in the U.S. and worldwide (Grube et al., 2011). In our Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) birth cohort study, we have reported associations of prenatal OP exposure with poorer cognitive function and executive function, and more attention and behavior problems from birth through age 18 years (Eskenazi et al., 2007; Marks et al., 2010; Sagiv et al., 2021, 2023). While we have observed adverse neurodevelopment with prenatal OP pesticide exposure with notable consistency in CHAMACOS, our knowledge of the neural mechanisms underlying these associations remains limited.

Applying neuroimaging to studying the impact of environmental exposures could help elucidate which structures and functions in the

* Corresponding author. Center for Environmental Research and Community Health (CERCH) School of Public Health University of California at Berkeley 1995 University Avenue, Suite 265 Berkeley, CA 94720, USA.
E-mail addresses: sagiv@berkeley.edu (S.K. Sagiv), eskenazi@berkeley.edu (B. Eskenazi), areiss1@stanford.edu (A.L. Reiss).
[1] Co- Senior authos

https://doi.org/10.1016/j.envres.2023.117756
Received 19 September 2023; Received in revised form 15 November 2023; Accepted 20 November 2023
Available online 26 November 2023

brain are targeted by these exposures and reveal associations with brain function that may have gone undetected in studies using standard neurobehavioral tests. To date, few studies have employed neuroimaging to examine the impact of early life OP pesticide exposure on the brain. A cohort of 40 New York City children found associations of prenatal exposure to the OP chlorpyrifos with reduced cortical thickness of frontal, temporal, and parietal regions using structural magnetic resonance imaging (MRI) (Rauh et al., 2012). In a previous study, conducted in a subset of 95 16-year-old CHAMACOS participants without prenatal biomarkers, we found that residential proximity to OP pesticide use during pregnancy was associated with altered brain activation patterns observed with functional near infrared spectroscopy (fNIRS) during tasks of executive function (Sagiv et al., 2019). Both of these studies of neuroimaging were small, however, and require corroboration of the relationship of exposure to OPs with neuroimaging endpoints in a larger number of participants.

In this study, we investigated associations of a non-specific OP metabolite, dialkylphosphates (DAPs), measured in urine collected twice during pregnancy (at 13 and 26 weeks), and five times in childhood (ages 6 months, 1, 2, 3, and 5 years), in relation to neural activation patterns among 291 Mexican American young adults (18–19 years old) from the CHAMACOS birth cohort study. We hypothesized that early life exposure to OPs, measured by urinary DAPs, is associated with alterations in cortical activation in young adulthood, as measured by fNIRS.

2. Methods

2.1. Study population and recruitment methods

We have reported an in depth description of recruitment and follow-up procedures for our CHAMACOS birth cohort study in previous work (Eskenazi et al., 2007; Bradman et al., 2005). In brief, between the years 1999 and 2000, we recruited pregnant women 18 years or older who were less than 20 weeks gestation, Spanish- or English-speaking, qualified for low-income health insurance, and attended prenatal care visits at one of six local community or hospital clinics serving farmworkers. Of the 601 women recruited, 532 were followed to delivery of 537 live born infants (including 5 twin sets) in 2000–2001. We lost a large proportion of families (19%) between birth and 6 months (many moved away from the area during this time). However, our cohort maintenance efforts resulted in an average of over 96% retention in subsequent biennial visits, with 317 youth (60% of the original live born cohort) participating in the 18-year study visit, when fNIRS data collection was conducted. We excluded one participant with hydrocephaly. We note that the youth in this analysis are separate from the subset of 95 youth who completed fNIRS at age 16 years (Sagiv et al., 2019).

We obtained written informed consent from all mothers and youth. Study activities were approved by the UC Berkeley Committee for the Protection of Human Subjects.

2.2. OP pesticide exposure assessment

We assessed exposure to OP pesticides by measuring dialkyl phosphate (DAP) metabolites in urine collected twice during pregnancy (at approximately 13- and 26-weeks gestation) and several times during early childhood (at the 6-month, 1-year, 2-year, 3.5-year, and 5-year visits). We have reported our urine collection, analysis, limits of detection (LOD), and quality control procedures in detail (Bradman et al., 2005). Briefly, we aliquoted and stored samples at −80 °C before shipping them on dry ice to the Centers for Disease Control and Prevention (CDC). At CDC, samples were analyzed using gas chromatography-tandem mass spectrometry (GC-MS/MS) (Bravo et al., 2002) and quantified using isotope dilution calibration (Bradman et al., 2005) for six DAP metabolites, including three dimethyl (DM) phosphate metabolites (dimethylphosphate, dimethylthiophosphate, dimethyldithiophosphate) and three diethyl (DE) phosphate metabolites (diethylphosphate, diethylthiophosphate, and diethyldithiophosphate). Because DM metabolite concentrations greatly exceeded DE concentrations in CHAMACOS, and were very similar to total DAP concentrations, we conducted analyses with only total DAPs. For metabolite values that fell below the LOD, we randomly imputed values based on a log-normal probability distribution and used maximum likelihood estimation, (Lubin et al., 2004) as we have described previously (Bradman et al., 2005, 2011). To account for urine dilution of pregnancy samples, we measured the specific gravity of urine samples with a refractometer calibrated with deionized water at room temperature and normalized DAP concentrations for specific gravity (Mahalingaiah et al., 2008) using the following formula: Measurement * (1.024–1)/(specific gravity-1), where 1.024 is the mean specific gravity from a large reference sample (Elkins and Pagnotto, 1969). We did not measure specific gravity of child urine samples, but rather measured urinary creatinine using a commercially available diagnostic enzyme method, and normalized child DAP concentrations for creatinine.

2.3. Cortical activation using functional near infrared spectroscopy (fNIRS)

We measured cortical activation using fNIRS while participants completed fNIRS-adapted computerized tasks. Participants were fitted with a cap configured with optodes that captured neural activity from 36 different channels grouped into 15 functional localization clusters on both the right and left hemispheres of the brain (Fig. 1). Clusters were positioned in the prefrontal (9 clusters), temporal (2 clusters), and parietal (4 clusters) neural regions. We used the NIRScout (NIRx, Germany) with a maximum sampling frequency of 62.5 Hz to collect data on hemodynamic activity. After optode calibration was complete, participants viewed PowerPoint-based instructions and reviewed with study staff how to complete each of three tasks, including two executive function tasks, adaptations of the Wisconsin Card Sort Test and the Sternberg working memory task, and of one semantic language task, the Pyramids and Palm Trees Test. Details on each of the tasks, including administration, trials, and duration are described in Supplementary Material. All tasks were programmed at Stanford University using Matlab 2014b and Psychtoolbox, optimizing trial order and timing to maximize detectable changes in hemodynamic response using OptSeq2 (Ashburner, 2009). We selected tasks based on epidemiologic evidence of OPs and neurodevelopment and our recent study of 95 CHAMACOS participants age 16 years, (Sagiv et al., 2019) that guided us in narrowing down from six tasks to three. Participants were administered tasks on a Macbook Pro connected to a 20" LED monitor and task responses were collected with a standard keyboard. We presented tests in a randomized order to account for test fatigue.

We processed and cleaned fNIRS data using the Homer2 Matlab package (Huppert et al., 2009). We corrected for motion artifact using a wavelet motion correction procedure and bandpass filter between 0.01 and 0.5 Hz, which removes artifacts related to physiologic fluctuations such as heartbeat and respiratory signals. We rejected individual channels from further analysis if they reached thresholds based on one on scalp coupling index (SCI) values derived from qt-nirs toolbox (SCI threshold = 0.8, Q threshold = 0.75; https://github.com/lpollonini/qt-nirs). (Pollonini et al., 2016) We converted corrected optical density data to HbO and HbR values using the modified Beers-Lambert law.

We estimated cortical activation using a generalized linear model (GLM) approach, a well-established method of analysis for event-related fNIRS protocols (Plichta et al., 2007). We submitted onset and duration of each condition to the GLM procedure as predictor variables and estimated standardized beta coefficients for each task and control condition across each channel. The sign and magnitude of each beta coefficient indicates the direction and intensity of blood oxygenation (cortical activation) in response to each condition. To estimate cortical activation in response to task demand, we contrasted beta coefficients

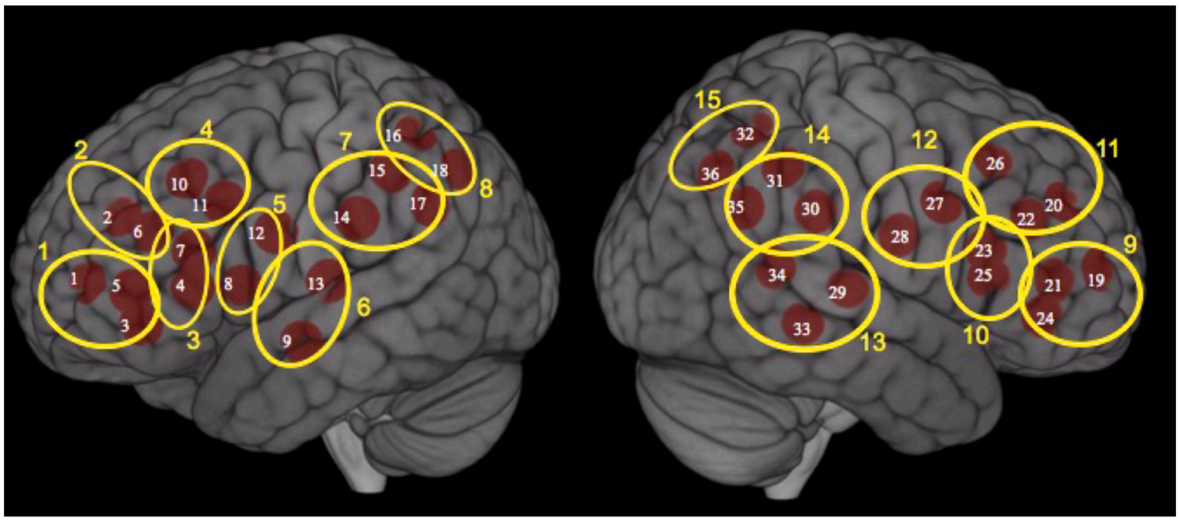

Fig. 1. Functional localization clusters (15) across the 36 fNIRS channels in regions of interest for the CHAMACOS cohort, Salinas Valley, California. Red circles represent channel locations visualized as the midpoint between each source and detector pair. Yellow circles are clusters based on proximity of channels and anatomy, and include 1 = left inferior frontal pole; 2 = left superior frontal pole; 3 = left Broca's/Brodmann areas 44 and 45; 4 = left dorsolateral prefrontal cortex; 5 = left Broca's/Brodmann areas 44 and 6; 6 = left superior/inferior temporal gyrus/postcentral gyrus; 7 = left inferior parietal lobule; 8 = left superior parietal lobule; 9 = right inferior frontal pole; 10 = right Broca's/Brodmann areas 44 and 45; 11 = right superior frontal pole/dorsolateral prefrontal cortex; 12 right premotor/somatosensory cortex; 13 = right posterior superior/middle temporal sulcus; 14 = right inferior parietal lobule; 15 = right superior parietal lobule.

between task and corresponding control conditions. We used a functional localization method, which allows for minor variability across individuals in the location of task-responsive brain regions and reduces Type II (false negative) errors by selecting maximum activation across channels in each region of interest (ROI). (Baker et al., 2018; Bruno et al., 2018; Hosseini et al., 2017).

2.4. Covariate assessment

We collected data from participants (mother-youth pairs) at approximately biennial visits (twice during pregnancy, shortly following delivery, and when youth were 6 months and 1, 2, 3.5, 5, 7, 9, 10.5, 12, 14, 16, and 18 years of age), including sociodemographic characteristics of the mother (age, years living in the U.S., educational attainment) and the youth (sex at birth, age at fNIRS), and poverty level at assessment. At the 6-month visit we administered the Home Observation for the Measurement of the Environment (HOME) to measure enrichment in the home environment, (Caldwell and Bradley, 1984) and the Peabody Picture Vocabulary Test (PPVT), (Dunn, 1997) or its Spanish equivalent, (Dunn et al., 1986) to the mothers to assess maternal receptive language.

2.5. Statistical analysis

For prenatal DAPs, we computed the mean total DAP concentrations across the two specific-gravity adjusted pregnancy (13 and 26 week) samples. For childhood DAPs, we computed the area under the curve (AUC) for the five creatinine-adjusted childhood (6-month, 1-year, 2-year, 3.5-year, and 5-year) samples (Borgatta et al., 2022; Teeguarden et al., 2011). We log10-transformed total DAP exposure concentrations to reduce the influence of extreme DAP values.

Using one-sample t-tests, we examined brain activation (increased HbO and decreased HbR) in each localization cluster for each of the contrasts of interest (i.e., match vs. control for Wisconsin Card Sort Test; encoding and maintenance vs. recall for the Sternberg working memory task; and semantic meaning vs. text size for the Pyramids and Palm Trees Test). We focused all cortical activation analyses on HbO.

In linear regression models, we estimated associations (β and 95% CI) for continuous log10-transformed total DAP concentrations (mean DAPs for prenatal measures, AUC DAPs for child measures) with cortical activation across task conditions for each localization cluster. Associations (β coefficients) represent the change in brain activity during a challenge vs. control task per 10-fold increase in total DAP concentrations. To account for multiple comparisons, we controlled for Type I error using the Benjamini–Hochberg False Discovery Rate (FDR) at <0.05 (Hochberg and Benjamini, 1990). We assessed linearity of DAPs-activation associations using generalized additive models (GAMs) with 3-degrees of freedom cubic splines and found that associations were generally linear; we therefore present results with continuous DAP concentrations.

We selected covariates *a priori* for inclusion in multivariable models based on our previous studies of DAPs and neurodevelopment, which relied on causal diagrams (Eskenazi et al., 2007; Sagiv et al., 2018, 2021). All models included youth's sex (at birth) and age at fNIRS (years; continuous), maternal age at delivery (years; continuous), maternal duration of residence in the United States at delivery (years; continuous), maternal educational level at delivery (categorical: <7th grade, 7–11th grade, completion of high school), maternal receptive language when the child was age 6 months (continuous PPVT score), HOME score at 6-months (continuous z-score), and federal poverty level at assessment (categorical: at or below the poverty level, above the poverty level) (U.S. Census Bureau, 2000). When data on covariates were missing, we used participants' data on that covariate from other study visits to replace the missing value.

We assessed whether associations differed for males vs. females by including an interaction term between DAPs and sex in our multivariable models. In secondary analyses, we examined associations of prenatal and childhood total DAP concentrations with fNIRS performance on each task, including total and perseverative errors for the Wisconsin Card Sort Test, and accuracy for both the Sternberg and Pyramids and Palm Trees tests. In addition, we investigated whether associations of DAPs with cortical activation differed depending on test performance by dichotomizing at the median score for each of the tasks and estimating associations of DAPs and cortical activation across strata of performance (high vs. low performance).

We conducted sensitivity analyses based on two variables that may have introduced additional variability in fNIRS performance, but which we chose not to include in multivariable models in the interest of parsimony: 1) we restricted to right-handed participants (93.5% of the cohort); 2) we restricted to those who reported no alcohol or marijuana use in 24 h prior to fNIRS testing (85.6%).

3. Results

Table 1 shows covariate distributions for the 291 CHAMACOS youth and their families included in the current analysis, which includes participants with DAPs at any time point (prenatal or childhood) who were also administered an fNIRS at the 18-year visit. Participants had mothers who were mostly born outside of the U.S. (90.4%; almost all were born in Mexico) and had not completed high school by the birth of their child (80.8%), and many were living at or below the poverty line at the 18-year visit (41.6%). Compared to the 246 participants not included in the current analysis (Table 1), included participants were more likely to be female, have mothers of slightly older age, and have slightly less enrichment in the home environment (lower HOME scores at the 6-month visit). Table 2 shows summary statistics for pregnancy and childhood DAP concentrations among participants with an 18y fNIRS.

3.1. Prenatal DAPs and cortical activation

Results from our analysis of maternal urinary DAP concentrations during pregnancy and fNIRS cortical activation are presented in Figs. 2–4. Fig. 2 displays associations of prenatal DAPs and cortical activation during the Wisconsin Card Sort Task, a task of cognitive flexibility, for the left and right hemisphere across the fifteen localization clusters. For this task, we found suggestive associations of prenatal DAPs with increased cortical activation in regions of the left hemisphere, particularly for localization clusters 1 and 7, the interior frontal cortex (β per ten-fold DAP increase = 3.37; 95% CI: −0.02, 6.77) and the inferior parietal lobule (β = 3.43; 95% CI: 0.64, 6.22), respectively. There was also altered activation with prenatal DAP concentrations during the Sternberg letter retrieval working memory task, but in the opposite direction (Fig. 3), with the most pronounced reductions in activation with prenatal exposure to DAPs in localization clusters 4, 9 and 11, the left dorsolateral prefrontal cortex (β = −3.49; 95% CI: −8.07, 1.09), the right interior frontal pole (β = −3.10; 95% CI: −6.43, 0.22), and the right superior frontal pole/dorsolateral prefrontal cortex (β = −3.67; 95% CI: −7.94, 0.59), respectively. We found no associations of prenatal DAPs with cortical activation during the Pyramids and Palm Trees task (Fig. 4), a test of semantic language.

There were few statistically significant sex differences for associations of prenatal total DAPs with fNIRS cortical activation (Supplementary Table 1), and no discernible pattern of differences across any of the three tasks.

Table 1
Demographic characteristics of study population, CHAMACOS.

Characteristics	Participants included in analysis (n = 291) Mean ± SD or N (%)	Participants not included in analysis (n = 246) Mean ± SD or N (%)
Youth characteristics		
Age at assessment (years)	18.2 ± 0.3	NA
Sex at birth		
Female	158 (54.3)#	113 (45.9)#
Male	133 (45.7)	133 (54.1)
Handedness		
Right	271 (93.8)	NA
Left	18 (6.2)	NA
Self-reported alcohol or marijuana use in 24 h before fNIRS		
No	248 (85.5)	NA
Yes	42 (14.5)	NA
Family characteristics		
Maternal age at delivery (years)	26.6 ± 5.2*	25.1 ± 5.0*
Maternal education at delivery		
<7th grade	131 (45.0)	103 (41.9)
7th-11th grade	104 (35.7)	90 (36.6)
Completed high school	56 (19.2)	53 (21.5)
Maternal years in the U.S. at delivery		
≤5 years	126 (43.3)	135 (54.9)
>5 years, non-native	137 (47.1)	79 (32.1)
Born in the U.S.	28 (9.6)	32 (13.0)
Maternal PPVT score	85.3 ± 20.8	85.6 ± 20.7
Poverty level at assessment		
At or below poverty level	121 (41.6)	24 (49.0)
Above poverty level	170 (58.4)	25 (51.0)
HOME z-score at 6-month visit	0.01 ± 1.01*	0.22 ± 1.12*

#p < 0.1.
*p < 0.05.

3.2. Childhood DAPs and cortical activation

Associations of the AUC of childhood total DAPs (ages 6 months, 1, 2, 3.5, and 5 years) and cortical activation were essentially null for all fNIRS tasks across all brain regions (Figs. 5–7). Sex-specific associations of childhood DAPs and activation were inconsistent and imprecise. There was some suggestion that associations of childhood DAPs with cortical activation during the Wisconsin Card Sort Task were in different directions for females vs. males across specific brain regions (e.g., for the left superior/inferior temporal/postcentral gyrus β per 10-fold increase in DAPs = 6.01; 95% CI: 0.52, 11.50 for females vs. β = −3.26; 95% CI: −10.12, 3.59 for males; p-for-interaction = 0.03), however, wide confidence intervals provided low certainty in these patterns (Supplementary Table 2). We also observed activation in different directions for females vs. males for the Sternberg, but the pattern was the opposite from the Wisconsin Card Sort Task and estimates were very imprecise (e.g., for the superior parietal lobe β = −12.09; 95% CI: −24.67, 0.48 for females vs. β = 5.12; 95% CI: −4.94, 15.18 for males; p-for-interaction = 0.01).

3.2.1. Task performance

In secondary analyses, we examined associations of prenatal and childhood DAPs with performance on the tasks administered during the fNIRS (Supplementary Table 3). Associations of DAPs (prenatal and childhood) with task performance (errors and accuracy) were essentially null, though prenatal DAPs were associated with marginally lower accuracy on the Sternberg letter retrieval working memory task (β = −0.04; 95% CI: −0.07, −0.01). Associations stratified by task performance (high vs. low performance), dichotomized at the median score for each of the tasks, were imprecise and showed no consistent pattern for prenatal DAPs (Supplementary Table 4) or childhood DAPs (Supplementary Table 5). There was a suggestion of decreased cortical activation with childhood DAPs among high vs. low performers of the Wisconsin Card Sort Task in regions of the left hemisphere (Supplemental Table 5), however this pattern of associations is hard to explain, and estimates were imprecise.

In sensitivity analyses, we found no meaningful changes when we: 1) restricted to right-handed participants, and 2) restricted to those who reported no alcohol or marijuana use in 24 h prior to fNIRS testing. FDR correction led to no statistically significant associations.

4. Discussion

In this sample of 291 CHAMACOS participants, we found some evidence for associations of prenatal DAPs with altered cortical activation in regions of the prefrontal cortex during tasks of executive function, including tasks of cognitive flexibility and working memory; associations with language comprehension were null, however. In addition, we found no associations of childhood DAP concentrations with cortical activation for any of the fNIRS tasks.

The current analysis extends prior research on a small subset of 95 CHAMACOS participants from a separate wave of the CHAMACOS cohort who were recruited at age 9 years and did not have pregnancy/early life OP biomarkers (Sagiv et al., 2019). For this previous study, we

Table 2
Summary statistics for pregnancy and childhood DAP concentrations among CHAMACOS participants with an 18y fNIRS.

Sample period	N	GM	GSD	Min	P25	P50	P75	Max
Pregnancy (specific gravity-corrected, nmol/L)								
13-week	290	133.7	4.6	4.4	44.6	127.6	416.7	9065.5
26-week	272	127.8	2.6	6.1	71.3	121.8	238.4	2366.1
Pregnancy mean	291	171.9	2.9	10.1	84.5	165.2	367.6	4628.3
Childhood (creatinine-adjusted, nmol/g)								
6-month	259	213.7	5.0	2.0	74.5	189.9	656.6	78234.6
1-year	270	229.3	4.7	3.6	86.2	224.5	636.3	10551.8
2-year	265	203.9	4.6	2.9	87.7	212.0	547.7	5942.5
3.5-year	218	153.1	4.8	1.9	54.6	181.6	464.0	9240.2
5-year	257	124.7	5.0	0.9	45.1	146.3	333.5	10084.5
Childhood area under the curve (AUC)	231	1652.3	2.6	147.6	835.7	1536.7	3095.1	18926.5

DAP, dialkylphosphate; GM, geometric mean; GSD, geometric standard deviation; Max, maximum; Min, minimum; P25, 25th percentile; P50, 50th percentile; P75, 75th percentile.

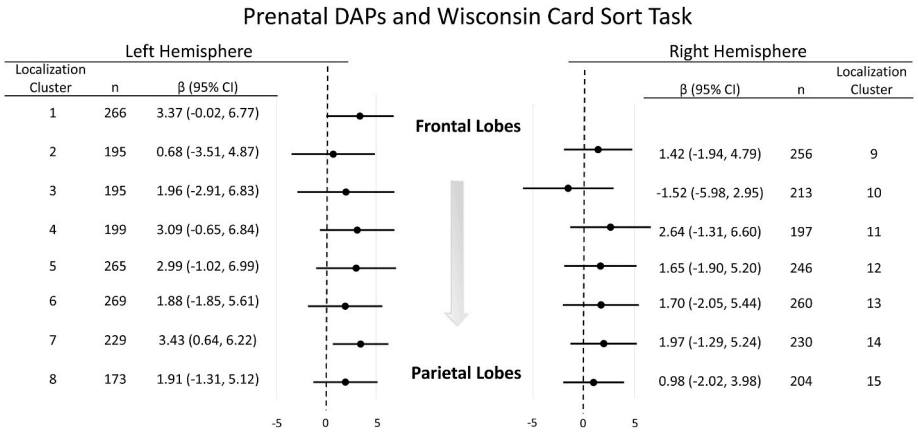

Fig. 2. Associations of maternal pregnancy DAPs (10-fold increase) with fNIRS brain activation during the Wisconsin Card Sort Task across 15 functional localization clusters, among participants of CHAMACOS, Salinas Valley, California. All models were adjusted for the youth's sex (at birth) and age at fNIRS (years; continuous), maternal age at delivery (years; continuous), maternal duration of residence in the United States at delivery (years; continuous), maternal educational level at delivery (categorical: <7th grade, 7–11th grade, completion of high school), maternal receptive language when the child was age 6 months (continuous PPVT score), HOME score at 6-months (continuous z score), and poverty level at assessment (categorical: at or below the poverty level, above the poverty level).

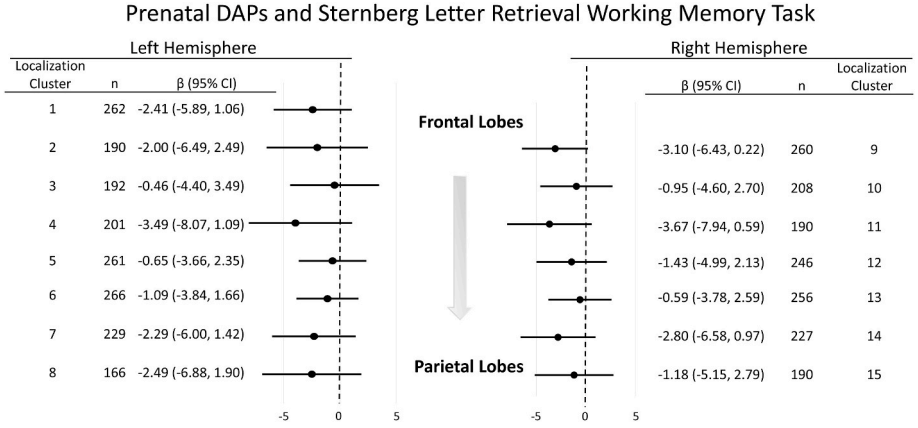

Fig. 3. Associations of maternal pregnancy DAPs (10-fold increase) with fNIRS brain activation during the Sternberg latter retrieval working memory task across 15 functional localization clusters, among participants of CHAMACOS, Salinas Valley, California. All models were adjusted for the youth's sex (at birth) and age at fNIRS (years; continuous), maternal age at delivery (years; continuous), maternal duration of residence in the United States at delivery (years; continuous), maternal educational level at delivery (categorical: <7th grade, 7–11th grade, completion of high school), maternal receptive language when the child was age 6 months (continuous PPVT score), HOME score at 6-months (continuous z score), and poverty level at assessment (categorical: at or below the poverty level, above the poverty level).

estimated prenatal OP exposure using California's Pesticide Use Report data, and found that a 10-fold increase in total OP pesticide use within 1 km of maternal residence during pregnancy was associated with a bilateral decrease in cortical activation in the interior frontal poles of the prefrontal cortex during the Wisconsin Card Sort Task ($\beta = -4.74$; 95% CI: −8.18, −1.31 and $\beta = -4.40$; 95% CI: −7.96, −0.84 for the left and right hemispheres, respectively) (Sagiv et al., 2019). This is in contrast to the current study, where we found that a 10-fold increase in prenatal

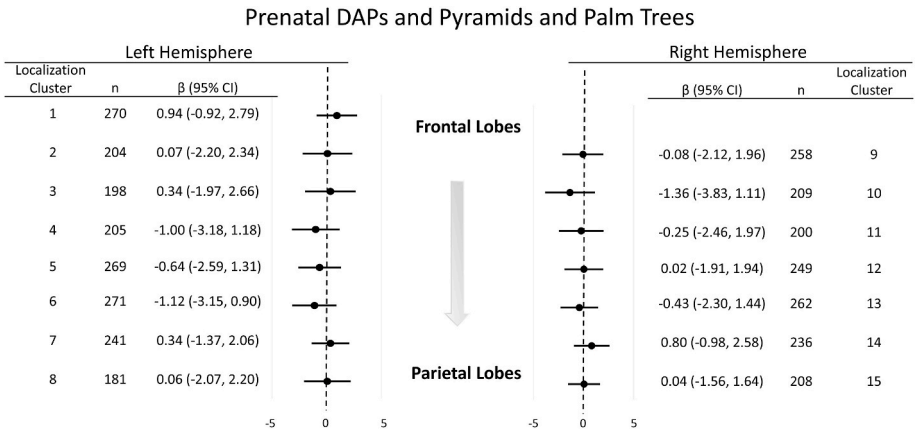

Fig. 4. Associations of maternal pregnancy DAPs (10-fold increase) with fNIRS brain activation during the Pyramid and Palm Trees task across 15 functional localization clusters, among participants of CHAMACOS, Salinas Valley, California. All models were adjusted for the youth's sex (at birth) and age at fNIRS (years; continuous), maternal age at delivery (years; continuous), maternal duration of residence in the United States at delivery (years; continuous), maternal educational level at delivery (categorical: <7th grade, 7–11th grade, completion of high school), maternal receptive language when the child was age 6 months (continuous PPVT score), HOME score at 6-months (continuous z score), and poverty level at assessment (categorical: at or below the poverty level, above the poverty level).

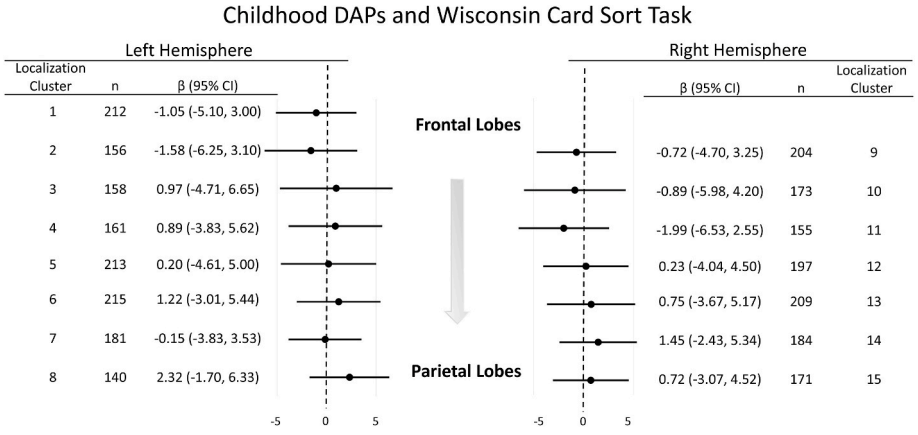

Fig. 5. Associations of AUC childhood DAPs (10-fold increase) with fNIRS brain activation during the Wisconsin Card Sort Task across 15 functional localization clusters, among participants of CHAMACOS, Salinas Valley, California. All models were adjusted for the youth's sex (at birth) and age at fNIRS (years; continuous), maternal age at delivery (years; continuous), maternal duration of residence in the United States at delivery (years; continuous), maternal educational level at delivery (categorical: <7th grade, 7–11th grade, completion of high school), maternal receptive language when the child was age 6 months (continuous PPVT score), HOME score at 6-months (continuous z score), and poverty level at assessment (categorical: at or below the poverty level, above the poverty level).

DAP concentrations was associated with an increase in cortical activation in only the left interior frontal pole during the Wisconsin Card Sort Task. We did, however, find associations of prenatal DAPs with decreased cortical activation for the Sternberg task in regions of the prefrontal cortex. While it is difficult to explain the opposing directions of these associations (decreased vs. increased activation), it is important to note that in both analyses increased OP exposure during pregnancy (proximity to application in the smaller subset, DAP biomarker in a larger sample in the current study) was associated with alterations in neural response during these executive function tasks.

The opposing directions in activation with exposure could have different mechanistic underpinnings, indicating compromised neural response with exposure to OP pesticides. For example, the increase in activation with urinary DAP exposure that we observed in the current study during the Wisconsin Card Sort Task could reflect a higher than typical increase in the recruitment of a neural response to meet the demands of the task. This has been shown among older adults with beta-amyloid deposition (Elman et al., 2014; Suskauer et al., 2008; Witiuk et al., 2014). Decreased activation in relation to exposure, as we found for the Sternberg task, and in our previous study, (Sagiv et al., 2019) may also indicate an altered neural response where that region is no longer able to produce a typical response to a task, which has also been shown previously in neurogenetic groups with cognitive impairment (Haberecht et al., 2001; Kwon et al., 2001).

Previous epidemiologic studies of prenatal OP exposure, including studies conducted in CHAMACOS, have reported associations with poorer neurodevelopment across a range of functions, including cognition, attention, hyperactive/impulsive behaviors, and other executive functions (Sagiv et al., 2021; Bouchard et al., 2011; Coker et al., 2017; Engel et al., 2011; Gunier et al., 2017; Rauh et al., 2011; Saeedi Saravi et al., 2016). Most of these previous studies rely on neuropsychological assessment or behavioral rating scales. In a recent CHAMACOS analysis, we detected associations of prenatal DAPs with behaviors related to executive function at ages 7–12 years (Sagiv et al., 2021). In this study, we reported that a 10-fold increase in prenatal DAPs was associated with Behavior Rating Inventory of Executive Function (BRIEF) scores, longitudinally reported by mothers when the children were 7, 9 and 12 years ($\beta = 4.0$; 95% CI: 2.1, 5.8, where a higher BRIEF score indicates more problems). We also reported that higher prenatal DAPs were associated with poorer Weschler Intelligence Scale for Children, Fourth Edition (WISC-IV) Working Memory scores assessed when children were 7 and 10.5 years ($\beta = -3.8$; 95% CI: −6.2, −1.3) and more total and

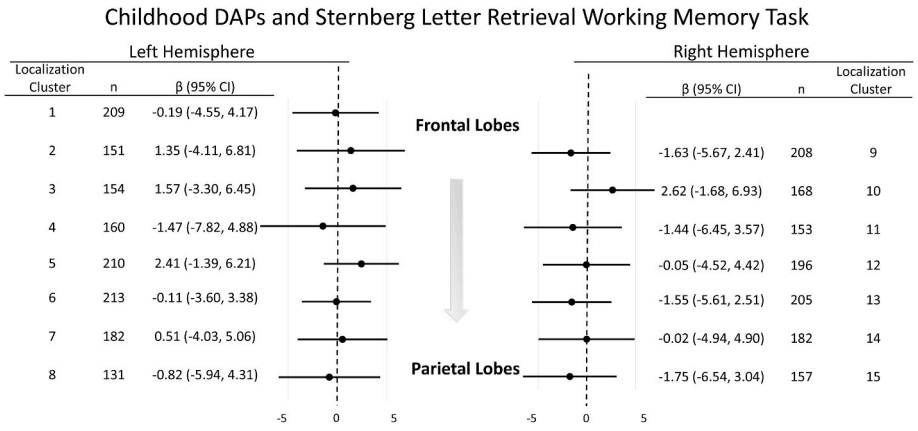

Fig. 6. Associations of AUC childhood DAPs (10-fold increase) with fNIRS brain activation during the Sternberg latter retrieval working memory task across 15 functional localization clusters, among participants of CHAMACOS, Salinas Valley, California. All models were adjusted for the youth's sex (at birth) and age at fNIRS (years; continuous), maternal age at delivery (years; continuous), maternal duration of residence in the United States at delivery (years; continuous), maternal educational level at delivery (categorical: <7th grade, 7–11th grade, completion of high school), maternal receptive language when the child was age 6 months (continuous PPVT score), HOME score at 6-months (continuous z score), and poverty level at assessment (categorical: at or below the poverty level, above the poverty level).

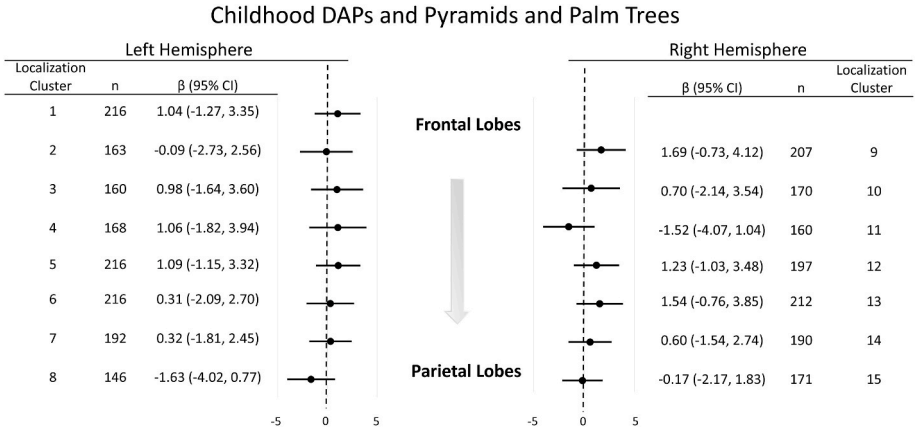

Fig. 7. Associations of AUC childhood DAPs (10-fold increase) with fNIRS brain activation during the Pyramid and Palm Trees task across 15 functional localization clusters, among participants of CHAMACOS, Salinas Valley, California. All models were adjusted for the youth's sex (at birth) and age at fNIRS (years; continuous), maternal age at delivery (years; continuous), maternal duration of residence in the United States at delivery (years; continuous), maternal educational level at delivery (categorical: <7th grade, 7–11th grade, completion of high school), maternal receptive language when the child was age 6 months (continuous PPVT score), HOME score at 6-months (continuous z score), and poverty level at assessment (categorical: at or below the poverty level, above the poverty level).

perseverative errors on the Wisconsin Card Sort Test ($\beta = -3.6$; 95% CI: −5.5, −1.7 and $\beta = -3.7$; 95% CI: −6.3, −1.2, respectively). Our observations in the current study, that prenatal DAPs were associated with altered cortical activation in regions of the prefrontal cortex during tasks of executive function, are consistent with the findings of these previous studies.

We did not see any associations of prenatal or childhood OPs with activation during the Pyramids and Palm Trees task, a test of semantic language. While this is consistent with what we found in our smaller subset of 95 participants without biomarkers, in this smaller study we did detect sex differences, with higher activation among males and lower activation among females across nearly all brain regions assessed; we did not detect any sex differences in the current study. Findings in the current study are also inconsistent with a previous CHAMACOS study of prenatal DAPs and the WISC-IV Verbal Comprehension, where we observed a 5.3 point decrease (95% CI: −8.6 to −2.0) per 10-fold increase in prenatal DAP concentrations in 329 participants age 7 years (Bouchard et al., 2011). However, these tests do assess language comprehension differently, which could explain this discrepancy in findings. It is possible that the weaker associations of prenatal OPs with cortical activation we report here, compared with earlier studies that report stronger associations with neurobehavioral outcomes in CHAMACOS, (Eskenazi et al., 2007; Marks et al., 2010; Sagiv et al., 2018, 2021, 2023; Bouchard et al., 2011) is due to the older age of assessment, and that these young adults may have outgrown any harm from prenatal OP exposure. However, the very different endpoints assessed in this neuroimaging study, compared with the functional endpoints in these previous studies, makes it difficult to know if this explanation is plausible.

We found no associations of childhood DAPs with activation in any of the regions of interest for any of the three tasks. This is consistent with our previous findings, which have shown no associations of childhood DAPs with any measures of neurodevelopment in CHAMACOS (Marks et al., 2010; Bouchard et al., 2011). While rapid brain development occurs in both the both the prenatal and early childhood period, (Grandjean and Landrigan, 2014) accumulating evidence from CHAMACOS and other cohorts suggests that fetal brain development may be the more sensitive window to OP exposure (Sapbamrer and Hongsibsong, 2019).

We did not observe any strong or consistent associations of prenatal or childhood DAPs with performance on any of the tasks administered with fNIRS, with the exception of slightly lower accuracy on the

Sternberg with prenatal DAPs (Supplementary Table 3). More notably, we did not find associations of prenatal DAPs with more total or perseverative errors on the fNIRS adapted Wisconsin Card Sort Task, which we observed in our previous study at ages 7–12 years (detailed above) (Sagiv et al., 2021). This may have been due to differences in the sensitivity of these tasks, which were optimized for fNIRS testing. And it is important to point out that despite the null associations with performance on the Wisconsin and Sternberg tasks, we did see some evidence for associations with cortical activation during these tasks, highlighting the potential for neuroimaging to detect very subtle impacts of pesticide exposure on the brain.

There are some limitations specific to using DAPs to estimate OP exposure. 1) As DAPs are non-specific metabolites of OP exposure, it is not possible to identify which specific OP pesticide (e.g., diazinon. chlorpyrifos, etc.) is driving associations with brain activity. 2) DAPs are rapidly metabolized in the body, and it is therefore difficult to accurately estimate exposure using a single spot urine. While we measured DAPs twice during pregnancy and several times in childhood in CHAMACOS, exposure measurement error was still a problem, which more likely masked associations with OPs. Measurement error and lack of specificity in window of exposure was also a problem when we estimated associations with the AUC of childhood DAP exposure and likely limited our interpretation of associations of childhood OP exposure and cortical activation. 3) DAP concentrations reflect both preformed metabolites and their parent pesticides. A recent study shows that preformed DAPs from diet may constitute the main source of urinary DAPs in urban settings, (Tsuchiyama et al., 2022) however in the agricultural CHAMACOS study, participants likely also derived exposure from local pesticide use (Bradman et al., 2015).

Another limitation of this study was that using fNIRS, we were only able to capture activation at the cortical surface of the brain. We therefore may have missed important impacts of OP exposure on subcortical, deep-brain regions. While fNIRS has been used to predict deep brain activity, (Liu et al., 2015) we did not conduct these analyses in the current study. Finally, we looked at many localization clusters across three different tasks raising concerns about multiple comparisons. Applying FDR correction resulted in no statistically significant associations.

In summary, we found suggestive associations of prenatal DAPs with alterations during tasks of executive function in regions of the prefrontal cortex. However, associations with childhood DAPs were null. These findings are consistent with what has been reported in previous studies of OP exposure and neurodevelopment and contribute to a larger literature showing the vulnerability of the developing brain to OP exposure. This research also demonstrates the utility of neuroimaging in epidemiologic studies of neurodevelopment.

Credit author statement

Sharon K. Sagiv: Substantial contributions to conception of fNIRS study, data analysis and interpretation of findings; drafted and revised manuscript. **Joseph M. Baker**: Substantial contributions to design, collection, and analysis of fNIRS data; drafted sections and reviewed manuscript. **Stephen Rauch**: Substantial contributions to exposure-outcome analysis; drafted sections and reviewed manuscript. **Yuanyuan Gao**: Contributions to interpretation, especially with respect to fNIRS data; reviewed manuscript. **Robert B. Gunier**: Contributions to collection, analysis, and interpretation of exposure data; reviewed manuscript. **Ana M. Mora**: Contributions to collection, analysis, and interpretation of data with respect to exposure and outcome; reviewed manuscript. **Asa Bradman**: Contributions to collection, analysis, and interpretation of exposure data; reviewed manuscript. **Brenda Eskenazi**: Substantial contributions to conception and design of CHAMACOS and fNIRS study, acquisition of data, analysis, and interpretation of data; reviewed manuscript. **Allan L. Reiss**: Substantial contributions to conception of fNIRS study, acquisition, analysis, and interpretation of fNIRS data; reviewed manuscript

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Asa Bradman reports a relationship with The Organic Center that includes: board membership.

Data availability

Some data can be made available on request and some is sensitive and therefore confidential. Data requests will be reviewed by our research team.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2023.117756.

References

Ashburner, J., 2009. Computational anatomy with the SPM software. Magn. Reson. Imaging 27 (8), 1163–1174. https://doi.org/10.1016/j.mri.2009.01.006. Oct.

Baker, J.M., Bruno, J.L., Gundran, A., Hosseini, S.M.H., Reiss, A.L., 2018. fNIRS measurement of cortical activation and functional connectivity during a visuospatial working memory task. PLoS One 13 (8), e0201486. https://doi.org/10.1371/journal.pone.0201486.

Borgatta, M., Wild, P., Hopf, N.B., 2022. Blood absorption toxicokinetics of glycol ethers after inhalation: a human controlled study. Sci. Total Environ. 816, 151637 https://doi.org/10.1016/j.scitotenv.2021.151637. Apr 10.

Bouchard, M.F., Chevrier, J., Harley, K.G., et al., 2011. Prenatal exposure to organophosphate pesticides and IQ in 7-year-old children. Environ Health Perspect. 119 (8), 1189–1195. https://doi.org/10.1289/ehp.1003185.

Bradman, A., Eskenazi, B., Barr, D.B., et al., 2005. Organophosphate urinary metabolite levels during pregnancy and after delivery in women living in an agricultural community. Research Support. N.I.H., Extramural Research Support, U.S. Gov't, Non-P.H.S. Environ Health Perspect. 113 (12), 1802–1807.

Bradman, A., Castorina, R., Barr, D.B., et al., 2011. Determinants of organophosphorus pesticide urinary metabolite levels in young children living in an agricultural community. Int J Environ Res Public Health. 8 (4), 1061–1083. https://doi.org/10.3390/ijerph8041061.

Bradman, A., Quiros-Alcala, L., Castorina, R., et al., 2015. Effect of organic diet intervention on pesticide exposures in young children living in low-income urban and agricultural communities. Environ. Health Perspect. 123 (10), 1086–1093. https://doi.org/10.1289/ehp.1408660. Oct.

Bravo, R., Driskell, W.J., Whitehead Jr., R.D., Needham, L.L., Barr, D.B., 2002. Quantitation of dialkyl phosphate metabolites of organophosphate pesticides in human urine using GC-MS-MS with isotopic internal standards. J. Anal. Toxicol. 26 (5), 245–252.

Bruno, J.L., Baker, J.M., Gundran, A., et al., 2018. Mind over motor mapping: driver response to changing vehicle dynamics. Hum. Brain Mapp. 39 (10), 3915–3927. https://doi.org/10.1002/hbm.24220. Oct.

Caldwell, B., Bradley, R., 1984. Home Observation for Measurement of the Environment - Revised Edition. University of Arkansas.

Coker, E., Gunier, R., Bradman, A., et al., 2017. Association between pesticide profiles used on agricultural fields near maternal residences during pregnancy and IQ at age 7 years. Int. J. Environ. Res. Publ. Health 14 (5). https://doi.org/10.3390/ijerph14050506. May 9.

Dunn, L.M., 1997. Examiner's Manual for the Peabody Picture Vocabulary Test, third ed. American Guidance Service, Circle Pines, MN.

Dunn, L.P.E., Lugo, D., Dunn, L., 1986. Test de Vocabulario en Imagenes Peabody: Adaptacion Hispanoamericana (Peabody Picture Vocabulary Test: Hispanic-American Adaptation) Circle Pines. Dunn Educational Services, Inc, MN.

Elkins, H.B., Pagnotto, L.D., 1969. The specific gravity adjustment in urinalysis. Arch Environ Health. 18 (6), 996–1001. https://doi.org/10.1080/00039896.1969.10665525.

Elman, J.A., Oh, H., Madison, C.M., et al., 2014. Neural compensation in older people with brain amyloid-beta deposition. Nat Neurosci. 17 (10), 1316–1318. https://doi.org/10.1038/nn.3806.

Engel, S.M., Wetmur, J., Chen, J., et al., 2011. Prenatal exposure to organophosphates, paraoxonase 1, and cognitive development in childhood. Environ Health Perspect. 119 (8), 1182–1188. https://doi.org/10.1289/ehp.1003183.

Eskenazi, B., Marks, A.R., Bradman, A., et al., 2007. Organophosphate pesticide exposure and neurodevelopment in young Mexican-American children. Environ Health Perspect. 115 (5), 792–798. https://doi.org/10.1289/ehp.9828.

Grandjean, P., Landrigan, P.J., 2014. Neurobehavioural effects of developmental toxicity. Research Support, N.I.H., Extramural Review. Lancet Neurol. 13 (3), 330–338. https://doi.org/10.1016/S1474-4422(13)70278-3. Mar.

Grube, A.D.D., Kiely, T., Wu, L., 2011. Pesticides Industry Sales and Usage: 2006 & 2007 Market Estimates. . U.S. Environmental Protection Agency, Washington, D.C. https://www.epa.gov/sites/production/files/2015-10/documents/market_estimates2007.pdf. (Accessed 28 May 2021).

Gunier, R.B., Bradman, A., Harley, K.G., Kogut, K., Eskenazi, B., 2017. Prenatal residential proximity to agricultural pesticide use and IQ in 7-year-old children. Environ. Health Perspect. 125 (5), 057002 https://doi.org/10.1289/EHP504. May 25.

Haberecht, M.F., Menon, V., Warsofsky, I.S., et al., 2001. Functional neuroanatomy of visuo-spatial working memory in Turner syndrome. Hum. Brain Mapp. 14 (2), 96–107. Oct.

Hochberg, Y., Benjamini, Y., 1990. More powerful procedures for multiple significance testing. Stat Med. 9 (7), 811–818.

Hosseini, S.M.H., Bruno, J.L., Baker, J.M., et al., 2017. Neural, physiological, and behavioral correlates of visuomotor cognitive load. Sci. Rep. 7 (1), 8866. https://doi.org/10.1038/s41598-017-07897-z. Aug 18.

Huppert, T.J., Diamond, S.G., Franceschini, M.A., Boas, D.A., 2009. HomER: a review of time-series analysis methods for near-infrared spectroscopy of the brain. Appl Opt. 48 (10), D280–D298. https://doi.org/10.1364/ao.48.00d280.

Kwon, H., Menon, V., Eliez, S., et al., 2001. Functional neuroanatomy of visuospatial working memory in fragile X syndrome: relation to behavioral and molecular measures. Am J Psychiatry. 158 (7), 1040–1051. https://doi.org/10.1176/appi.ajp.158.7.1040.

Liu, N., Cui, X., Bryant, D.M., Glover, G.H., Reiss, A.L., 2015. Inferring deep-brain activity from cortical activity using functional near-infrared spectroscopy. Biomed Opt Express. 6 (3), 1074–1089. https://doi.org/10.1364/BOE.6.001074.

Lubin, J.H., Colt, J.S., Camann, D., et al., 2004. Epidemiologic evaluation of measurement data in the presence of detection limits. Environ. Health Perspect. 112 (17), 1691–1696. https://doi.org/10.1289/ehp.7199. Dec.

Mahalingaiah, S., Meeker, J.D., Pearson, K.R., et al., 2008. Temporal variability and predictors of urinary bisphenol A concentrations in men and women. Environ Health Perspect. 116 (2), 173–178. https://doi.org/10.1289/ehp.10605.

Marks, A.R., Harley, K., Bradman, A., et al., 2010. Organophosphate pesticide exposure and attention in young Mexican-American children: the CHAMACOS study. Environ. Health Perspect. 118 (12), 1768–1774. https://doi.org/10.1289/ehp.1002056. Dec.

Plichta, M.M., Heinzel, S., Ehlis, A.C., Pauli, P., Fallgatter, A.J., 2007. Model-based analysis of rapid event-related functional near-infrared spectroscopy (NIRS) data: a parametric validation study. Neuroimage 35 (2), 625–634. https://doi.org/10.1016/j.neuroimage.2006.11.028. Apr 1.

Pollonini, L., Bortfeld, H., Oghalai, J.S., 2016. PHOEBE: a method for real time mapping of optodes-scalp coupling in functional near-infrared spectroscopy. Biomed. Opt Express 7 (12), 5104–5119. https://doi.org/10.1364/BOE.7.005104. Dec 1.

Rauh, V., Arunajadai, S., Horton, M., et al., 2011. Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide. Environ Health Perspect. 119 (8), 1196–1201. https://doi.org/10.1289/ehp.1003160.

Rauh, V.A., Perera, F.P., Horton, M.K., et al., 2012. Brain anomalies in children exposed prenatally to a common organophosphate pesticide. Proc. Natl. Acad. Sci. U. S. A. 109 (20), 7871–7876. https://doi.org/10.1073/pnas.1203396109. May 15.

Saeedi Saravi, S.S., Amirkhanloo, R., Arefidoust, A., et al., 2016. On the effect of minocycline on the depressive-like behavior of mice repeatedly exposed to malathion: interaction between nitric oxide and cholinergic system. Metab Brain Dis. 31 (3), 549–561. https://doi.org/10.1007/s11011-015-9764-z.

Sagiv, S.K., Harris, M.H., Gunier, R.B., et al., 2018. Prenatal organophosphate pesticide exposure and traits related to autism spectrum disorders in a population living in proximity to agriculture. Environ. Health Perspect. 126 (4), 047012 https://doi.org/10.1289/EHP2580. Apr 25.

Sagiv, S.K., Bruno, J.L., Baker, J.M., et al., 2019. Prenatal exposure to organophosphate pesticides and functional neuroimaging in adolescents living in proximity to pesticide application. Proc Natl Acad Sci U S A. 116 (37), 18347–18356. https://doi.org/10.1073/pnas.1903940116.

Sagiv, S.K., Kogut, K., Harley, K., Bradman, A., Morga, N., Eskenazi, B., 2021. Gestational exposure to organophosphate pesticides and longitudinally assessed behaviors related to attention-deficit/hyperactivity disorder and executive function. Am. J. Epidemiol. 190 (11), 2420–2431. https://doi.org/10.1093/aje/kwab173. Nov 2.

Sagiv, S.K., Mora, A.M., Rauch, S., et al., 2023. Prenatal and childhood exposure to organophosphate pesticides and behavior problems in adolescents and young adults in the CHAMACOS study. Environ. Health Perspect. 131 (6), 67008.

Sapbamrer, R., Hongsibsong, S., 2019. Effects of prenatal and postnatal exposure to organophosphate pesticides on child neurodevelopment in different age groups: a systematic review. Environ Sci Pollut Res Int. 26 (18), 18267–18290. https://doi.org/10.1007/s11356-019-05126-w.

Suskauer, S.J., Simmonds, D.J., Caffo, B.S., Denckla, M.B., Pekar, J.J., Mostofsky, S.H., 2008. fMRI of intrasubject variability in ADHD: anomalous premotor activity with prefrontal compensation. J Am Acad Child Adolesc Psychiatry. 47 (10), 1141–1150. https://doi.org/10.1097/CHI.0b013e3181825b1f.

Teeguarden, J.G., Calafat, A.M., Ye, X., et al., 2011. Twenty-four hour human urine and serum profiles of bisphenol a during high-dietary exposure. Toxicol Sci. 123 (1), 48–57. https://doi.org/10.1093/toxsci/kfr160. Sep.

Tsuchiyama, T., Ito, Y., Oya, N., et al., 2022. Quantitative analysis of organophosphate pesticides and dialkylphosphates in duplicate diet samples to identify potential sources of measured urinary dialkylphosphates in Japanese women. Environ. Pollut. 298, 118799 https://doi.org/10.1016/j.envpol.2022.118799. Apr 1.

U.S. Census Bureau, 2000. Poverty Thresholds: Poverty Thresholds by Size of Family and Number of Children. Google Scholar. https://www.census.gov/data/tables/time-series/demo/income-poverty/historical-poverty-thresholds.html. (Accessed 25 May 2022).

Witiuk, K., Fernandez-Ruiz, J., McKee, R., et al., 2014. Cognitive deterioration and functional compensation in ALS measured with fMRI using an inhibitory task. J. Neurosci. 34 (43), 14260–14271. https://doi.org/10.1523/JNEUROSCI.1111-14.2014. Oct 22.