
بسم الله الرحمن الرحیم

A journal club presentation on

Environmental exposures to pesticides, phthalates, phenols and trace elements are associated with neurodevelopment in the CHARGE study

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Environment International 161 (2022) 107075

Contents lists available at ScienceDirect

Environment International

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

No.	Title	Subject Category	Publisher/ Holder	IF	IF Quartile	CiteScore	CiteScore Quartile	H-Index	Indexed in	Details
1	Environment International ISSN/ISBN: 0160-4120, 1873-6750	5% Environmental ...	Elsevier	9.621	Q1	11.60	Q1	191	ISI, Scopus, PubMed, Embase	

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Introduction

ASD

- Autism spectrum disorder
- Major public health concern
- Affects about 1 in 54 children

Risk Factors

- Nutrition
- Genetics
- Environmental exposures
  - Such as air pollution
  - Pesticides (organophosphate, pyrethroid, and/or organochlorine)

Litrature review

Study	Pollutants	Outcome
A birth cohort	Pyrethroid metabolite (3-PBA) OP	ASD ASD in girls
Robust research	Phthalates	Neurodevelopment
Animal studies	Phthalates	Neuro-developmental toxicities
A canadian study	Phthalates	Autistic behaviors in boys
A high-risk ASD birth cohort	Phenols	Asd
Bellinger 2019	Metals such as pb and hg	Adverse neurological effects
Wang et al 2008	Metalloid arsenic	Metalloid arsenic

Aims

The aim of this study was to evaluate :

If exposures to pesticides, phthalates, phenols and trace elements at 2–5 years of age are associated with neurodevelopmental outcomes

For this purpose, three main comparisons are made,

- ASD vs TD (typical development),
- DD (developmental delays other than ASD) vs TD,
- Other Early Concerns (OEC) vs TD.

Material and methods

Study design

- A part of the CHARGE study
- A case control study that recruits participants from three groups:
  - Children with ASD
  - Children with DD but not ASD,
  - General population controls

Recruitment

- ASD and DD children ⇒ from the California Department of Developmental Services (coordinates services for people with developmental disabilities, and includes all residents of California regardless of place of birth, religion, or financial resources)

- Controls are sampled from California birth files, with frequency-matching to ASD cases on age, sex and broad geographic regions encompassing up to 10 counties

Inclusion criteria

a) Aged 24–60 months at recruitment

b) Living with a biologic parent who speaks English or Spanish

c) Born in California

d) Residing in the study catchment area

Study participants

- 627 children participated this study
- Participants were recruited to the study between 2006 and 2017 and had at least 16 mL of urine

Developmental Assessment

ASD diagnosis

- Two gold standard psychometric instruments
  - The autism diagnostic interview-revised (ADI-R) (semi-structured interview for the primary caregiver that reviews the child's development)
  - Autism Diagnostic Observation Schedules (ADOS) (semi-structured assessment in which the researcher observes the social interaction, communication, play and imaginative use of materials by children suspected of having ASD)
- To assign final diagnoses of ASD, DSM-5 was used

Developmental Assessment

All children were administered

- the Mullen Scales of Early Learning (MSEL)
- the Vineland Adaptive Behaviors Scores (VABS).

DD diagnosis

Children who did not meet criteria for ASD and had:

- Scores on either the MSEL or VABS that fell below 1.5 SD lower than the mean

- Scores on the other instrument <2.0 SD lower than the mean were

OEC diagnosis

- Children in both DD and control group were screened for ASD using the Social Communications Questionnaire to confirm that they do not have ASD

- For those who screened positive, the ADI-R and ADOS was then administered to determine whether or not they have ASD

- Other children who were diagnoses with ASD or DD, but were not confirmed for either of these two diagnoses, were grouped together as Other Early Concerns (OEC)

Control group

- Children enrolled as general population controls who did not meet criteria for either ASD or DD were classified as TD

- All classification groups are mutually exclusive

Exposure Assessment

Urine samples

- Urine samples were collected at the time of the visit
- Immediately frozen at -20 ◦C
- Samples remained frozen until analysis (mean 7.4 years)
- They were then shipped on dry ice to the New York State Department of Health (NYSDOH)

Measurements of phenolic compounds

2.5 ng of isotopically labeled internal standards+ 250µL urine

400 µL of 1 M ammonium acetate+200 unit/mL of β-glucuronidase

incubated at 37 ◦C for 12 h

Ethyl acetate (10 mL)

shaken for 60 min and centrifuged at 4500 rpm for 5 min

Supernatant washed with water

Concentrated with nitrogen stream + 250 µL methanol

Injected to HPLC-MS/MS

Measurements of phthalates metabolites

2.5 ng of internal standards+ 250µL urine

300 µL of 1 M ammonium acetate containing 100 unit/mL of β-glucuronidase

incubated at 37 ◦C for 12 h

ABS Elut-NEXUS SPE cartridges

conditioned with methanol (2 mL) and water (2 mL)

250 µL of acetonitrile/ 20 mM ammonium acetate (90:10, v/v)

evaporated to near-dryness

Filtred

Injected to HPLC-MS/MS

Measurements of dialkylphosphate metabolites

2.5 ng of isotopically labelled internal standards+ 250µL urine

2% formic acid (750 µL)

incubated at 37 ◦C for 12 h

WAX cartridges

conditioned with methanol (2 mL) and water (2 mL)

250 µL of acetonitrile/ 20 mM ammonium acetate (90:10, v/v)

evaporated to near-dryness

Filtred

Injected to HPLC-MS/MS

Measurements of elements

Diluted 1 + 19 with a reagent containing nitric acid, Triton X-100 + internal standards

Injected to ICP-MS

Concentration correction

- Urinary biomarkers were specific gravity (SG) corrected using the following formula:

$$Pc = P \times [(SGp - 1)/(SG - 1)]$$

Pc : the SG-corrected metabolite concentration (ng/mL)

P : the measured metabolite concentration in ng/mL

SG : the specific gravity of the urine sample

SGp : the median specific gravity across CHARGE participants providing urine for this study = (1.0223)

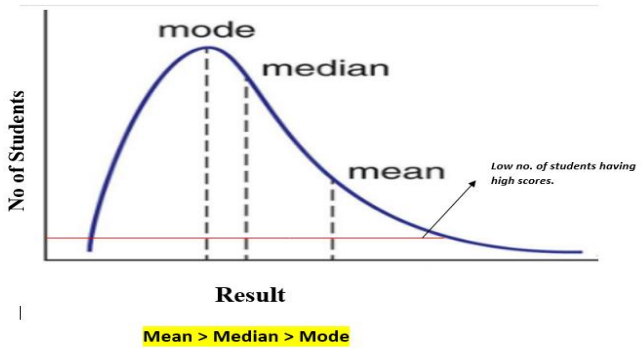
***Specific gravity correction factors greater than 2 were replaced with 2 and for values below 0.5 were replaced with 0.5**

Data analysis

- Negative values can arise legitimately at or below the limit of detection (LOD)
- The calculation of the LOD represents a theoretical RSD of ± 33% of the signal, and this is equivalent to a relative uncertainty of ± 94% in the concentration number reported, resulting in some negative values
- For each urinary chemical with a minimum SG-corrected concentration of 0 or less (i.e. negative), the minimum concentration and a value of 0.01 were added to all values

Data analysis

- All chemicals were measured in ng/ml.
- To account for right skewedness of biomarker data, natural log transformed values were used.



Data analysis

Two regression approaches were used for assessing the chemicals in relation to child diagnoses:

- Single chemical
- Mixture models.

Mixture models were applied in two ways:

- to combine individual compounds within a chemical class,
- to combine across all classes of chemicals

Chemicals used in regression analyses had at least 60% of measured concentrations above the study wide LOD prior to SG correction.

CONFOUNDERS

- All models were adjusted for covariates selected a priori or that were related to the exposure and outcome ($p < 0.20$)
- These included child's sex, year of birth, age in months at time of diagnosis, and race, as well as parental homeowner status, and maternal metabolic conditions during pregnancy.
- They strove to use the most parsimonious model that still adjusted for important confounders, and thus selected a single variable, parental homeowner status, to represent socioeconomic status.
- Year of birth was centered by subtracting the mean birth year.

Data analysis

Models	Purpose
Multinomial logistic regression models	For each individual chemical, to simultaneously estimate three regression coefficients, representing the strength of associations with ASD, DD, or OEC versus TD
False discovery rate (FDR) correction	Applied to p-values per outcome and chemical class to account for multiple comparisons
Weighted quantile sum (WQS) regression	Mixture effect of chemicals on the outcome while accommodating for a complex correlation pattern among the chemical components
5th percentile (PCT)	Used to define the lower limit
Random subset WQS (rswqs)	Total chemical mixture was tested, combining across all urinary chemical classes

Data analysis

- 1/c was refered to the chemical of concern threshold moving forward
- An alpha of 0.05 was the criterion for statistical significance
- All statistical analyses were conducted with SAS statistical analysis software version 9.4

Results

Results

- The population included 237 TD, 224 ASD, 81 DD, and 85 OEC participants
- The population had a greater fraction of male participants, as ASD is more prevalent in males
- Methyl paraben (MEPB), was selected as a representative compound as it was widely detected and had an association with all three outcomes to help select model covariates
- Chemicals which met the threshold of above 60% of LOD included in the remaining analyses.

	Typical Development N = 237	Autism Spectrum Disorder N = 224	Developmental Delay N = 81	Other early concerns or high risk N = 85	Mean MEPB (ng/ml)	Association with ln MEPB p-value[a]	Outcome p-value[b]
	Freq (%)	Freq (%)	Freq (%)	Freq (%)			
Sex						0.680	<0.001
Male	193 (81.43)	181 (80.80)	60 (74.07)	50 (58.82)	568.0		
Female	44 (18.57)	43 (19.20)	21 (25.93)	35 (41.18)	389.9		
Race						0.010	0.140
White (non-Hispanic)	128 (54.01)	106 (47.32)	32 (39.51)	41 (48.24)	416.6		
Non-White (non-Hispanic)	46 (19.41)	55 (24.55)	16 (19.75)	15 (17.65)	904.7		
Hispanic (any race)	63 (26.58)	63 (28.13)	33 (40.74)	29 (34.12)	443.5		
Highest education in household						0.756	<0.001
High school diploma/GED or less	6 (2.53)	28 (12.50)	18 (22.22)	10 (11.76)	760.2		
Some college (inc. vocational, 2 yr degree)	82 (34.60)	62 (27.68)	28 (34.57)	31 (36.47)	606.5		
Bachelor's degree	90 (37.97)	81 (36.16)	26 (32.10)	28 (32.94)	440.3		
Graduate or professional degree	59 (24.89)	53 (23.66)	9 (6.57)	16 (18.82)	448.0		
Homeowner						0.154	<0.001
No	57 (24.05)	73 (32.59)	36 (44.44)	38 (44.71)	706.4		
Yes	180 (75.95)	151 (67.41)	45 (55.56)	47 (55.29)	441.1		
Prenatal Vitamin Use in 3 months before or during 1st month of pregnancy (31 Missing)						0.473	0.190
No	67 (29.13)	74 (34.58)	32 (41.56)	22 (29.33)	431.7		
Yes	163 (70.87)	140 (65.42)	45 (58.44)	53 (70.67)	537.7		
Maternal metabolic conditions						0.074	0.006
Healthy weight and no metabolic conditions	119 (50.21)	101 (45.09)	31 (38.27)	39 (45.88)	508.9		
Overweight and no metabolic conditions	58 (24.47)	43 (19.20)	18 (22.22)	10 (11.76)	519.7		
Obese and no other metabolic conditions	34 (14.35)	30 (13.39)	15 (18.52)	10 (11.76)	566.8		
Any hypertensive disorder or diabetes at any BMI	26 (10.97)	50 (22.32)	17 (20.99)	26 (30.59)	551.4		
Mother's birthplace						0.557	0.024
USA	202 (85.23)	164 (73.21)	64 (79.01)	68 (80.00)	565.4		
Mexico	10 (4.22)	18 (8.04)	9 (11.11)	8 (9.41)	239.5		
Outside the US and Mexico	25 (10.55)	42 (18.75)	8 (9.88)	9 (10.59)	456.6		
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)			
Child age at assessment (months)	46.02 (9.04)	48.67 (8.70)	47.88 (7.99)	48.00 (9.25)		0.015	0.013
Child's year of birth	2006.5 (2.90)	2007.3(3.22)	2006.1 (2.73)	2006.4 (2.87)		0.172	0.006
Mother's age at time of child's birth (years)	30.23 (5.27)	30.67 (5.74)	30.11 (6.41)	31.00 (5.79)		0.160	0.617
Pre-pregnancy BMI	25.71 (5.46)	26.40 (6.17)	26.64 (6.23)	27.35 (7.83)		0.569	0.183

[a] For categorical variables with only two levels, p-values are shown from t-tests; for categorical characteristics with more than two levels, p-values are shown from ANOVA tests; for continuous characteristics, p-value are shown from univariate linear models.

[b] For categorical characteristics, p-values are shown from Chi square test; for continuous characteristics, p-values are shown from univariate multinomial logistic models.

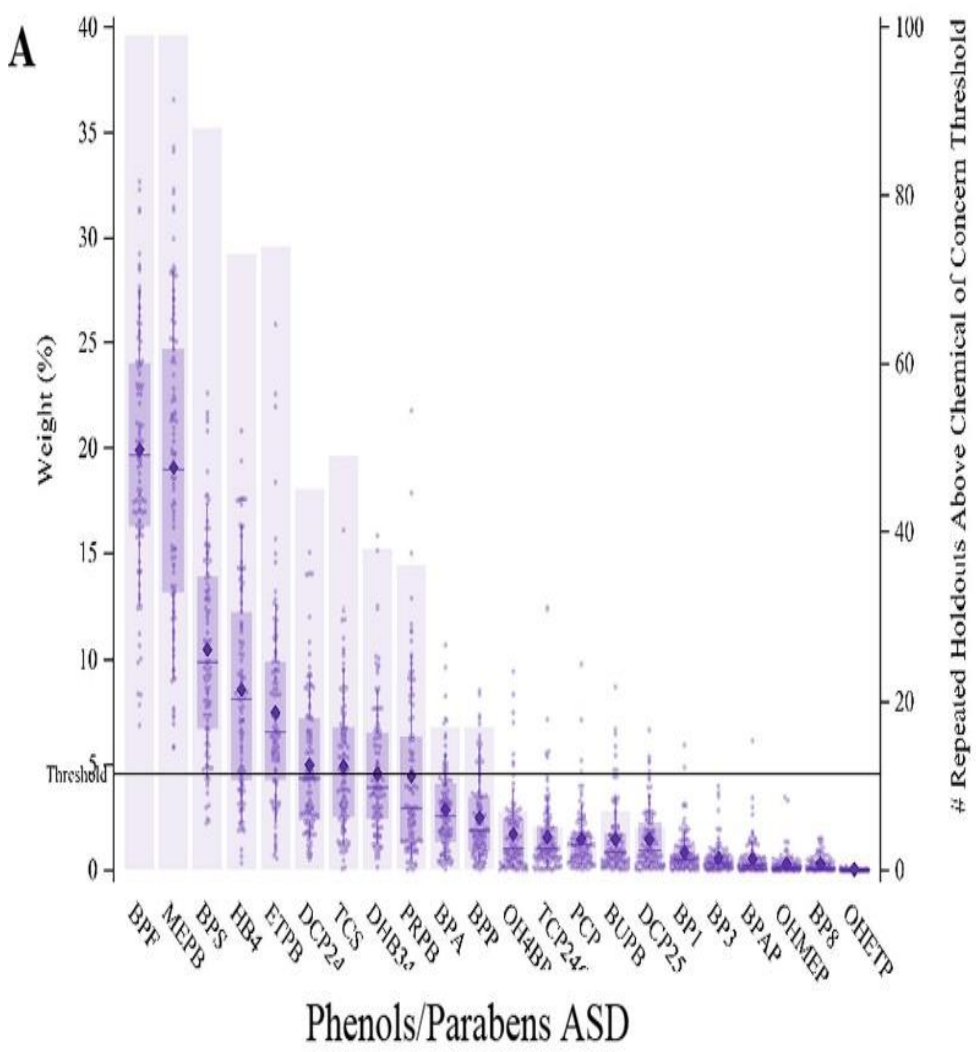
Results

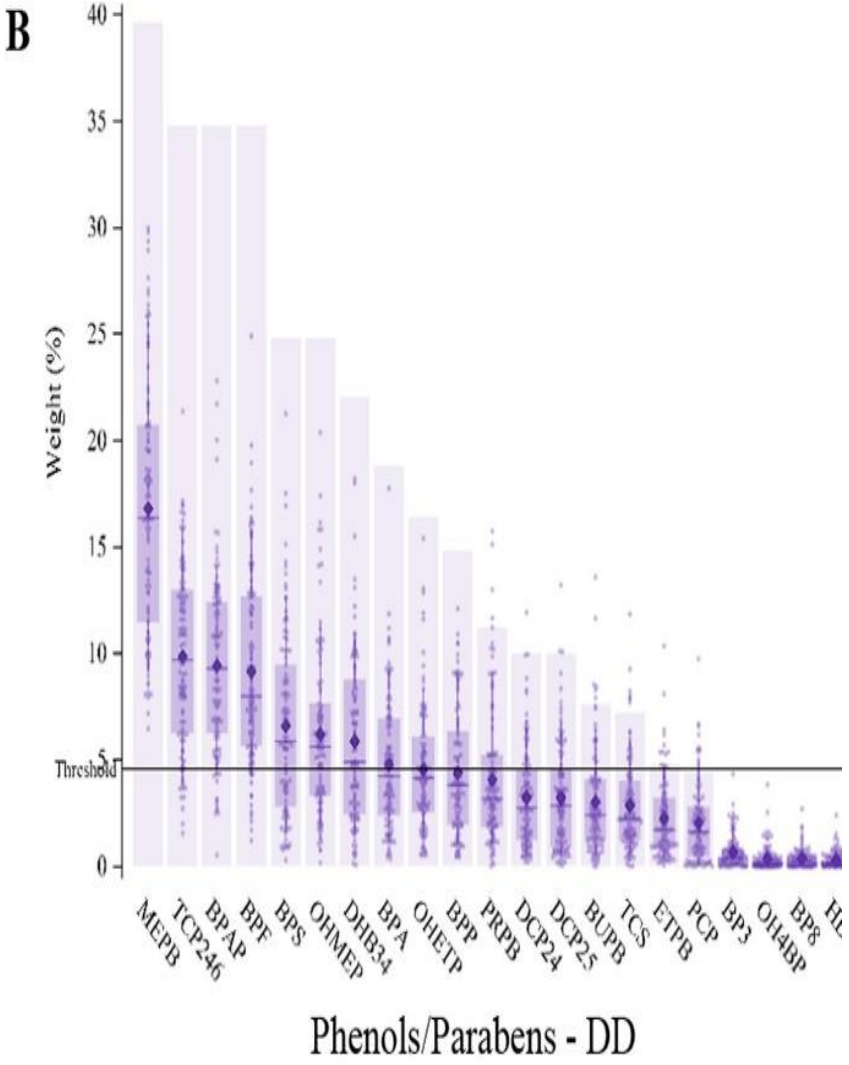
- Due to the large number of results of the single chemical analysis, results that were statistically significant were focused at $p < 0.05$, or had sizable (non-null) effect size (OR > 1.1 or < 0.9).

Phenols and parabens

- The repeated holdout WQS indicated a significant association between the phenol/paraben index and DD diagnosis [average OR = 2.40, (5th PCT = 1.05)]
- A weaker but still significant mixture effect was also found for diagnosis of ASD with the phenol/paraben index [average OR = 1.50, (5th PCT =1.04).
- A positive but wak trend with the phenol index and OEC diagnosis [average OR = 1.65, (5th PCT = 0.94)]

Phenols and parabens





Phenols and parabens

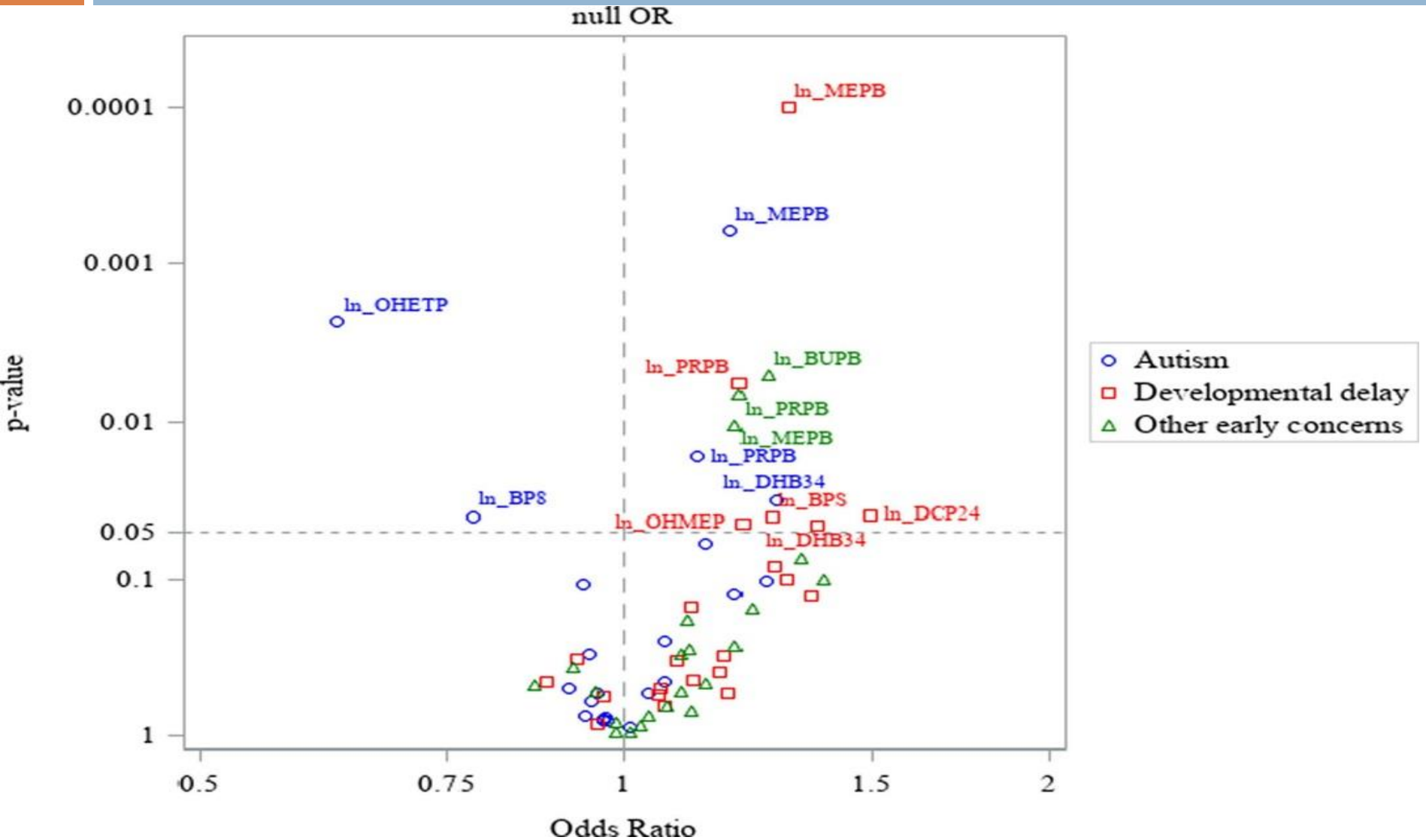
- MEPB was a probable contributor to the mixture effect for the relationship between the phenol/paraben index and ASD diagnosis
- Single chemical analysis with quartiles supported associations with MEPB [OR = 1.40 (1.18–1.68), FDR-corrected p-value 0.004]
- Bisphenol F (BPF) was both a probable contributor to the mixture effect on ASD and was borderline significant in the single chemical linear model [OR = 1.14 (0.99–1.31)].
- Probable contributor ethyl paraben (ETPB) was not significant in the linear model [OR = 1.07 (0.95–1.20)] but was significant in the quartile analysis [OR = 1.19 (1.01–1.42)]. This relationship was not significant after FDR correction.

Phenols and parabens

- Bisphenol S (BPS) was a possible contributor to the mixture effect but was not significant in linear or quartile single chemical analysis [linear OR = 1.07 (0.90–1.28)]

- For the DD diagnosis, MEPB, BPS, DHB34, and TCP246 had probable, or possible contributions in the mixture analysis

Volcano plot of the association of phenols and parabens with ASD, DD AND OEC



Phthalates

- The WQS analysis resulted in no significant mixture effect for phthalate metabolites on any of the outcomes

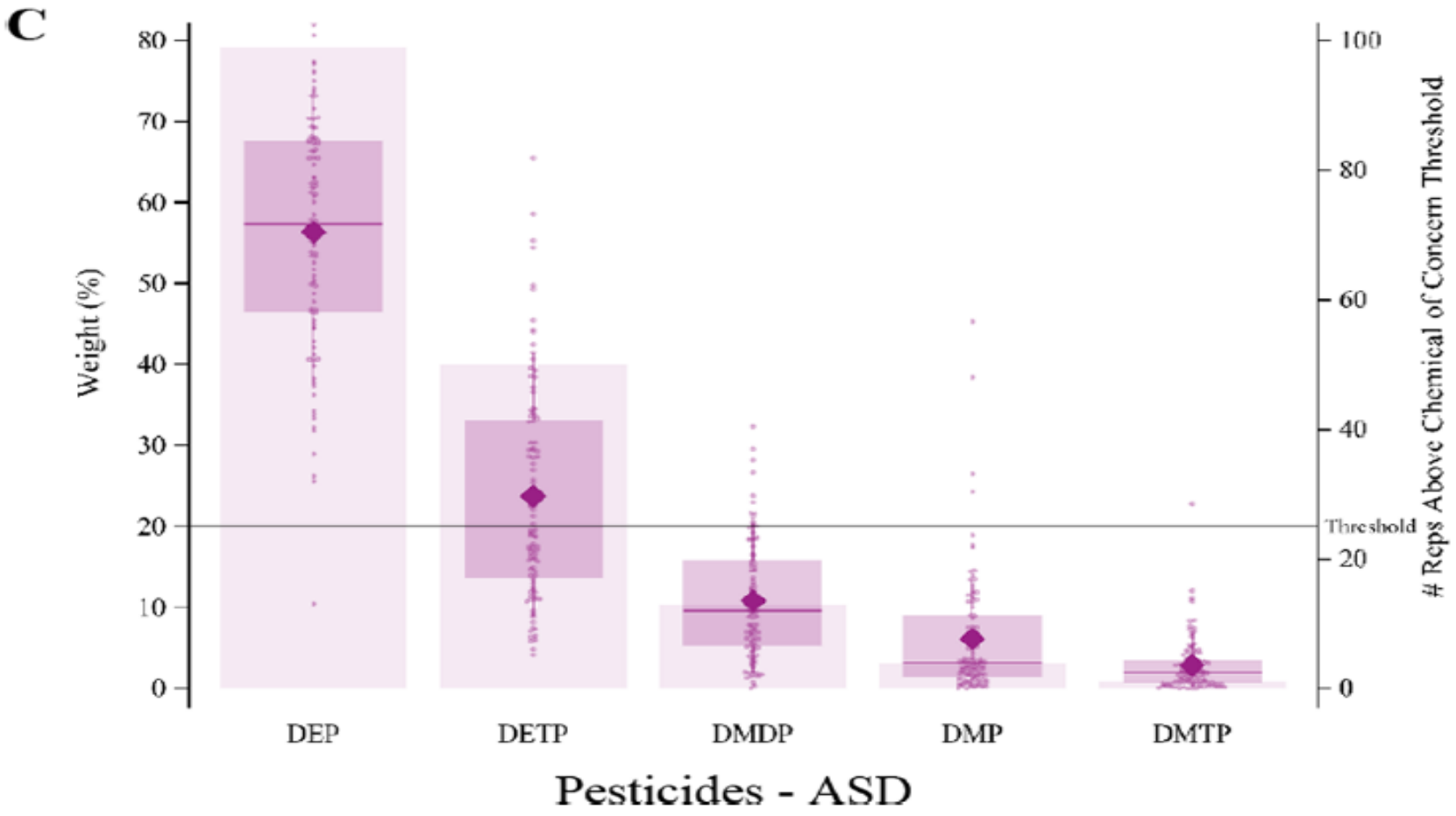
- Quartile results shows that higher levels of MCINP were associated with increased odds of ASD [OR = 1.19 (1.00–1.41)]

		ASD vs TD				DD vs TD				OEC vs TD			
Chemical Class Mixture		Mean	Median	5thPCT	95thPCT	Mean	Median	5thPCT	95thPCT	Mean	Median	5thPCT	95thPCT
Phenols/ Parabens	OR	1.50	1.46	1.04	2.10	2.40	2.25	1.05	4.59	1.65	1.58	0.94	2.50
Phthalates	OR	1.12	1.08	0.88	1.42	1.33	1.29	0.89	1.93	1.30	1.26	0.90	1.74
Pesticides	OR	1.10	1.11	1.02	1.19	1.05	1.05	0.93	1.19	1.11	1.11	0.99	1.25
Trace Elements	OR	1.17	1.16	0.88	1.46	1.75	1.68	1.19	2.51	1.22	1.18	0.80	1.77
Total Mixture	OR	1.84	1.86	1.08	2.70	3.44	3.09	1.43	7.04	2.20	2.07	1.07	3.93

Pesticides

- Mixture analysis resulted in a statistically significant association between the DAP (organophosphate pesticides) mixture and ASD [(average OR = 1.10, 5th PCT = 1.02)
- The pesticide mixture was positive, on average, for DD but the confidence interval overlaps with 1, likewise for OEC
- DETP is a possible contributor to the mixture effect and was also significant in the single chemical analyses [linear: OR = 1.16 (1.00–1.35), quartile: OR = 1.19 (1.00–1.42)]

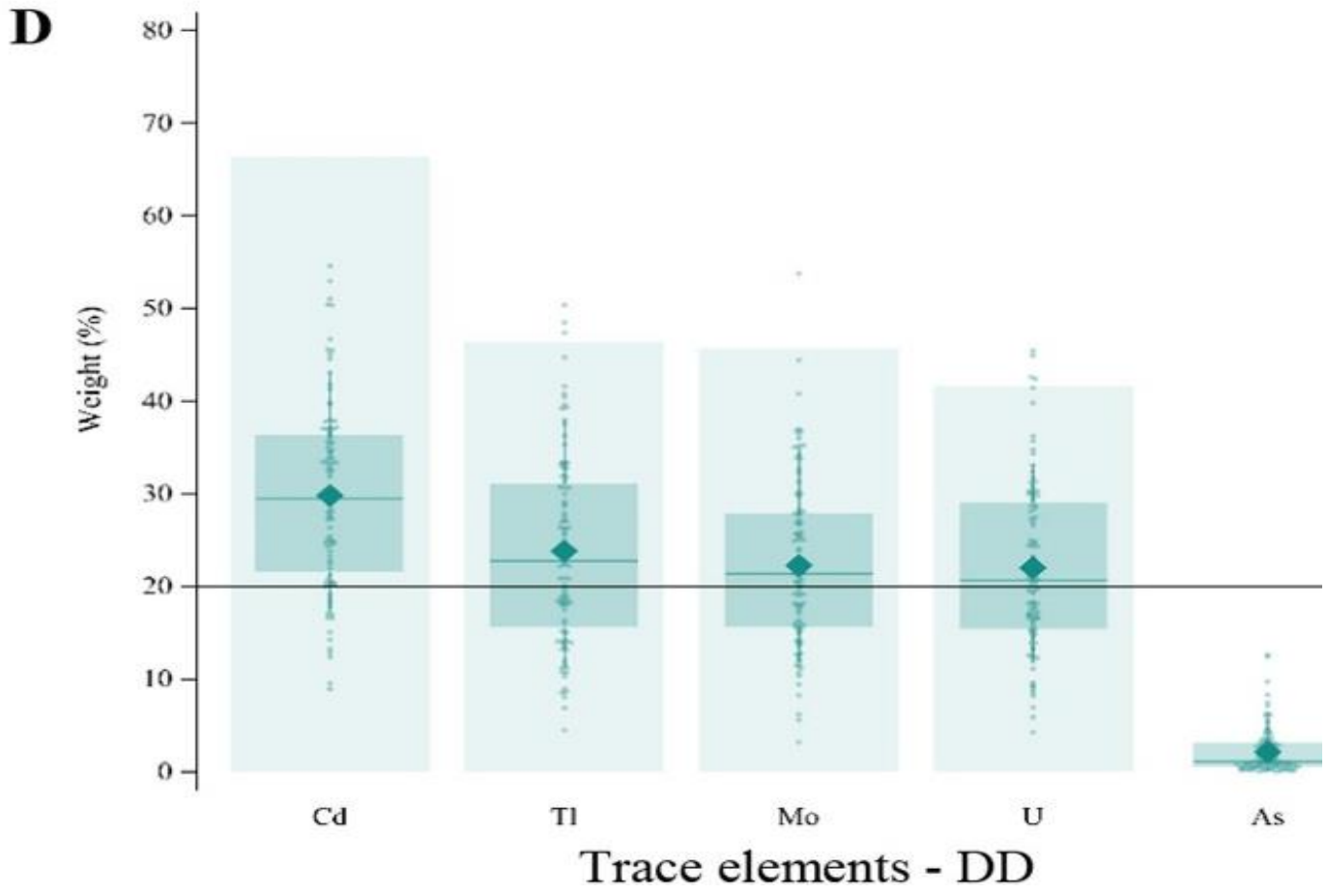
Pesticides


C
Weight (%)
80
70
60
50
40
30
20
10
0
100
80
60
40
Threshold
20
0
Reps Above Chemical of Concern Threshold
DEP
DETP
DMDP
DMP
DMTP
Pesticides - ASD

Trace Elements

- The trace element mixture was significantly associated with increased odds of DD compared to TD [(average OR = 1.75, 5th PCT = 1.19)
- Four different individual elements each contribute only modestly, but together the impact is noticeable
- The mixture effect was not significant for ASD, U was associated with increased odds of ASD compared to TD and was borderline significant after FDR correction in the linear model

Trace Elements



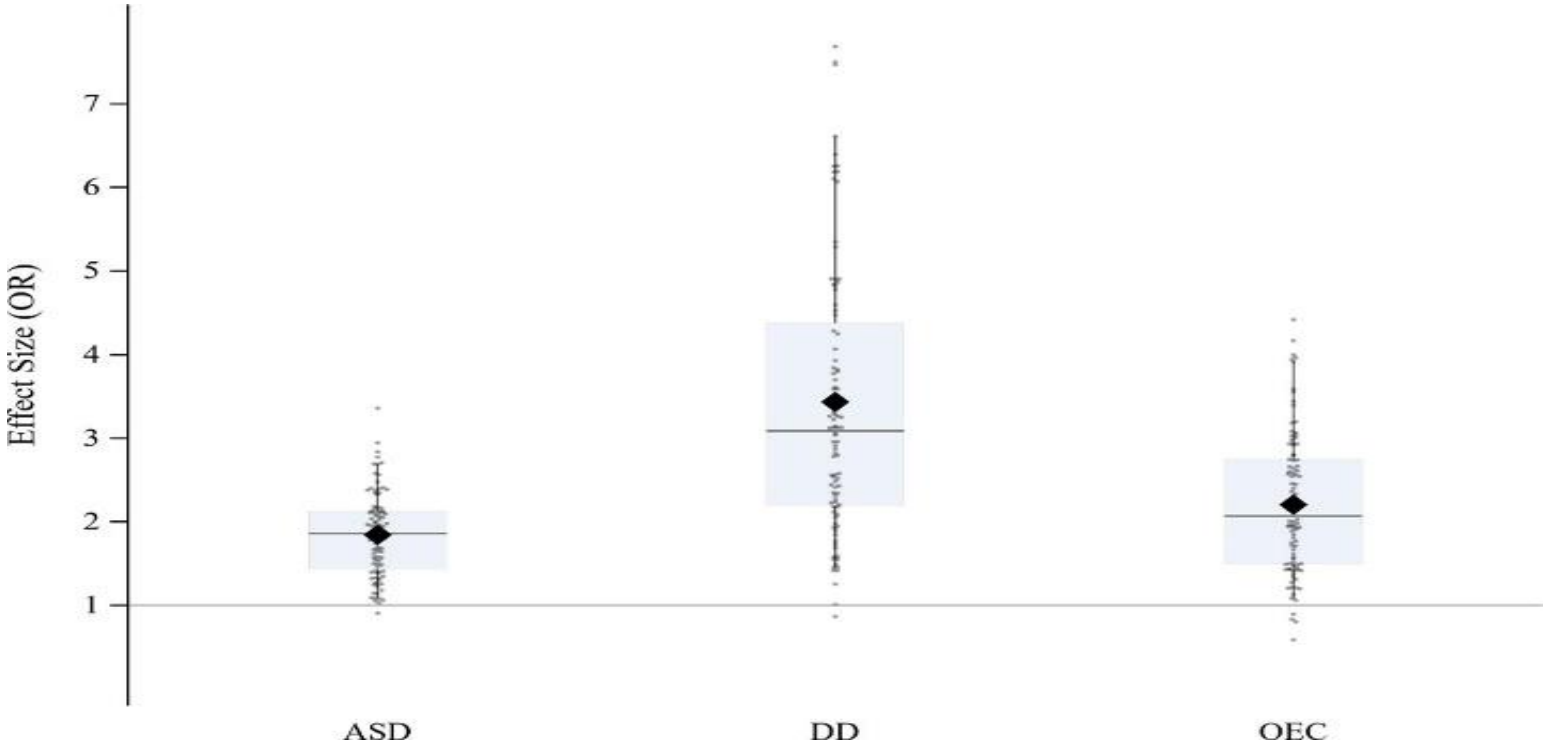
Mixtures

- A total mixture effect, combining all urinary chemicals (44 chemicals in total) was tested with each outcome

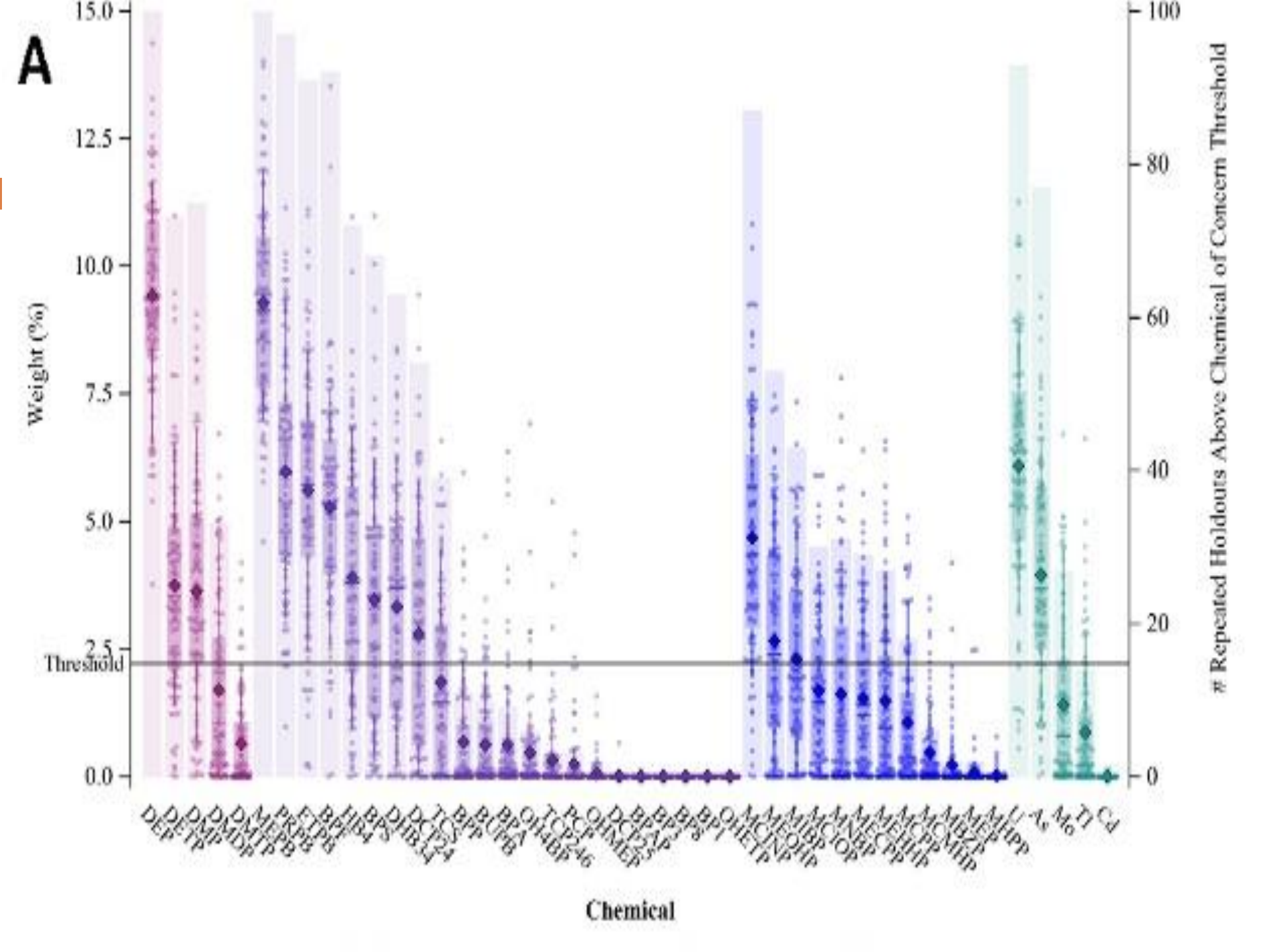
- The mixture effect was positively associated with ASD (average OR = 1.84, 5th PCT =1.08), DD (average OR = 3.44, 5th PCT = 1.43), and OEC (average OR =2.20, 5th PCT = 1.07) diagnoses

		ASD				DD				OEC[I]		
Chemical Class	Chemical Abbreviation	Linear	Quartile	Class Mixture	Total Mixture	Linear	Quartile	Class Mixture	Total Mixture	Linear	Quartile	Total Mixture
Phenols/ Parabens	BP1						-(*)				-	
	BP3		-				-				-(*)	
	BP8	-(*)	-			-	-				-	
	BPA						+		possible		+	
	BPAP					+	+	possible	possible	+		
	BPF	+	+	probable	possible			possible	possible		-(*)	
	BPP					+	+		possible	+	+	possible
	BPS		+	possible	possible	*	+	possible	possible			
	BUPB						+		possible	*	+	possible
	DCP24	+	+		possible	*	+		possible	+	+	possible
	DHB34	*	+			*	*	possible	possible	+	+	possible
	ETPB		*	possible	probable	+	+			+	+	possible
	HB4	+	*	possible	possible	+	+			+	*	possible
	MEPB	**	**	probable	probable	**	**	probable	probable	*	*	probable
	OH4BP					+				+	+	
	OHETP	-(**)	-(**)									
	OHMEP					*	+	possible	possible	+	+	possible
	PCP					+						
	PRPB	*	*		probable	*	*		probable	*	*	possible
	TCP246					+	*	possible	probable			
	TCS						+				+	possible
Phthalates	MCINP		*		probable	+	+		possible	+	+	possible
	MCIOP					+	+			+	*	probable
	MCPP					*	*		probable	+		
	MECPP	+				+	+		possible	+	+	possible
	MEHHP	+				+	*		probable	+	+	
	MEOHP	+			possible	+	*		possible	+	+	
	MHPP					+						
	MIBP	+	+		possible					+	+	possible
Pesticides	DEP	*	**	probable	probable					+	*	probable
	DETP	*	*	possible	possible		+				+	
	DMP		+		possible		+			+	+	possible
	DMTP									+	+	possible
Trace Elements	As	+	+		possible	+						
	Cd		-			+	+	possible	possible	-		
	Mo					+	+	possible	possible	+		
	Tl					+	*	possible	probable	+	+	possible
	U	*	*		probable	*	*	possible	possible	+	+	possible

Visual representation of OR distributions



The box represents 25th and 75th percentiles, the line represents the median, the closed diamond represents the mean, and the whiskers show the 5th and 95th percentiles


A
Weight (%)
15.0
12.5
10.0
7.5
5.0
2.5
0.0
Threshold
Repeated Holdouts Above Chemical of Concern Threshold
100
80
60
40
20
0
DEP
DETP
DMP
DMTP
DMDP
DMTTP
MEPB
PRPB
ETPB
BPF
HIB4
BPS
PHB14
DCP24
TCS
BPP
BUMB
BPA
OH4BP
TCP246
PCP
OHMEP
DCP25
BPAP
BPS3
BPI
OHETP
MCNP
MEOHP
MIBP
MCIOP
MNBP
MECPP
MEHHP
MCPP
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MBZP
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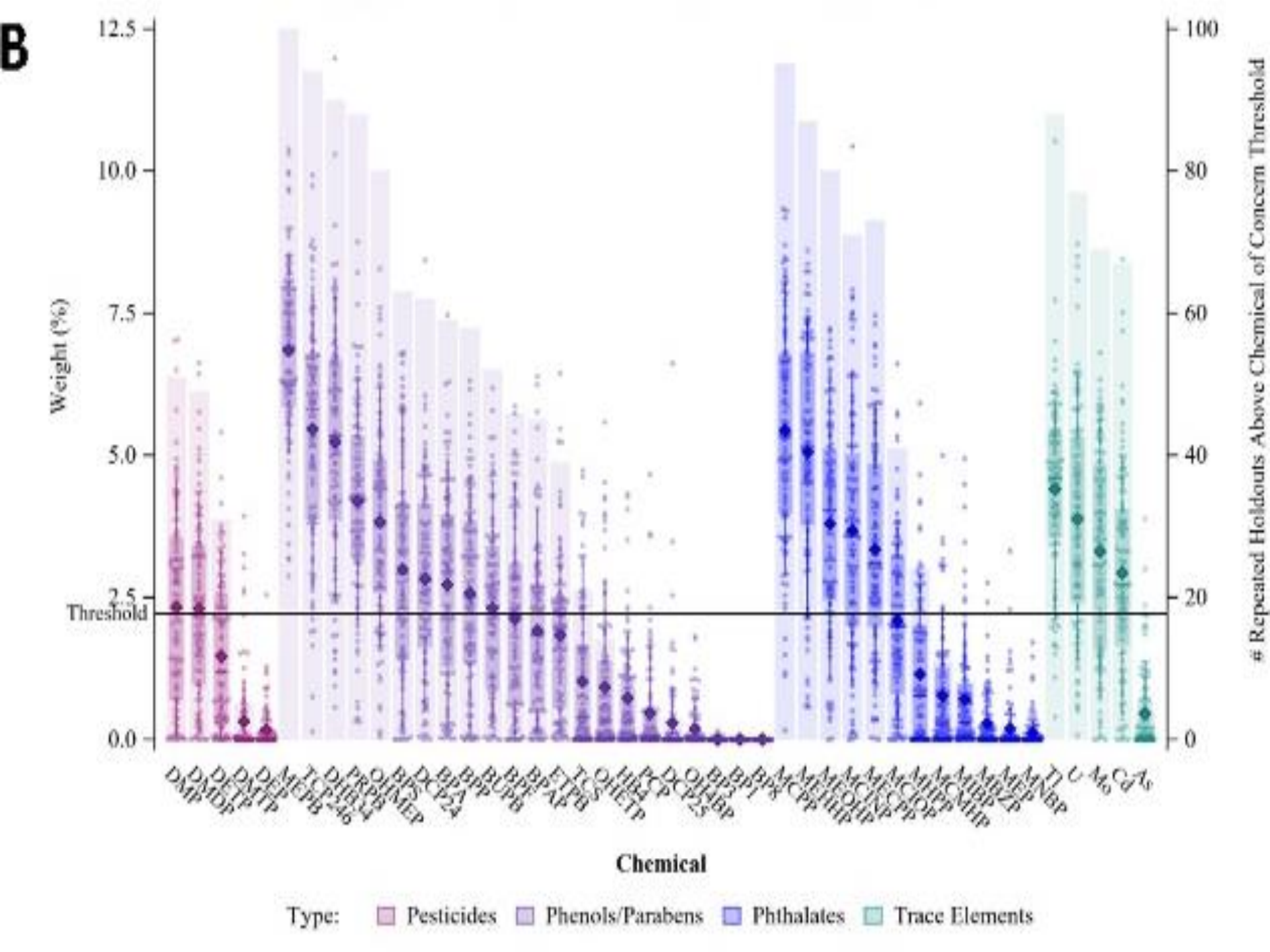
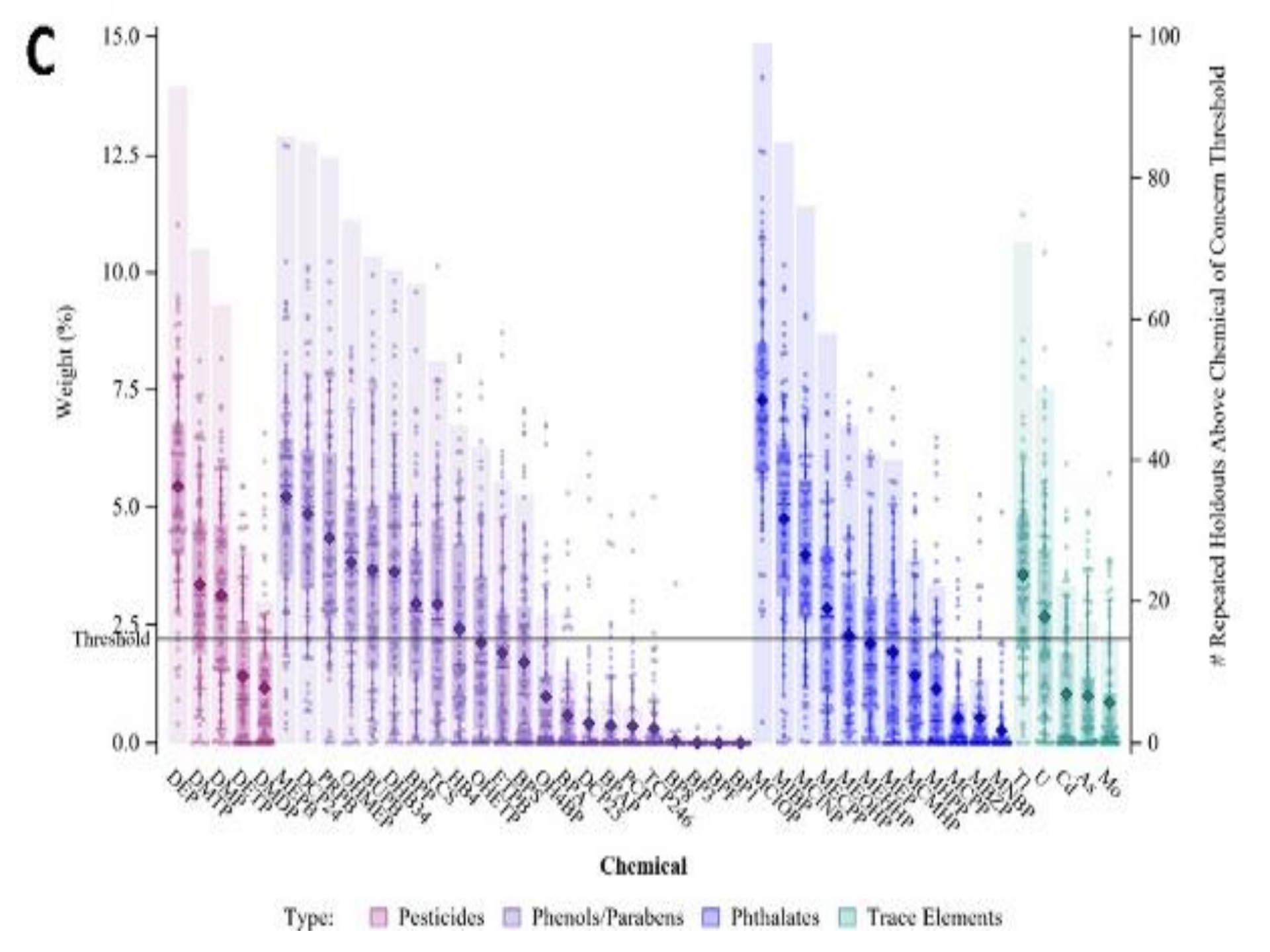
MHPP
As
Mo
Tl
Cd
Chemical


B
12.5
10.0
7.5
5.0
2.5
0.0
Threshold
Weight (%)
100
80
60
40
20
0
Repeated Holdouts Above Chemical of Concern Threshold
DMP
DMOP
DETP
DMTP
DEP
MEPB
TCPY46
DHB14
PRPB
OPMEP
BP3
DCP24
BPA
BPP
BUPB
BPF
BPAP
ETPB
TCS
OHETP
HIBP
PCP
DCP25
OH4BP
BP3
BPP
BPS
MCPP
MEHHP
MEOHP
MCNP
MECPP
MCIOP
MHPP
MCMHP
MBP2P
MEP
MNBP
Tl
U
Mo
Cd
As
Chemical
Type:
Pesticides
Phenols/Parabens
Phthalates
Trace Elements


C
Weight (%)
15.0
12.5
10.0
7.5
5.0
2.5
0.0
Threshold
Repeated Holdouts Above Chemical of Concern Threshold
100
80
60
40
20
0
Chemical
Type:
Pesticides
Phenols/Parabens
Phthalates
Trace Elements

Discussion

Main findings

- Mixture analysis demonstrates associations of phenols/parabens, pesticide metabolites, and trace metals associated with increased odds of ASD and/or DD

- When all 44 individual chemicals were analyzed many of the same chemicals that were prominent in their contribution to their respective chemical class mixture's association with outcome also appeared to be the probable important contributors to the total mixture associations

Main findings

- The same chemicals were often associated with all three outcomes

- Findings were weaker for the OEC group, which is somewhat expected as this group was closer to the typically developing group.

- The compound with the most consistent results was MEPB, with associations with both ASD and DD that were significant after FDR-correction for both linear and quartile models, and probable significance in both the phenol and total mixture.

- ETPB, a structurally similar compound, was also a probable contributor to ASD

Limitations

- Measurements were made after the child initially received the diagnoses
- Urinary measurements of the organic compounds examined here represent recent exposures and should ideally be accessed with multiple repeated urine samples
- For trace elements, the half-lives range from days to years, therefore, the study does not provide evidence as to whether these chemicals contributed to the diagnoses of concern.
- For some of these chemicals there can be considerable day-to-day variation in metabolite levels

Limitations

- Confounders were selected for the analysis based on them being confounders for only one chemical (specifically MEPB).

- There could be other confounders that were important in other associations, but it was beyond the scope of this study to select a different set of confounders for each model.

- Therefore, there is the possibility of residual confounding due to unmeasured confounders

Suggestions

- Further work characterizing toxicokinetic differences by neurodevelopmental outcome would shed light on the mechanisms and directionality underlying the associations that emerged from our mixture analyses

- As the children continue to mature, an additional future direction is to examine the chemicals measured in early childhood for potential influence on their long-term trajectories, which may diverge over time

Conclusion

- Higher concentrations of urinary biomarkers increased the odds of ASD, DD, and OEC compared to TD for several compounds
- Findings were particularly consistent for MEPB for both ASD and DD, ETPB for ASD, and DEP for ASD
- Biospecimens used for chemical analysis were collected many months after diagnoses were made the direction of any causal association is unknown

Ethics

- The CHARGE study protocol and this study were approved by the institutional review boards for the State of California and the University of California-Davis (UC-Davis)
- Participants provided written informed consent before collection of any data


Thanks for
your attention


Thank You.