Preliminary Results: Urinary Biomarkers of Response in Adults and Children from the San Joaquin Valley

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Disclosure

- The content is solely the responsibility of the authors and does not necessarily represent the official views of the collaborators involved.

- The authors declare no conflict of interest.

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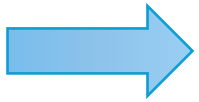
Background – air pollution

- Exposure to air pollutants such as particulate matter 2.5 ($PM_{2.5}$), nitrogen dioxide, and ozone has been linked to adverse health effects.
- The San Joaquin Valley is burdened by high air pollution.
- Indoor air quality (IAQ) is especially important because adults and children spend most of their time indoors.
- Several factors contribute to poor IAQ, including:
  - Smoking, cooking, heating, the use of candles or incense, poor ventilation, the infiltration of traffic-related and other outdoor air pollutants.

Background – study design



- San Joaquin Valley Pollution and Health Environmental Research (SPHERE) Study
  - Environmental measurements
- Participant eligibility:
  - Adults aged 18+ with a child between the ages 3- 13 yrs.
  - N= 64 parent-child pairs
- Residents of Stockton or Fresno
- Spanish or English speakers
- February to November of 2023

- BiomSPHERE added a biomonitoring component to SPHERE
  - 64 parent-child urine samples
  - Subset of 8 families
    - Daily samples were collected over four consecutive days.
- Urine sample measurements:
  - Biomarkers of exposure (VOCs, PAHs, tobacco smoke).
  - Biomarkers of response indicating oxidative stress, inflammation, and airway injury.

Oxidative stress biomarkers

- 8-Isoprostane (8-Isop)
  - 8-Isop indicates lipid peroxidation caused by reactive oxygen species.
  - High levels of 8-Isop reflect oxidative stress.
- 8-hydroxy-2'-deoxyguanosine (8-OHdG)
  - Reflects DNA damage.
  - Increased levels of 8-OHdG have been associated with oxidative damage to genetic material.

References: (Hashemzadeh et al., 2019; Stapleton et al., 2022; Havet et al., 2018; Svecova et al., 2009; Mann et al., 2021)

Inflammation biomarker

- Prostaglandin E2 (PGE2)
  - PGE2 reflects inflammation and may indicate the body's response to environmental stressors.
  - High levels of air pollution exposure have been associated with increased inflammatory responses.

References: (Schneider et al., 2005; Yan et al., 2016)

Airway injury biomarker

- Clara Cell Protein 16 (CC16)
  - Biomarker of airway injury to the respiratory tract lining.
  - Several studies showing that long term exposure to air pollutants damages CC16-producing club cells, leading to decreased levels of CC16, which may result in decrease lung function in adults and children.
  - Other studies have demonstrated that increased concentrations of CC16 can also indicate airway injury due to short-term air pollution exposure.

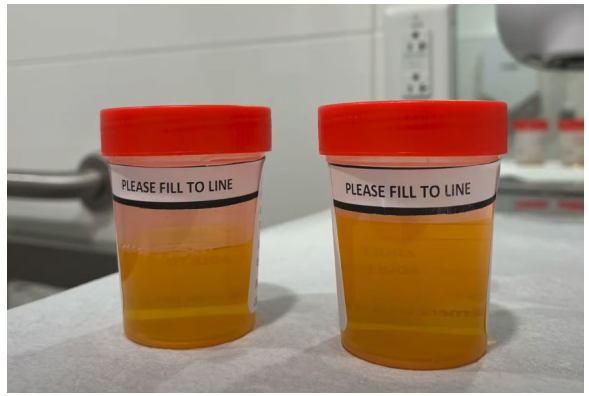
References: (Guerra et al., 2015; Guerra et al., 2013; Heldal et al., 2013; Nauwelaerts et al., 2022; Arsalane et al., 1999; Provost et al., 2014; Stockfelt et al., 2012; Zhang et al., 2022)

Study objectives

1. To examine the distribution of four urinary biomarkers of response in adults and children from the San Joaquin Valley.
2. To evaluate the association of the biomarkers with measurements of air pollutants in participant's homes.
3. To characterize the temporal variability in the biomarker measurements over several days.

Study design – urine samples

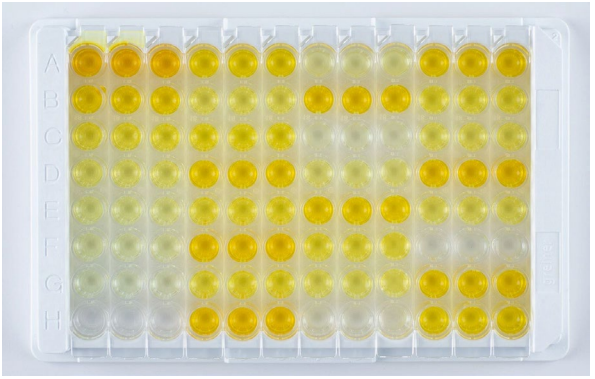
- 64 parent-child pairs
  - Morning samples, most were first morning voids
- Daily samples were collected over four consecutive days for a subset of 8 families
- Urine sample measurements (Holland Lab):
  - 8-Isop, 8-OHdG, PGE2 and CC16
- All biomarker measurements were adjusted for specific gravity[a] (SG) and $\log_2$ transformed

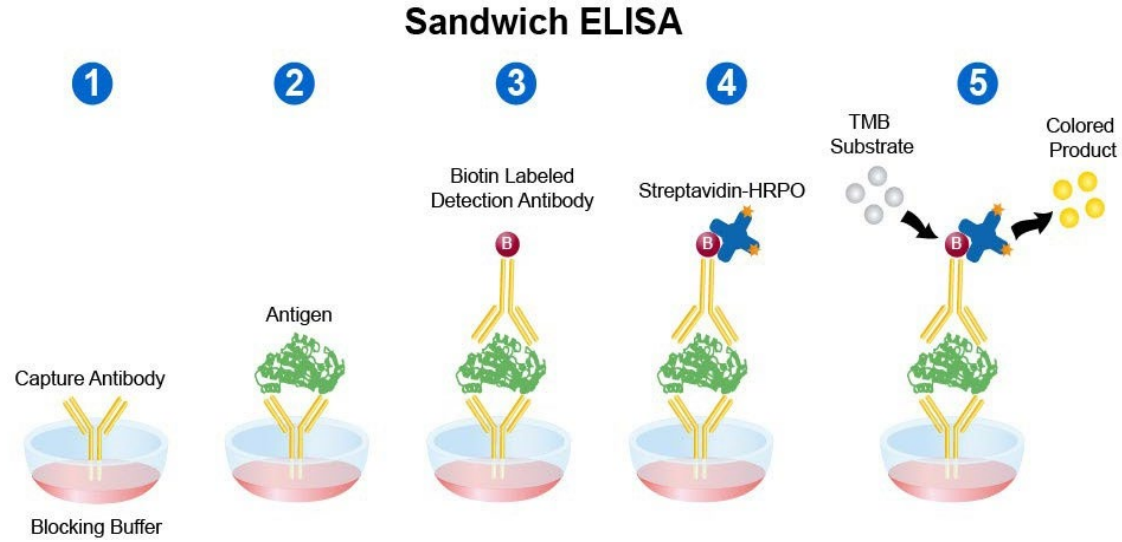


[a] Specific gravity was measured by the Environmental Health Laboratory Branch (EHLB), CDPH.

Biomarkers laboratory analysis

- Enzyme-Linked Immunosorbent Assays (ELISA)
- Holland laboratory at UC Berkeley
- ELISA kits used:
  - 8-Isop: Oxford Biomedical Research
  - 8-OHdG: Thermo Fisher Scientific
  - PGE2: R&D Systems
  - CC16: Abcam

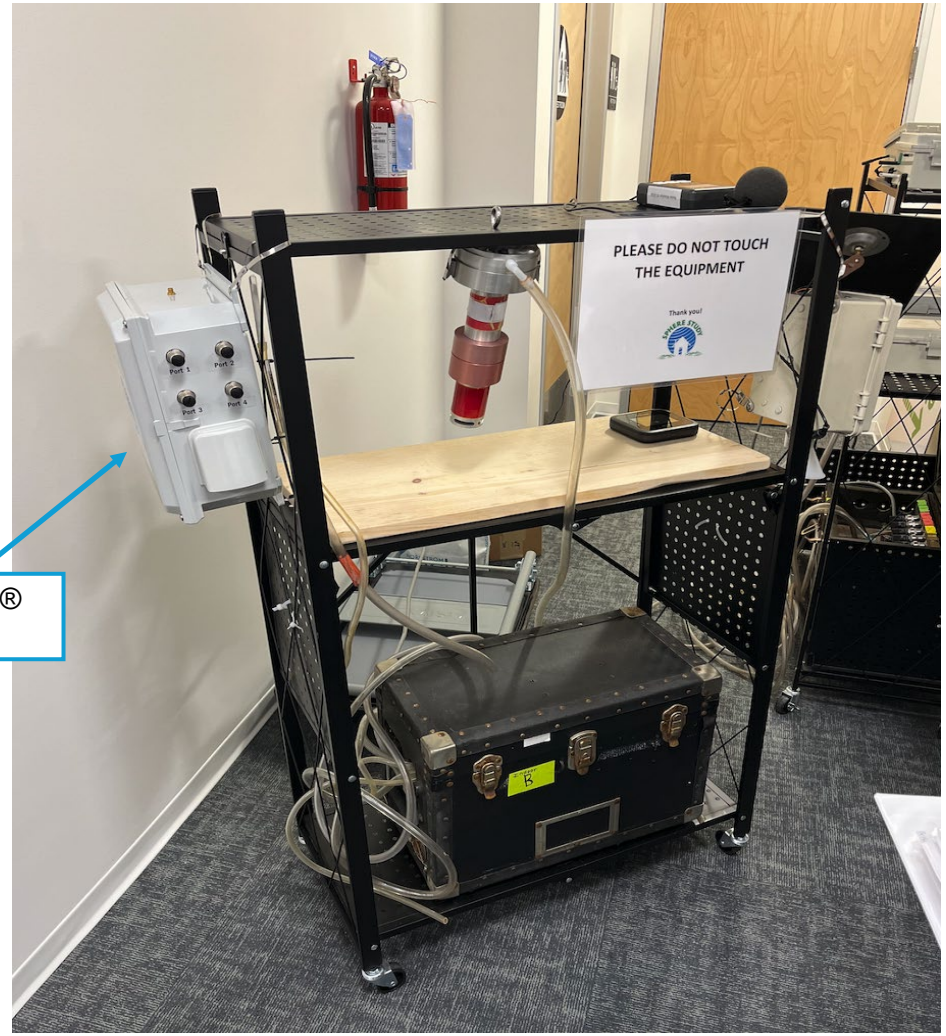




https://www.leinco.com/sandwich-elisa-protocol/

Study design – indoor air monitoring

- Average computed for 12-hours prior to urine sample collection
  - SENSIT® RAMP (SENSIT Technologies)
    - Nitrogen dioxide (NO_2)
    - Ozone (O_3)
    - Particulate Matter 2.5 ($PM_{2.5}$)
- Air pollutants were $\log_2$ transformed for data analysis



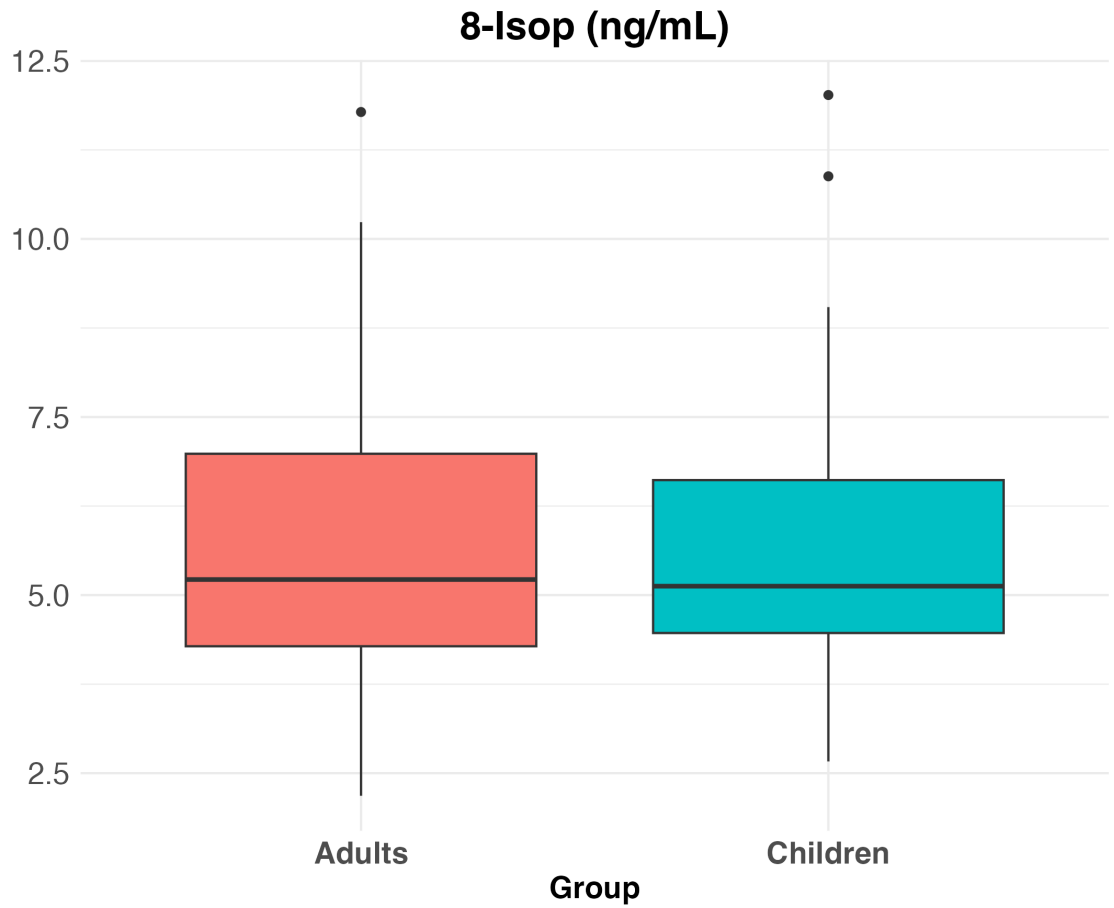
Parent/guardian demographic characteristics (n=63)

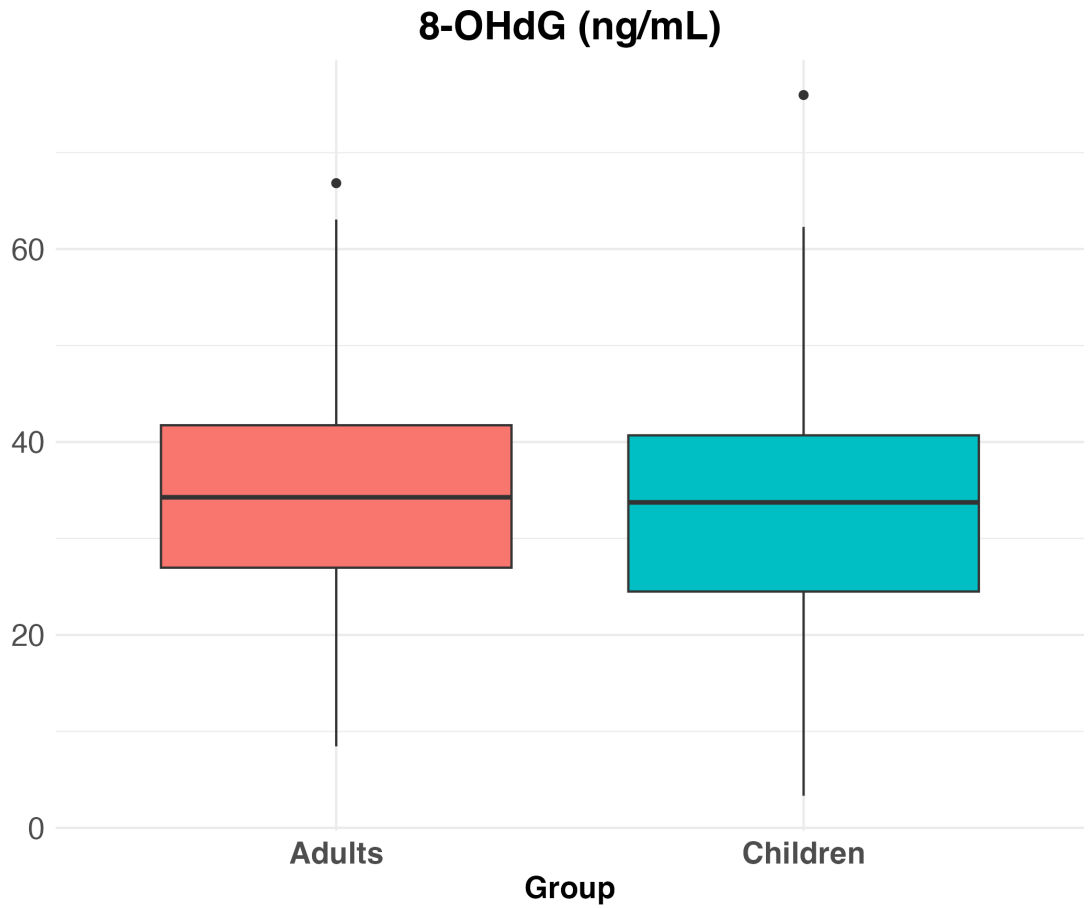
		n (%) or Mean ± SD
Language	English	32 (50.8)
	Spanish	31 (49.2)
Sex	Male	2 (3.2)
	Female	61 (96.8)
Race / Ethnicity	Asian	2 (3.2)
	Black/African American	3 (4.8)
	Hispanic/Latino	43 (68.3)
	White	8 (12.7)
	Two or more races	7 (11.1)
Relationship to child	Parent	60 (95.2)
	Grandparent	3 (4.8)
Age (years)		42 (± 7.6)
Education Level	Have not graduated from high school	23 (36.5)
	High school graduate, GED, or equivalent	8 (12.7)
	Some college or AA degree	14 (22.2)
	College graduate or above	18 (28.6)
Income	Up to $30,000	35(55.6)
	More than $30,000 up to $50,000	9 (14.3)
	More than $50,000 up to $75,000	19 (30.1)

Child demographic characteristics (n=62)

		n (%) or Mean ± SD
Sex	Male	32 (51.6)
	Female	30 (48.4)
Age (years)		8.9 (± 2.6)
BMI Category	Underweight	7 (11.3)
	Normal weight	17 (27.4)
	Overweight	16 (25.8)
	Obese	22 (35.5)

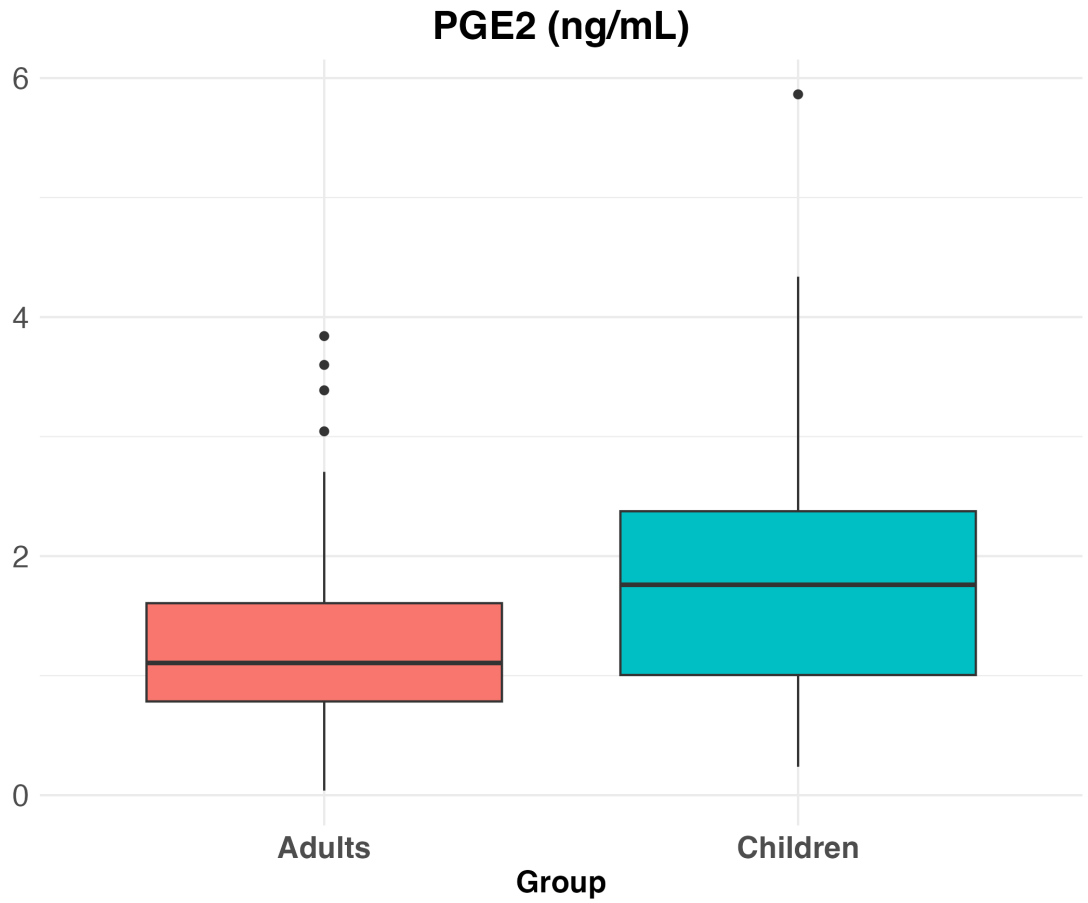
Distributions of oxidative stress biomarkers in adults and children

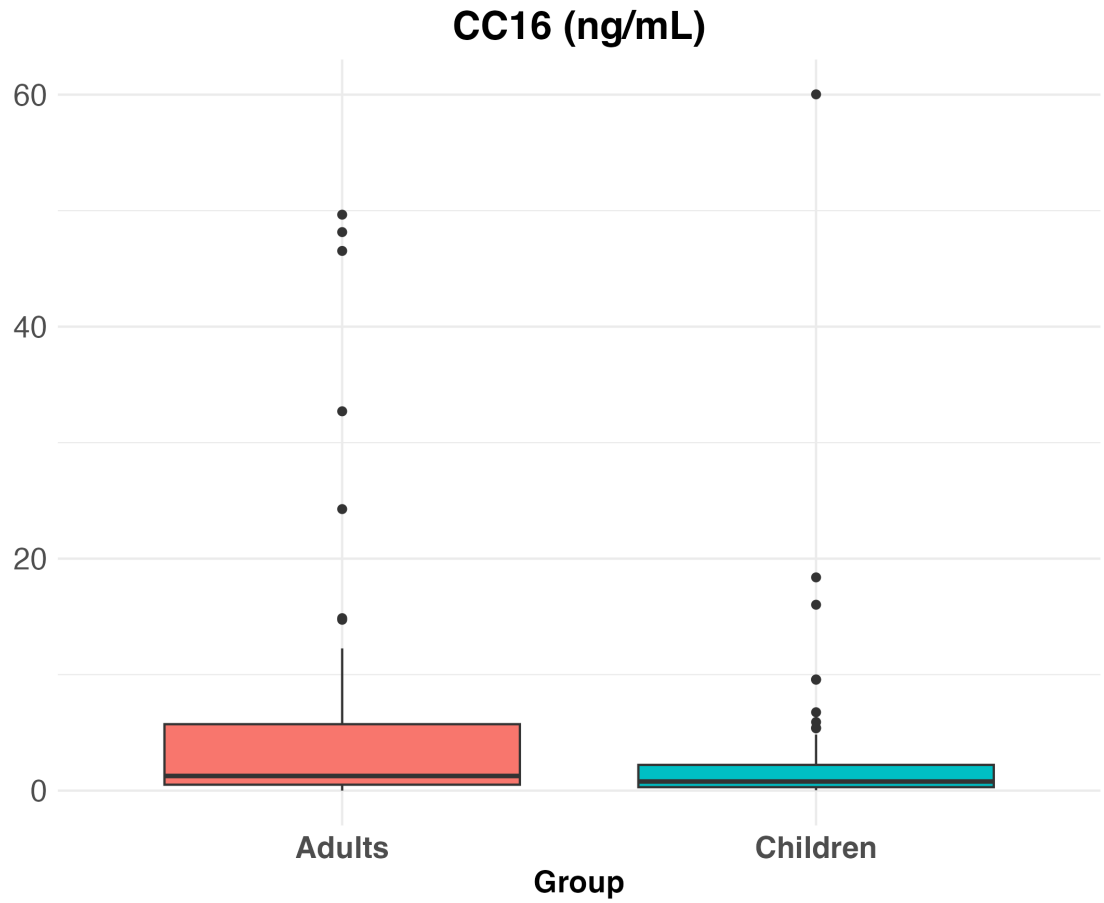




Summary statistics calculated using all reported values for 63 adults and 62 children.
Specific gravity-adjusted urinary biomarkers of response.

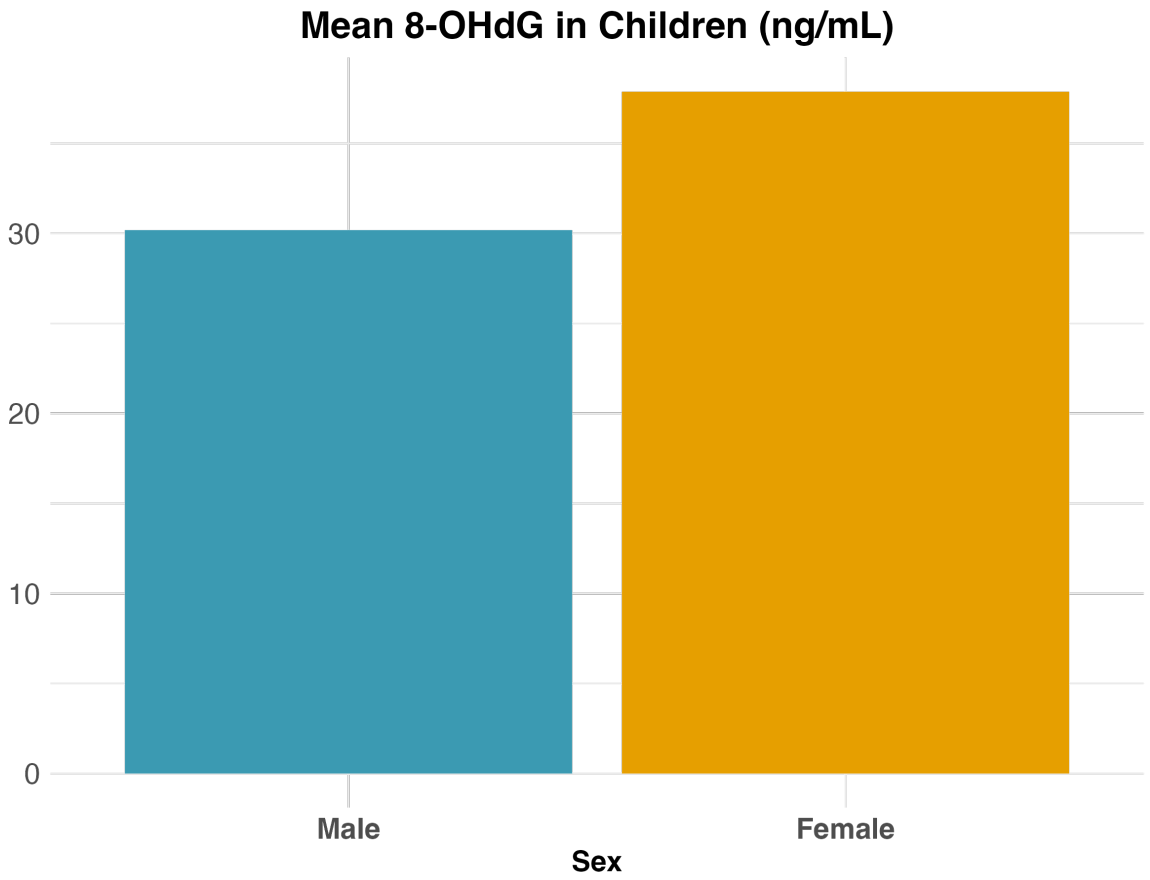
Distributions of inflammation (PGE2) and airway injury (CC16) biomarkers in adults and children



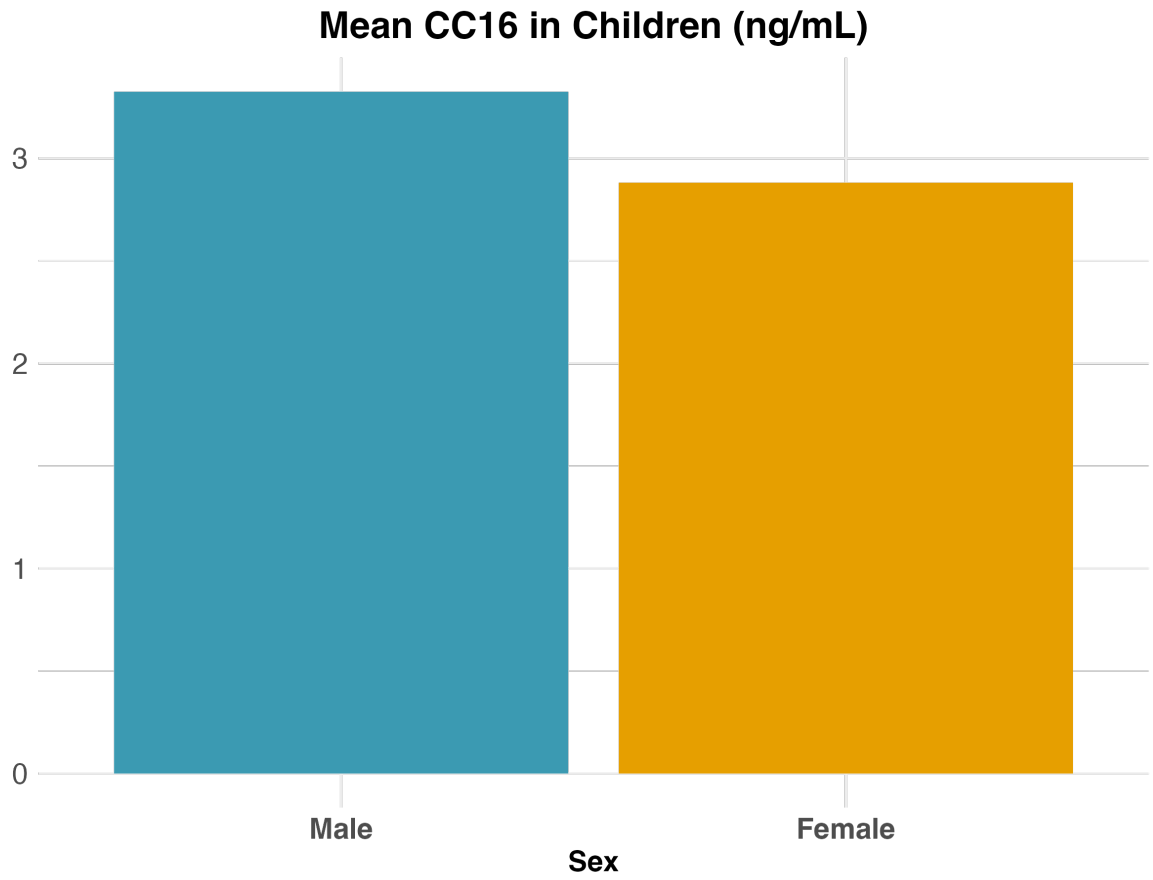


Summary statistics calculated using all reported values for 63 adults and 62 children.
Specific gravity-adjusted urinary biomarkers of response.

Significant mean differences in biomarkers of oxidative stress and airway injury in children



Wilcoxon signed-rank test results: p-value = 0.04.
(n=62)



Wilcoxon signed-rank test results: p-value < 0.001.
(n=62)

Short-term temporal variability of biomarkers of response (daily samples over four days)

Participant	n	Variance	8-Isop	8-OHdG	PGE2	CC16
Adult	30	Between	47%	32%	29%	66%
		Within	53%	68%	71%	34%
Child	31	Between	0%	19%	33%	53%
		Within	100%	81%	67%	47%

The adult samples include 23 first morning voids and 7 spot samples.

The children samples include 25 first morning voids and 6 spot samples.

Variance computed using linear mixed-effects models, with participant set as a random effect variable and sampling day set as a fixed effect variable.

Summary – biomarkers of response

- No significant differences among parent-child pairs for biomarkers of oxidative stress and airway injury.
- PGE2 (inflammation) was higher in children compared with adults.
- Female children had higher levels of 8-OHdG compared with male children.
- Female children had lower levels of CC16 compared with male children.
- There was higher within-subject variability compared with between-subject variability among adults and children for biomarkers of oxidative stress and inflammation.
- There was higher between-subject variability compared with within-subject variability among adults and children for CC16.

Relationship between biomarkers of response and indoor air quality

Distribution of pollutants in participating homes 12 hours prior to urine sample collection

Participant[a]	Pollutant	Units[b]	Mean	SD	Min	Max
Adult (n=56)	NO_2[c]	ppb	15.1	2.4	9.9	21.4
	O_3[d]	ppm	0.012	0.005	0.004	0.022
	$PM_{2.5}$[e]	µg / m^3	9.5	14.2	0.01	91.8
Child (n=57)	NO_2[c]	ppb	15.0	2.3	9.6	20.5
	O_3[d]	ppm	0.012	0.005	0.004	0.022
	$PM_{2.5}$[e]	µg / m^3	12.4	19.3	0.01	112.6

[a] Values differ due to difference in n for adult and children.

[b] Units based on United States National Air Quality Standards.

[c] Ozone (O_3)

[d] Nitrogen dioxide (NO_2)

[e] Particulate Matter 2.5 ($PM_{2.5}$)

Relationship between biomarkers of response and air pollutants

- A two-fold increase in NO_2 exposure was significantly associated with a 2.4-fold increase in adult urinary PGE2 concentrations (p-value<0.05).
- A two-fold increase in O_3 exposure was significantly associated with a 2.6-fold increase in adult urinary CC16 concentrations (p-value<0.05).
- No significant associations between indoor air pollutants and biomarkers of response in children.

Overall summary

- Few studies have examined these biomarkers in communities disproportionately impacted by air pollution.
  - None have examined short term temporal variability.
- Among adults, we found positive associations between indoor NO_2 levels and PGE2, and indoor O_3 levels and CC16.
- No significant associations between air pollutants and the child biomarkers were observed.
- Except for CC16, the higher within-subject variability suggests that single measurements may not characterize longer-term oxidative stress or inflammation status.
- The high short-term variability could point to impacts of short-term exposures, although we did not observe associations between the measured air pollutants and response biomarkers in children.
- Additional studies are needed to better understand the nuances and utility of these biomarkers as indicators of air pollution exposure and morbidity.

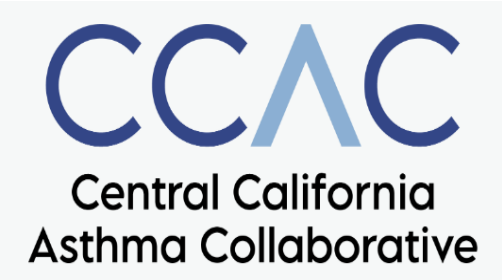
Extensive additional laboratory measurements are complete or in progress

Additional laboratory measurements:	Analyzed by:	Status:
Urinary Volatile Organic Compounds (VOCs) metabolites	UCSF	In progress
Urinary Polycyclic Aromatic Hydrocarbons (PAHs) metabolites	UCSF	Complete; Preparing results return
Cotinine – nicotine metabolites in urine	UCSF	Complete; Preparing results return
PAHs in air	BEAR lab (UCB)	QA/QC in progress
VOCs in air	EHLB	QA/QC complete

Next steps

- Evaluating biomarkers measurements in relation to:
  - Urinary biomarkers of VOCs, PAHs, and tobacco smoke
  - Outdoor 24-hour monitoring for NO_2, O_3, and $PM_{2.5}$
  - PAHs in air (indoor and outdoor)
  - VOCs in air
  - Nearby traffic metrics (Tracking California Traffic Tool)
  - Ambient levels of criteria air pollutants from community-science and regulatory monitoring over short to long- term periods (days, weeks, month) before urine collection
  - Health status (asthma diagnoses, medication use, etc.)

Community partners



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