

Peripheral Blood Gene Expression in Subjects with Coronary Artery Disease and Exposure to Particulate Air Pollutant Components and Size Fractions.

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Outline

- **Background and Rationale**
- Tasks
- Parent Study / Methods and Measurements
- Tasks 1-2 Methods and Results
- Task 3 Methods and Results
- Discussion

Background and Rationale

- Associations between cardiovascular morbidity and mortality outcomes with ambient exposure to $PM_{2.5}$ and PM_{10} have been consistently observed.
- There is still a need to better understand the sources and components of $PM_{2.5}$ and PM_{10} underlying the observed associations. This includes characterization of $PM_{2.5}$ ultrafine vs. accumulation mode fractions.

Background and Rationale

- In the present cohort panel of elderly subjects with coronary artery disease, we previously reported that exposure markers of traffic-related air pollution and quasi-ultrafine PM (<0.25 μm) were positively associated with plasma biomarkers of systemic inflammation and platelet activation, blood pressure, electrocardiographic ST segment depression and ventricular tachycardia.
- Changes in the expression of genes linked to these inflammatory and cardiovascular responses may underlie our previous findings:
 - *Environ Health Perspect*, 2008;116:898-906;
 - *Environ Health Perspect*, 2009;117:1232-38;
 - *Epidemiol*, 2010;21:396-404;
 - *Environ Health Perspect*, 2010;118:756-62;
 - *Epidemiol* , 2010;21:892-902;
 - *Environ Health Perspect*, 2011;119:196-202;
 - *Environ Health Perspect*, 2013;121:1135–1141.

Background and Rationale

- *In vitro* cell culture and *in vivo* animal experiments have shown that air pollutants (including diesel exhaust and urban ultrafine particles) can induce gene expression representing antioxidant response, inflammation, coagulation, endothelial function, endoplasmic reticulum stress, and apoptosis.
- However, there is little gene expression data from human subjects to support the relevance of these experimental results to public health.

Hypothesis

- Expression of 35 candidate genes in biological pathways relevant to cardiovascular responses will be altered in subjects following air pollutant exposures that are linked to products of fossil fuel combustion.

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Exposure Assessment Tasks

- **Task 1.** To conduct a chemical speciation of organic components in indoor and outdoor accumulation mode* filters (47 weeks) collected at retirement communities of study subjects in CHAPS.
- **Task 2.** To use accumulation mode composition data from Task 1 and existing metals data [and quasi-ultrafine PM data**] to conduct exposure analysis and source apportionment using chemical mass balance models.

* PM 0.25-2.5 μm (PM_{0.25-2.5})

** PM < 0.25 μm (PM_{0.25})

Epidemiologic Task

- **Task 3.** To conduct an epidemiologic analysis of the relation between gene expression and exposure to particle mass, components, and source tracers of $PM_{0.25}$ and $PM_{0.25-2.5}$ from Tasks 1-2 and PM mass and metals in $PM_{2.5-10}$.
- Genes selected *a priori*. Expression data was available from NIEHS-funded work.
- 35 candidate genes involved in oxidative stress, antioxidant defense, xenobiotic metabolism, inflammation, coagulation, and endoplasmic reticulum (ER) stress response (to buildup of unfolded or misfolded proteins).

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Parent Study

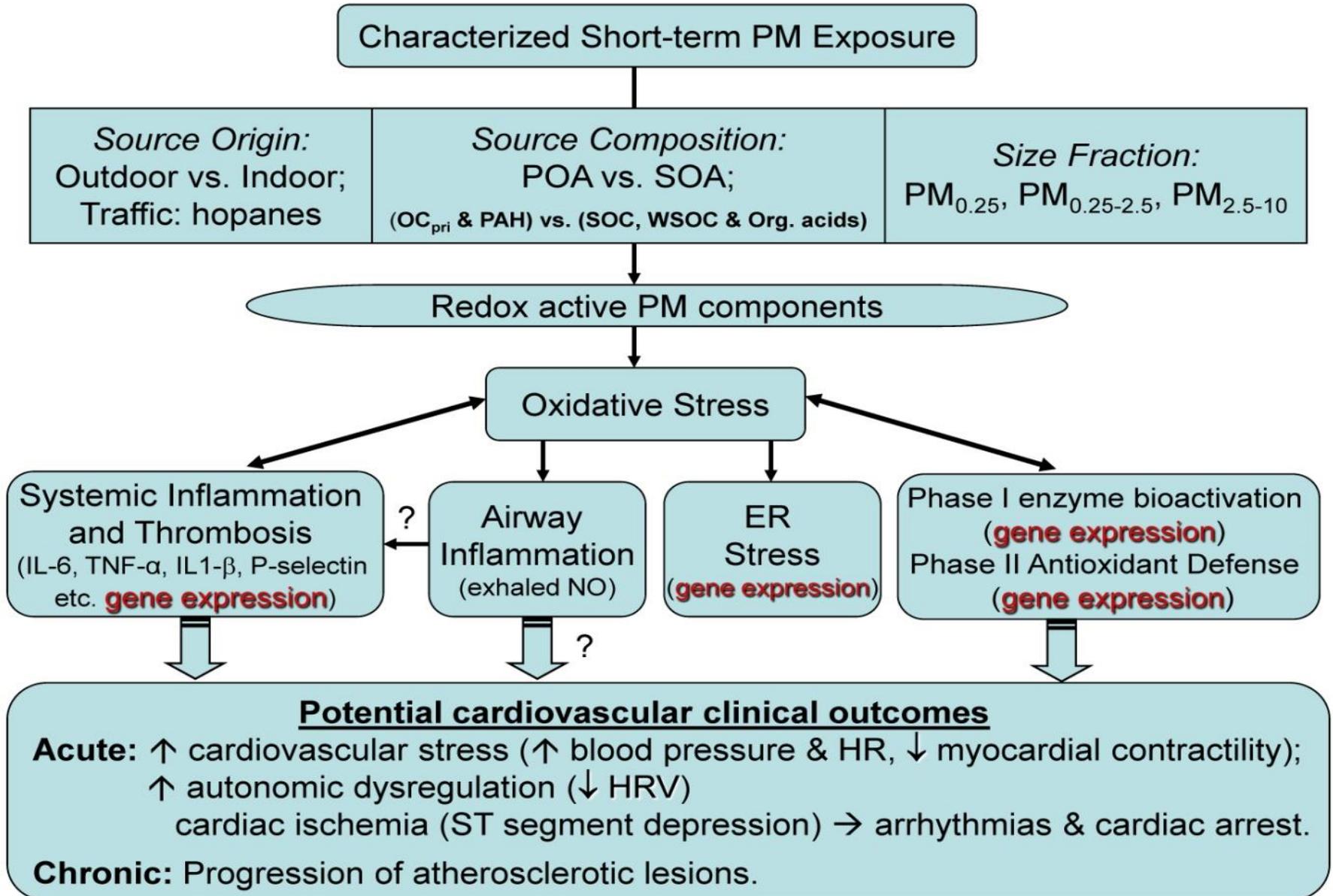
Cardiovascular Health and Air Pollution Study 1 (**CHAPS1**)

- Previous Funding:
NIH, NIEHS; CARB/AQMD; EPA SCPC.
- Design: Cohort panel study with repeated measures to evaluate within-subject acute cardiorespiratory health effects of PM exposure.
- PM_{0.25} data was available to supplement PM_{0.25-2.5} data in the present study (Tasks 1-2).
- PaxGene RNA was collected, extracted and processed for gene expression by qPCR.

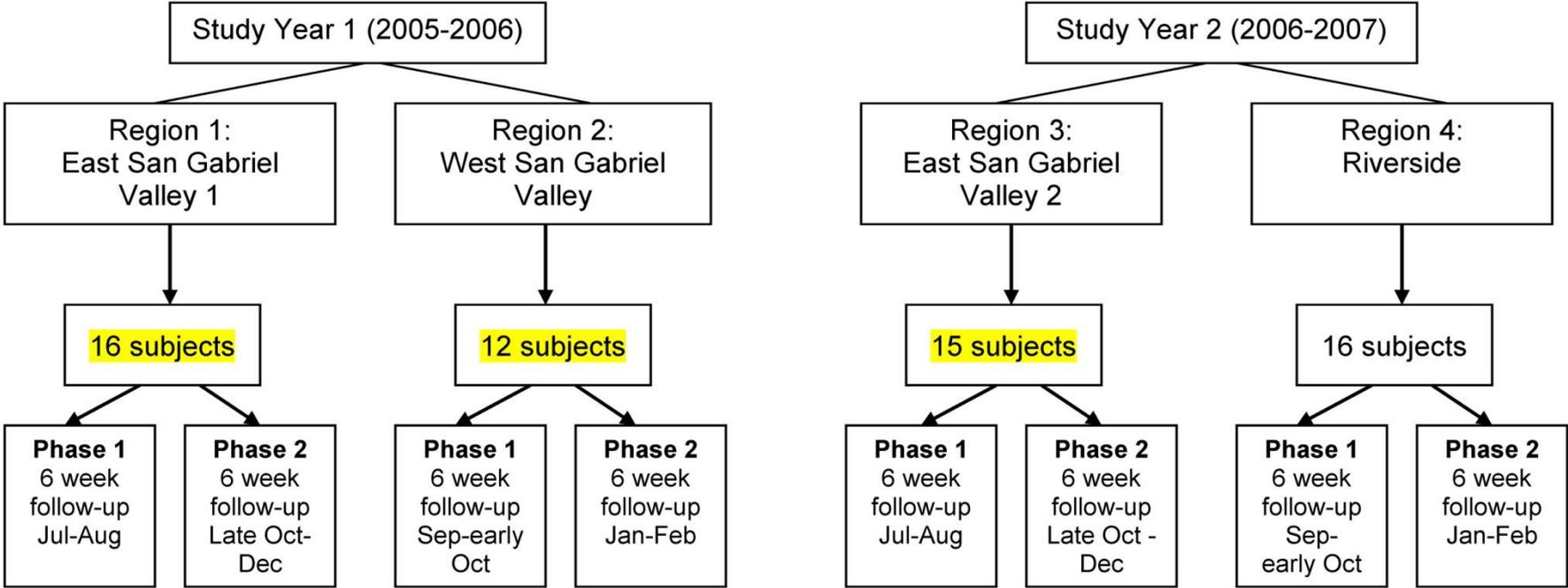
Design Overview

- Population: age ≥ 65 yr; history of coronary artery disease; nonsmoking; unexposed to passive smoke
- 3 of 4 retirement communities in the LA basin
- Weekly follow-up:
 - 12 weeks (6-week warm + 6-week cool phases in each community (2005-2007))
 - Blood draws for protein biomarkers and gene expression.
 - Exhaled NO (biomarker of airway inflammation)
- Ambulatory follow-up: 10 days with hourly blood pressure and Holter Monitoring (ST depression, arrhythmias, HRV)

Hypothesized pathways from PM exposure to effects relevant to cardiovascular health.



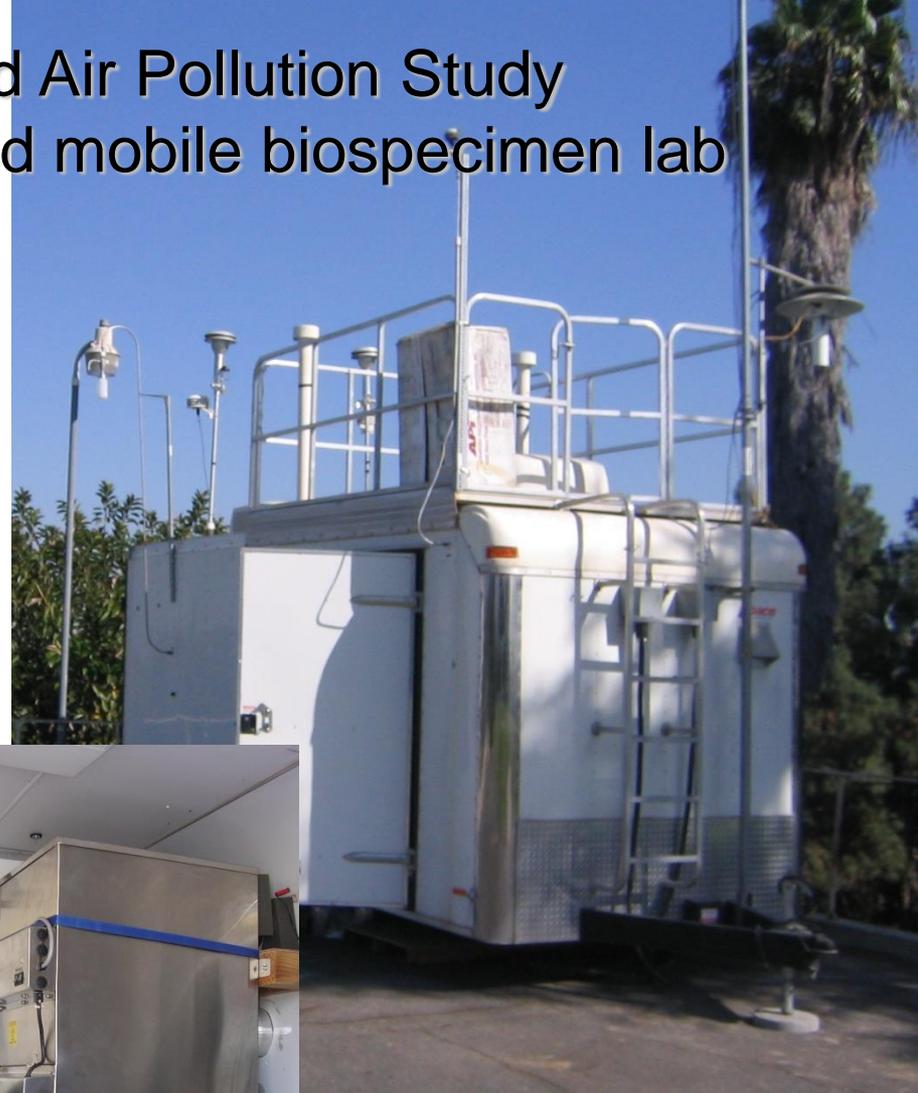
CHAPS Panel Study Flow Chart



Characteristics of Subjects in the Gene Expression Analyses (N=43).

Characteristic	Value
Age (years Mean \pm SD)	84.7 \pm 5.83
Female	53.5%
Race	
Hispanic	2.3%
White	97.7%
Cardiovascular History	
Confirmation of CAD	
Myocardial Infarction	44.2%
Coronary artery bypass graft or angioplasty	32.6%
Positive angiogram or stress test	16.3%
Clinical diagnosis	7.0%
Congestive heart failure	27.9%
Hypertension (by history)	76.7%
Hypercholesterolemia (by history)	67.4%
Medications	
ACE inhibitors and Angiotensin II receptor antagonists	41.9%
HMG-CoA reductase inhibitors (statins)	51.2%

Cardiovascular Health and Air Pollution Study air monitoring trailer (CARB) and mobile biospecimen lab



Example of an indoor air monitoring location



Exposure measurement summary

- Indoor and outdoor retirement community:
 - daily mean $PM_{0.25}$, $PM_{0.25-2.5}$, $PM_{2.5-10}$ mass (**Sioutas Impactor**) and chemical components (**5 days** of filters were composited – lag 0-4 days)
 - Hourly Exposures (**7 days** before blood draw)
 - $PM_{2.5}$ mass (**BAM**)
 - total particle number conc. (PN) (**CPC**)
 - black carbon (BC) (**Aethelometer**), elemental and organic carbon (EC and OC) (**Sunset Labs**)
 - NO_2 / NO_x , CO, O_3 (**Federal reference methods**)
 - Meteorology

PM components

- “EC tracer method” to estimate of hourly primary OC (OC_{pri}) \approx POA and secondary OC (SOC) \approx SOA

Polidori et al. *J Air Waste Manage Assoc*, 2007; 57:366-379.

- $PM_{0.25}$, $PM_{0.25-2.5}$, and $PM_{2.5-10}$ transition metals (ICP-MS)
- $PM_{0.25}$, and $PM_{0.25-2.5}$ filter extracts for water soluble organic carbon (WSOC) (GE Sievers TOC Analyzer)
- $PM_{0.25}$ and $PM_{0.25-2.5}$ filter extracts for organic components (**Task 1**), chemical analyses by GC/MS.

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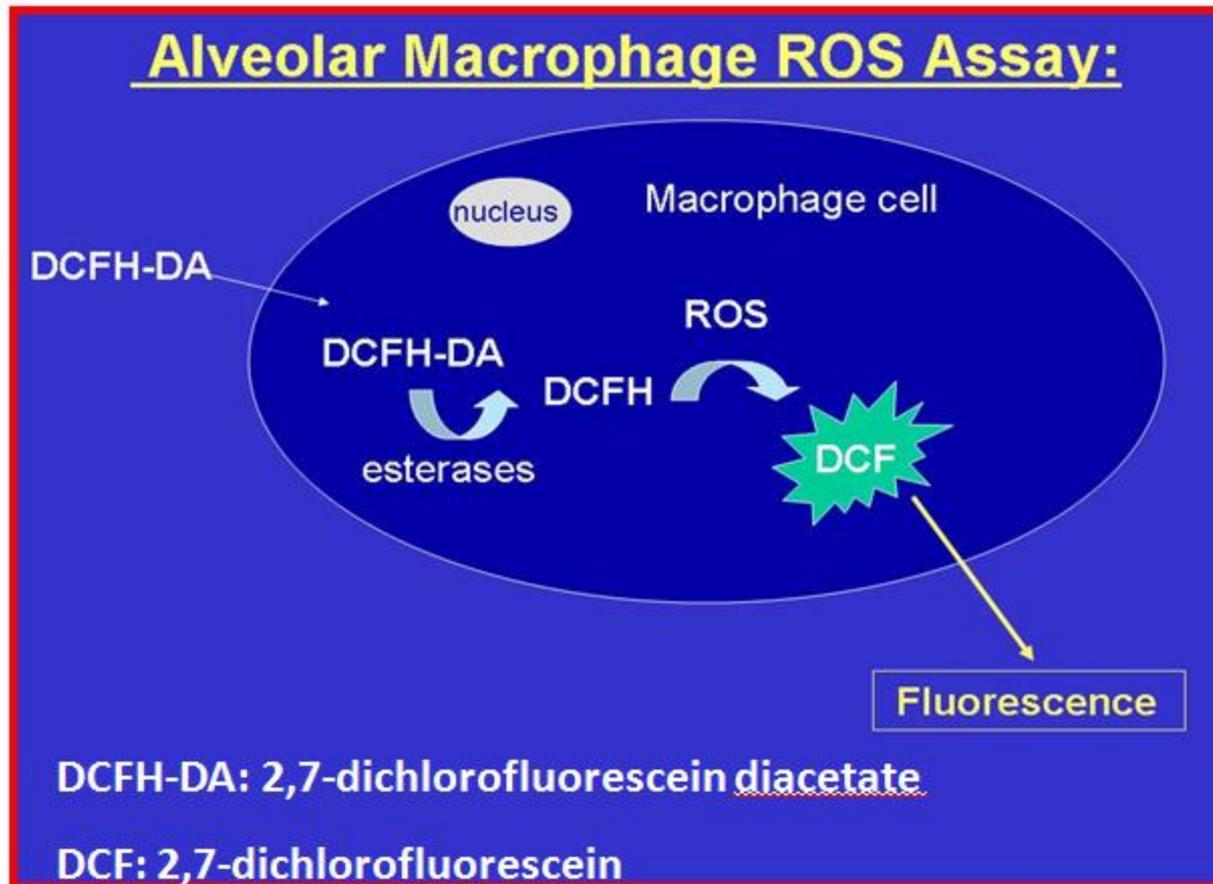
Task 1: New Air Pollutant Measurements

- Five PM_{0.25-2.5} (accumulation mode) filters composited for chemical analyses (JJ Schauer / M Shafer):
 - more than 80 different organic compounds using GC/MS, including
 - PAH, marker of POA
 - organic (n-alkanoic) acids, marker of SOA
 - Hopanes: tracers of primary vehicular aerosols from lubricant oils
 - Levoglucosan: tracer of biomass burning and used to adjust WSOC for an estimate of SOA (Task 2).
- *In vitro* generation of reactive oxygen species (ROS) by alveolar (lung) macrophages exposed to extracts of the 5-day PM_{0.25-2.5} samples.

***In Vitro* Reactive Oxygen Species (ROS) Assay (Martin Shafer):** Rat alveolar macrophage cells (NR8383) were exposed to aqueous extracts of PM_{0.25} and PM_{0.25-2.5} quartz filters from 5-day periods days preceding each subject's blood draw. After the incubation of cells with the PM extract and the ROS probe DCFH-DA, fluorescence intensity was measured to represent the oxidative generating capacity of particle extracts.

ROS results are reported in units of a glucan positive control, Zymosan equivalents/m³ air = (μg Zymosan equivalents/μg sample) x (5-day average PM_{0.25} or PM_{0.25-2.5} in μg/m³ air).

For details see: Landerman AP et al. *Aerosol Science Technol.* 2008;42:946-957.



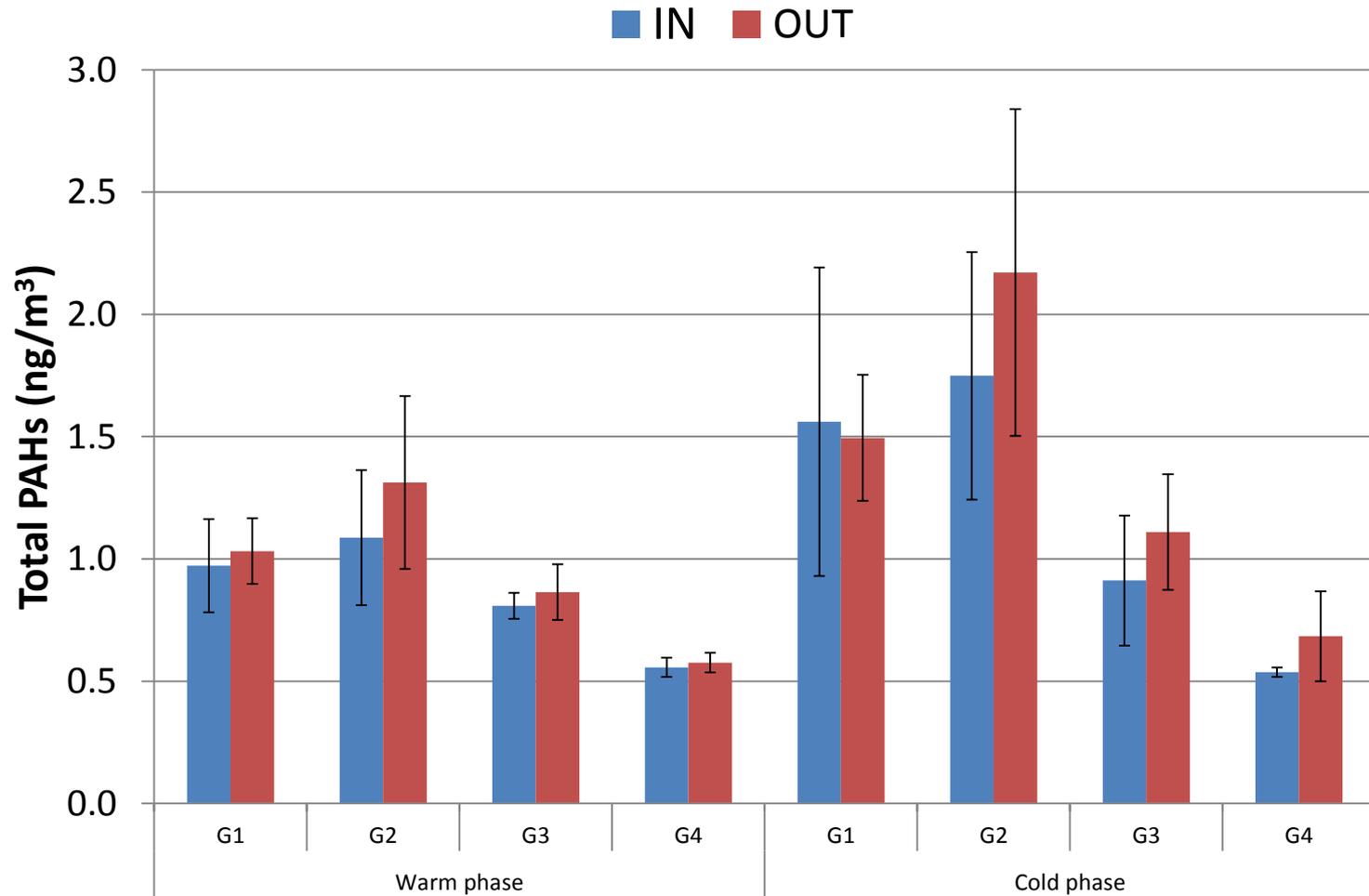
Task 2

- Chemical Mass Balance (CMB) model (CMB8.2, US EPA) for apportionment of total OC.
 - Samples from Riverside were excluded because they were affected by organic adsorption artifacts
- Focus on $PM_{2.5}$ combining Task 1 $PM_{0.25-2.5}$ with previously analyzed $PM_{0.25}$ in both indoor and outdoor environments of all four CHAPS1 retirement communities in the LA basin.
- Main objectives:
 - a) To determine the degree to which outdoor $PM_{2.5}$ infiltrates indoors.
 - b) To quantify source contributions and identify major sources of $PM_{2.5}$ at the indoor and outdoor environments throughout the two study years.

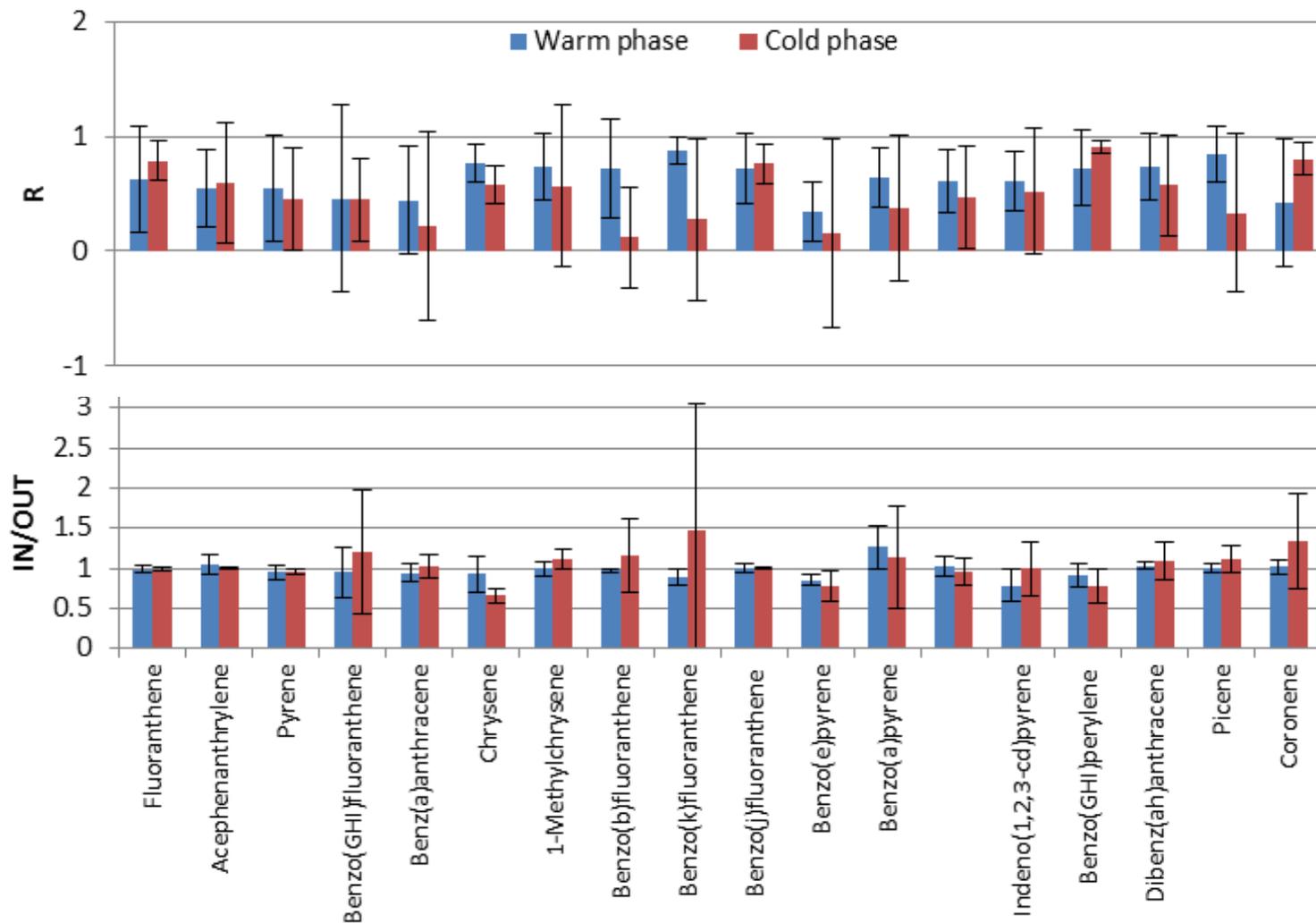
Task 2 Molecular marker compounds

- EC, 22S-homohopane, 22R-homohopane, 17 α (H)-21 β (H)-hopane, 17 α (H)-22,29,30-trisnorhopane, benzo(e)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(ghi)perylene, levoglucosan, indeno(1,2,3-cd)pyrene, nonacosane, hentriacontane, tritriacontane, vanadium, nickel aluminum, and sulfate (estimated from S).
- Other water-soluble organic carbon ("other WSOC") = total WSOC minus WSOC from biomass burning, estimated as 71% of OC apportioned to biomass burning from CMB output.
- SOA surrogate: multiplied "other WSOC" by:
1.8 (μg organic matter / μg OC)
(Turpin and Lim, 2001 *Aerosol Science & Technology* 35, 602-610)

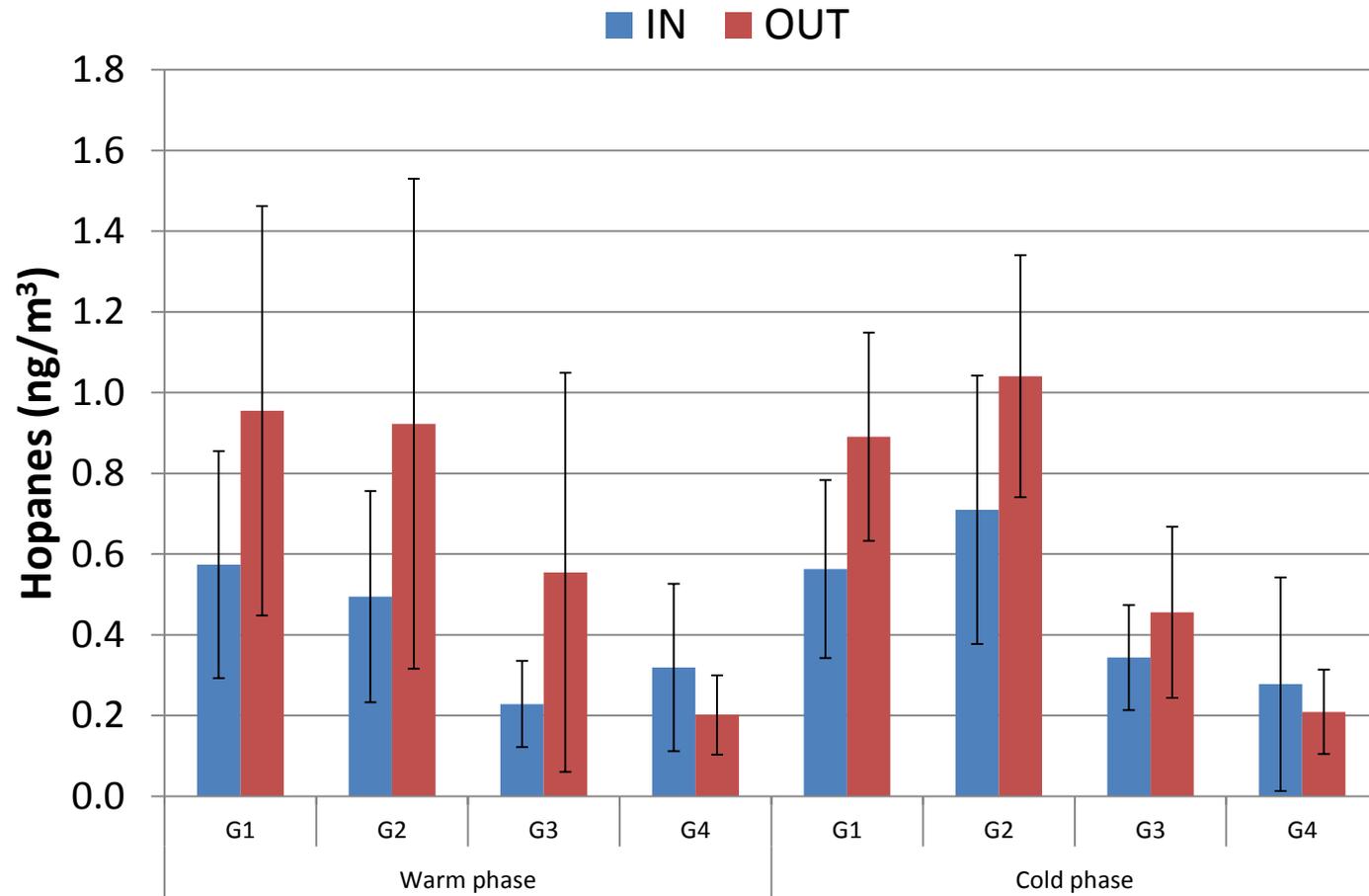
Average concentration of PAHs at the indoor and outdoor sampling sites during the warm and cold phases.



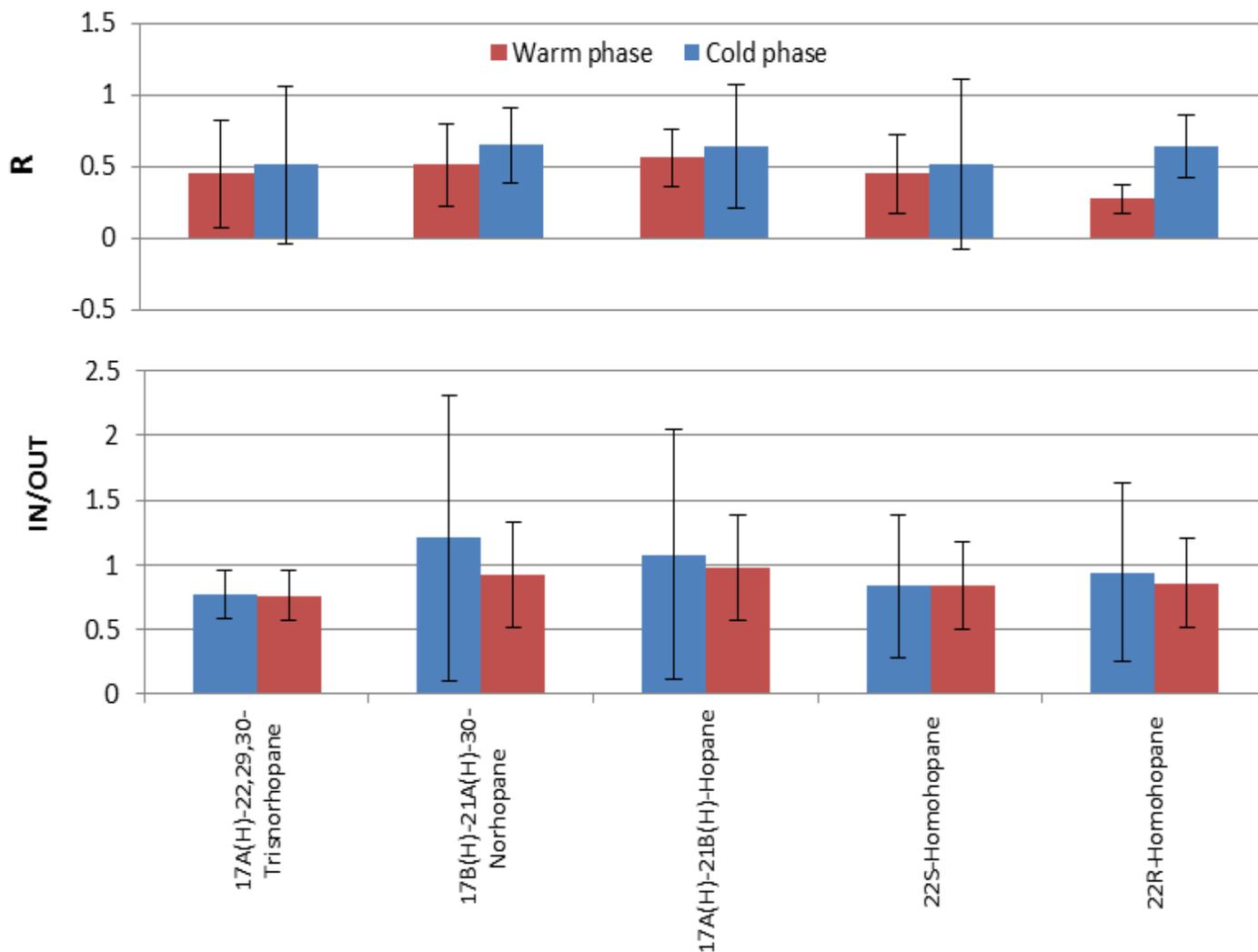
Average Pearson correlation coefficients (R) and indoor-to-outdoor ratios between indoor and outdoor concentrations of PAHs during the warm and cold phases.



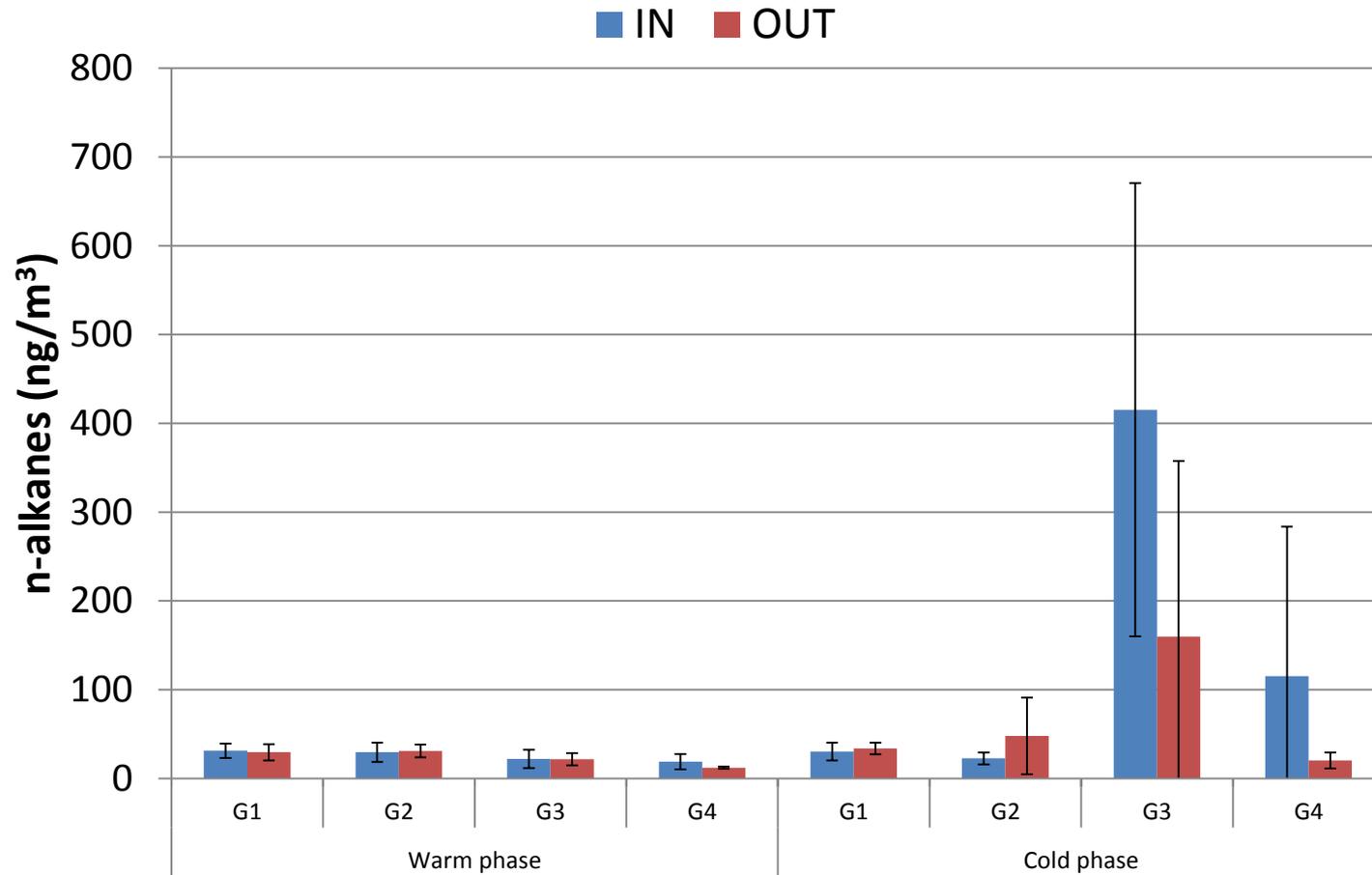
Average concentration of hopanes at the indoor and outdoor sampling sites during the warm and cold phases.



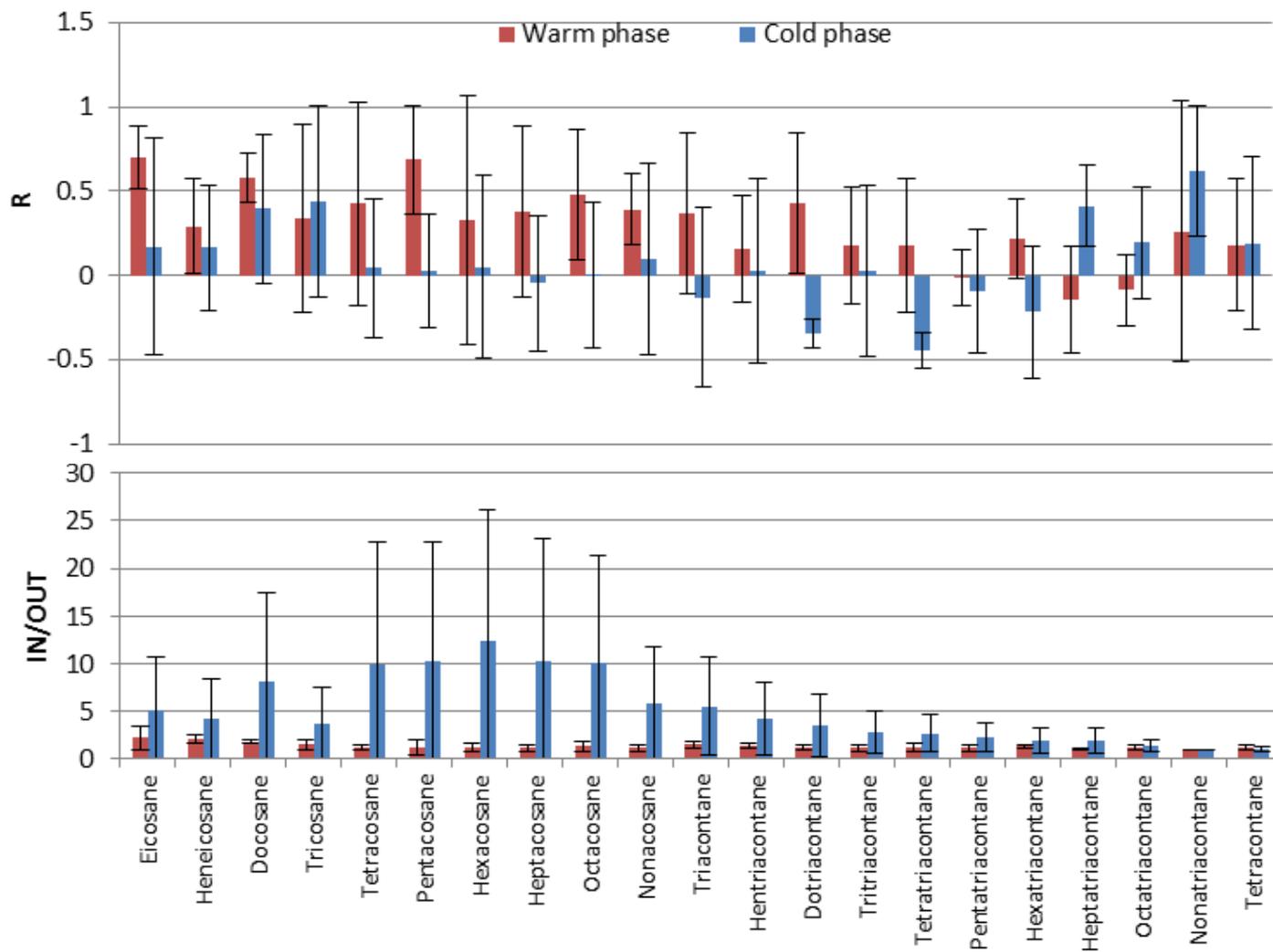
Average Pearson correlation coefficients (R) and indoor-to-outdoor ratios between indoor and outdoor concentrations of hopanes during the warm and cold phases.



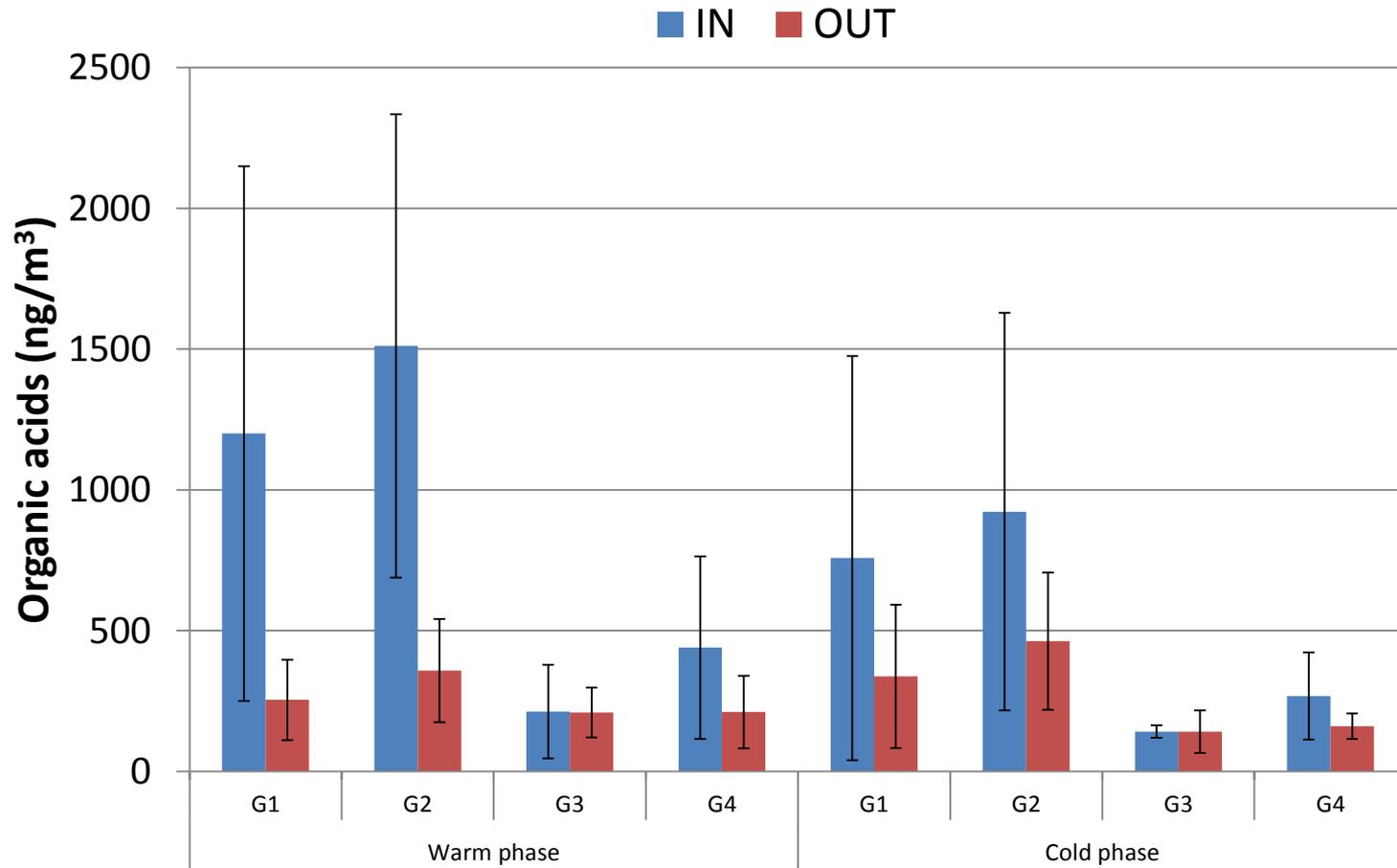
Average concentration of n-alkanes at the indoor and outdoor sampling sites during the warm and cold phases.



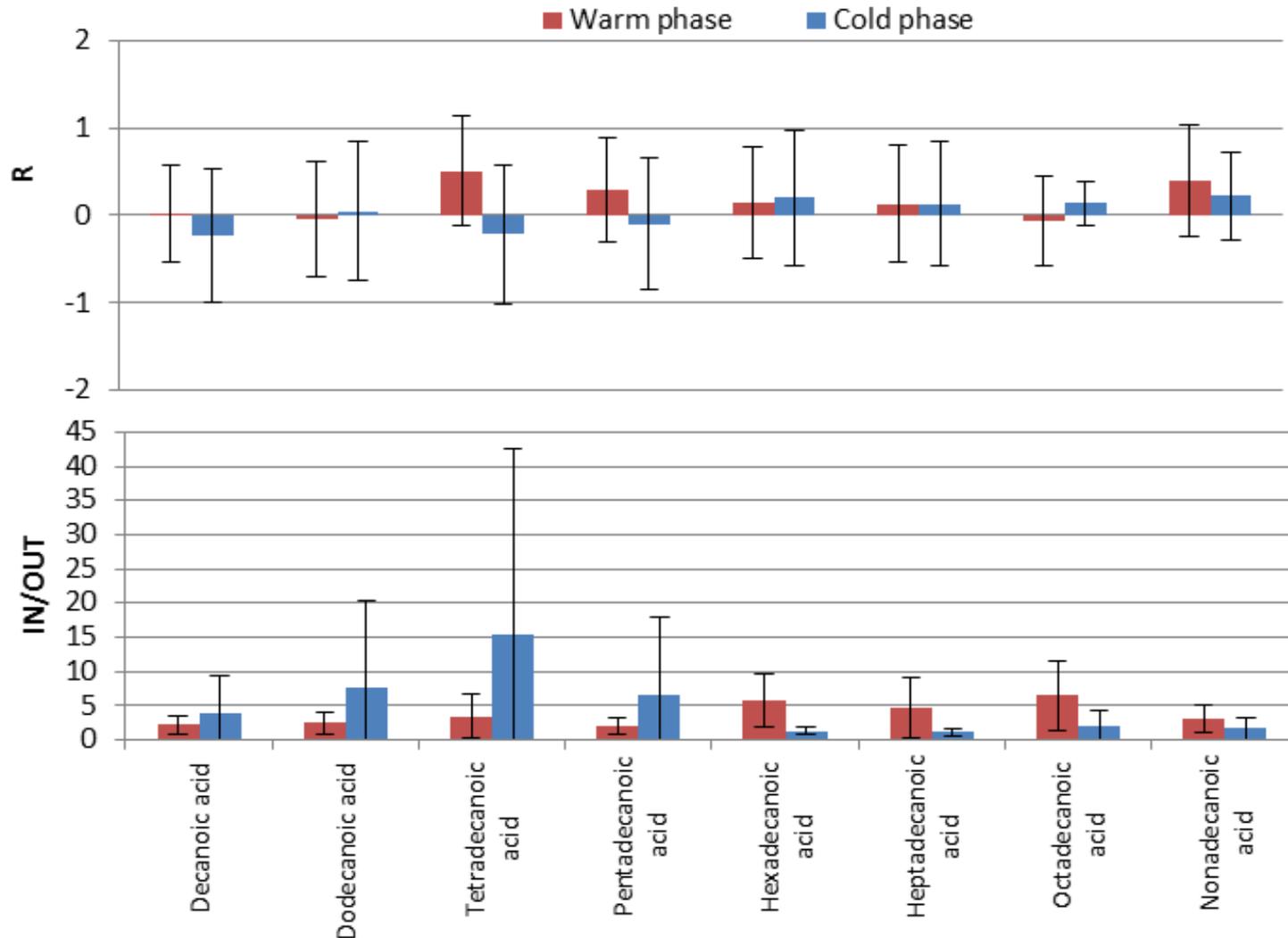
Average Pearson correlation coefficients (R) and indoor-to-outdoor ratios indoor and outdoor concentrations of n-alkanes during the warm and cold phases.



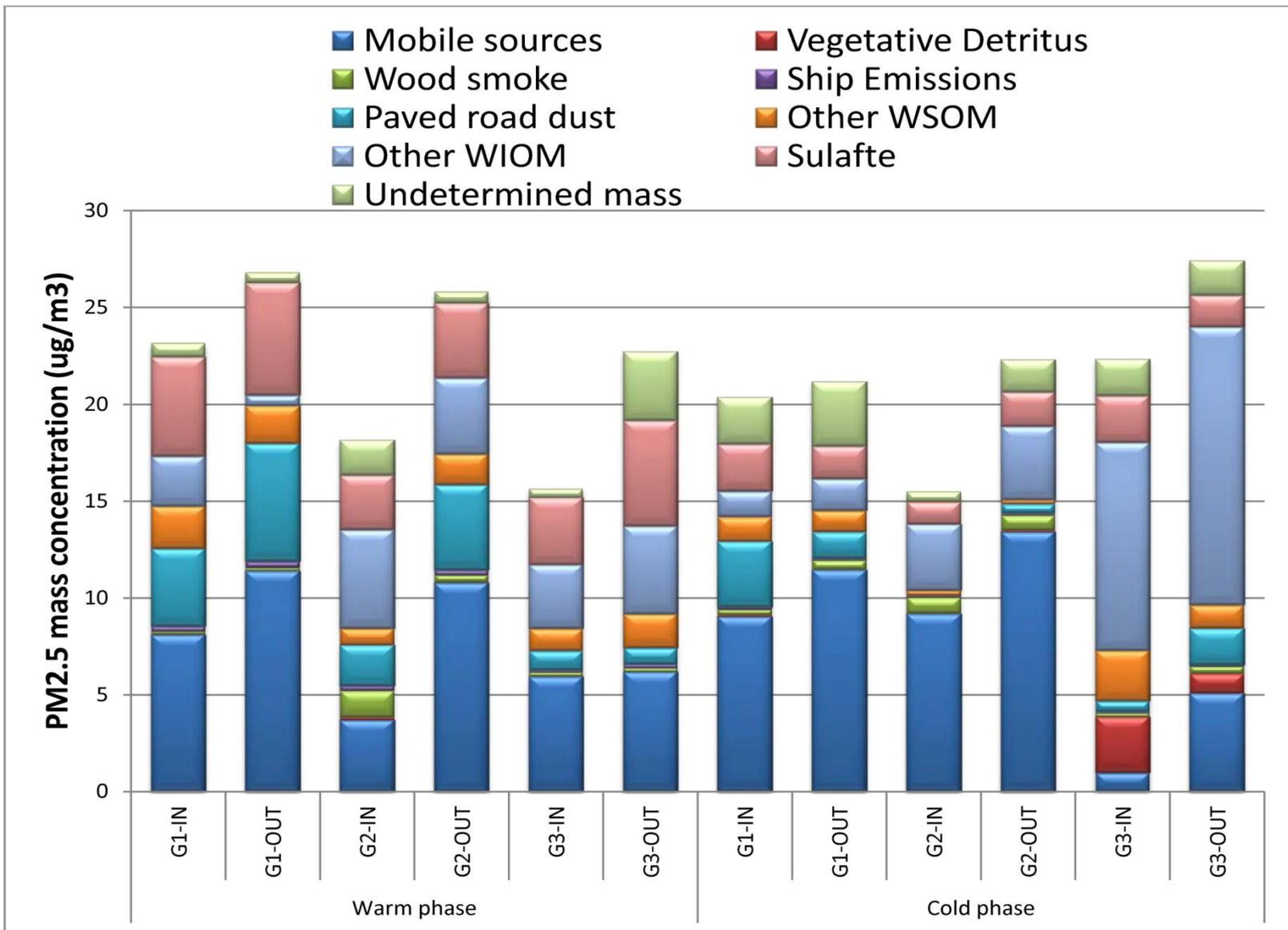
Average concentration of organic acids at the indoor and outdoor sampling sites during the warm and cold phases.



Average Pearson correlation coefficients (R) and indoor-to-outdoor ratios between indoor and outdoor concentrations of organic acids during the warm and cold phases.



Contribution of different sources to fine PM mass at the sampling sites during the warm and cold phases.



Overall Conclusions

- Indoor PAHs and hopanes showed moderately strong correlations with outdoor counterparts, with indoor/outdoor ratios close to unity, pointing to the influence of outdoor sources (mainly vehicular emissions) on indoor levels.
- Higher concentrations of n-alkanes and organic acids inside the retirement communities were likely dominated by indoor sources (e.g. cooking).
- Source apportionment results showed that mobile sources were a dominant contributor to both indoor and outdoor PM_{2.5} at all sites.

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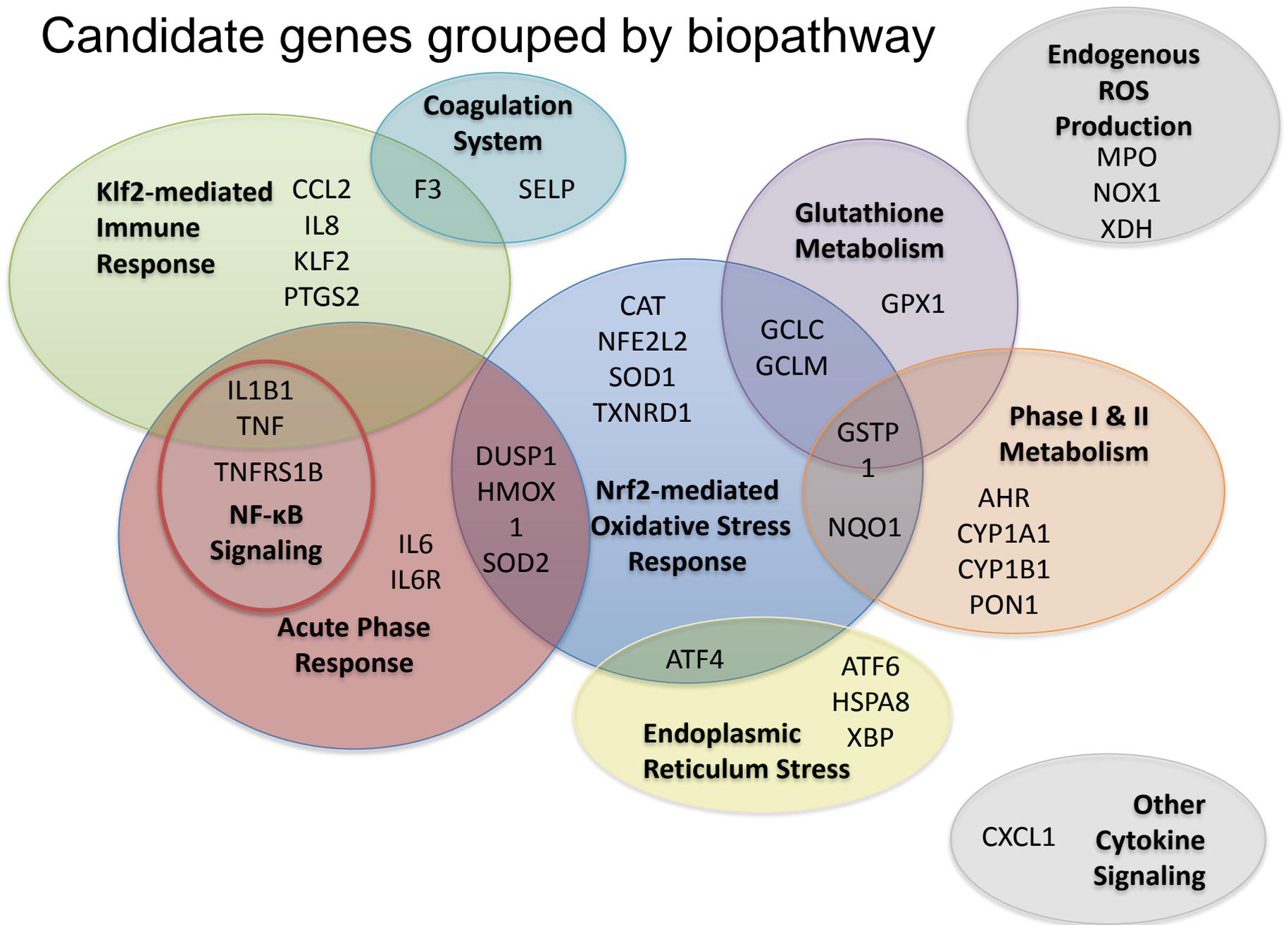
Task 3

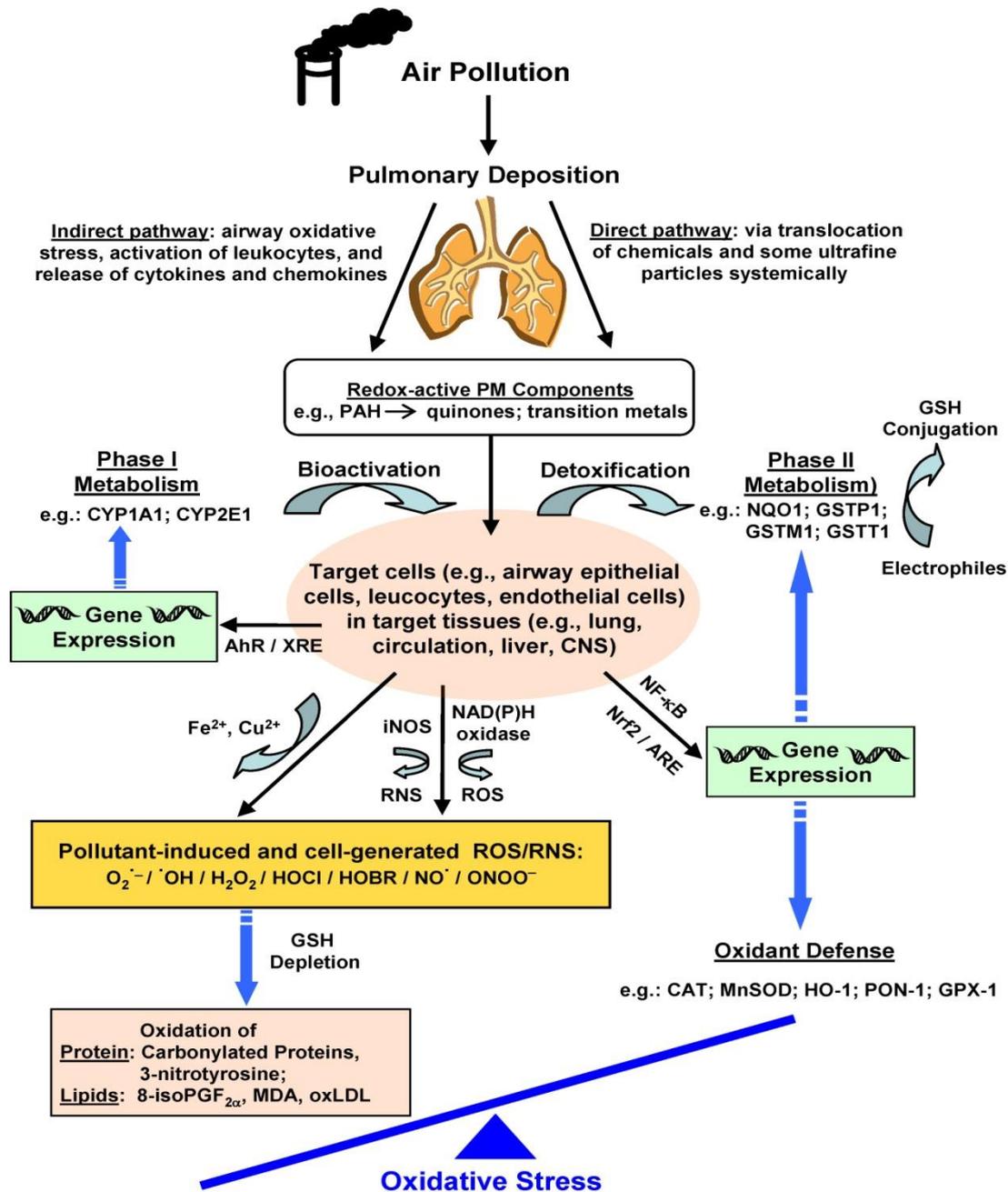
- Gene expression data for 35 genes selected *a priori* (NIH, NIEHS R21ES016420): genes involved in oxidative stress, antioxidant defense, xenobiotic metabolism, inflammation, coagulation, and ER stress.
- $PM_{0.25-2.5}$ data from Tasks 1-2 for this analysis was combined with exposure data already available for $PM_{0.25}$ and other air pollutant data.
- Compare associations of gene expression with organic components in $PM_{0.25}$ and $PM_{0.25-2.5}$
- Evaluate the relation of gene expression to size-fractionated particle mass and metals concentrations (in all size fractions including coarse).

Target Genes, Task 3

- 1.) CYP1A1 / cytochrome P450, subfamily A1 / oxygenase
- 2.) AHR / transcription factor (regulates xenobiotic-metabolic enzymes)
- 3.) GSTP1 / glutathione S-transferase pi / transferase
- 4.) NQO1 / NAD(P)H dehydrogenase, quinine / 2-electron reductase
- 5.) PON1 / paraoxonase / peroxidase, esterase
- 6.) TNF / tumor necrosis factor superfamily, member 2
- 7.) TNFRS1B / tumor necrosis factor receptor 2
- 8.) IL6R / interleukin 6 receptor
- 9.) IL6 / interleukin 6
- 10.) HMOX1 / heme oxygenase (decycling) 1 / oxygenase
- 11.) SOD2 / superoxide dismutase [Mn], mitochondrial / oxidoreductase
- 12.) IL1B / interleukin-1beta
- 13.) CCL2 / chemokine (C-C motif) ligand 2
- 14.) F3 / tissue factor (coagulation factor III)
- 15.) COX2 / prostaglandin-endoperoxide synthase 2
- 16.) IL8 / interleukin 8
- 17.) KLF2 / Kruppel-like factor 2 / Zinc finger transcription factor
- 18.) ATF4 / CREB activating transcription factor 4
- 19.) SOD1 / superoxide dismutase [Cu-Zn] / oxidoreductase
- 20.) TXNRD1 / thioredoxin reductase 1 / reductase
- 21.) CAT / catalase / peroxidase
- 22.) NRF2 / nuclear factor-erythroid 2 p45 related factor 2 / transcription factor
- 23.) HSPA8 / heat shock 70kDa protein 8 / Hsp 70 family chaperone
- 24.) ATF6 / CREB activating transcription factor 6
- 25.) XBP1 / X-box binding protein 1 / transcription factor
- 26.) SELP / p-selectin (antigen CD62), / cell adhesion molecule
- 27.) GPX1 / glutathione peroxidase 1 / peroxidase
- 28.) MPO / myeloperoxidase / peroxidase
- 29.) NOX1 / NADPH oxidase 1 / oxidase
- 30.) XDH / xanthine dehydrogenase
- 31.) CYP1B1 / cytochrome P450, subfamily B1 / oxygenase
- 32.) MKP-1 / dual specificity phosphatase 1 / kinase inhibitor
- 33.) CXCL1 / chemokine (C-X-C motif) ligand 1 / chemokine
- 34.) GCLC / glutamate-cysteine ligase, catalytic subunit / ligase
- 35.) GCLM / glutamate-cysteine ligase, modifier subunit / synthetase

Candidate genes grouped by biopathway





METHODS: Gene Expression Measurements

- PAXgene Blood RNA System (BD Diagnostics) and immediate freezing used to stabilize RNA in the field.
- Total RNA isolated by using a robotic workstation (QIAcube).
- Gene expression was quantified with competitive PCR coupled with MALDI-TOF mass spectrometry (Sequenom Mass ARRAY Quantitative Gene Expression).
 - Timothy Stinchcombe and Immune Sciences Lab (David H. Murdock Research Institute, Kannapolis, NC)

Housekeeping and potential control genes

- **House Keeping Genes:**

- A) **GAPDH**
- B) **UBC**
- C) **ACTB** 
- D) **B2M**
- E) **TBP**

Stable expression determined as the average pairwise variation: *ACTB*, *B2M*, and *GAPDH*. Gene expression **normalization** of copy numbers came from the geometric mean of the 3 reference genes using geNORM software (Biogazelle™).

- **Cell Specific Surface Markers**

- CD19 B-cell
- CD3G T-cell 
- SELL Granulocytes
- RPS24 Lymphocytes
- CD14 Monocytes

Pilot Study:

CBC vs. gene expression
CHAPS1 baseline and repeated measures in 10 asthma subjects.
Results: relations nonsignificant, suggesting cell surface markers are not sufficiently representative of cell type distribution.

Gene Expression Measurements

- Expression levels of *CYP1A1*, *PON1*, *SOD1*, *NOX1*, and *XDH* were too low for analysis.
- This left 30 genes to analyze from 43 subjects,
- Average sample size:
360 person-observations per gene.

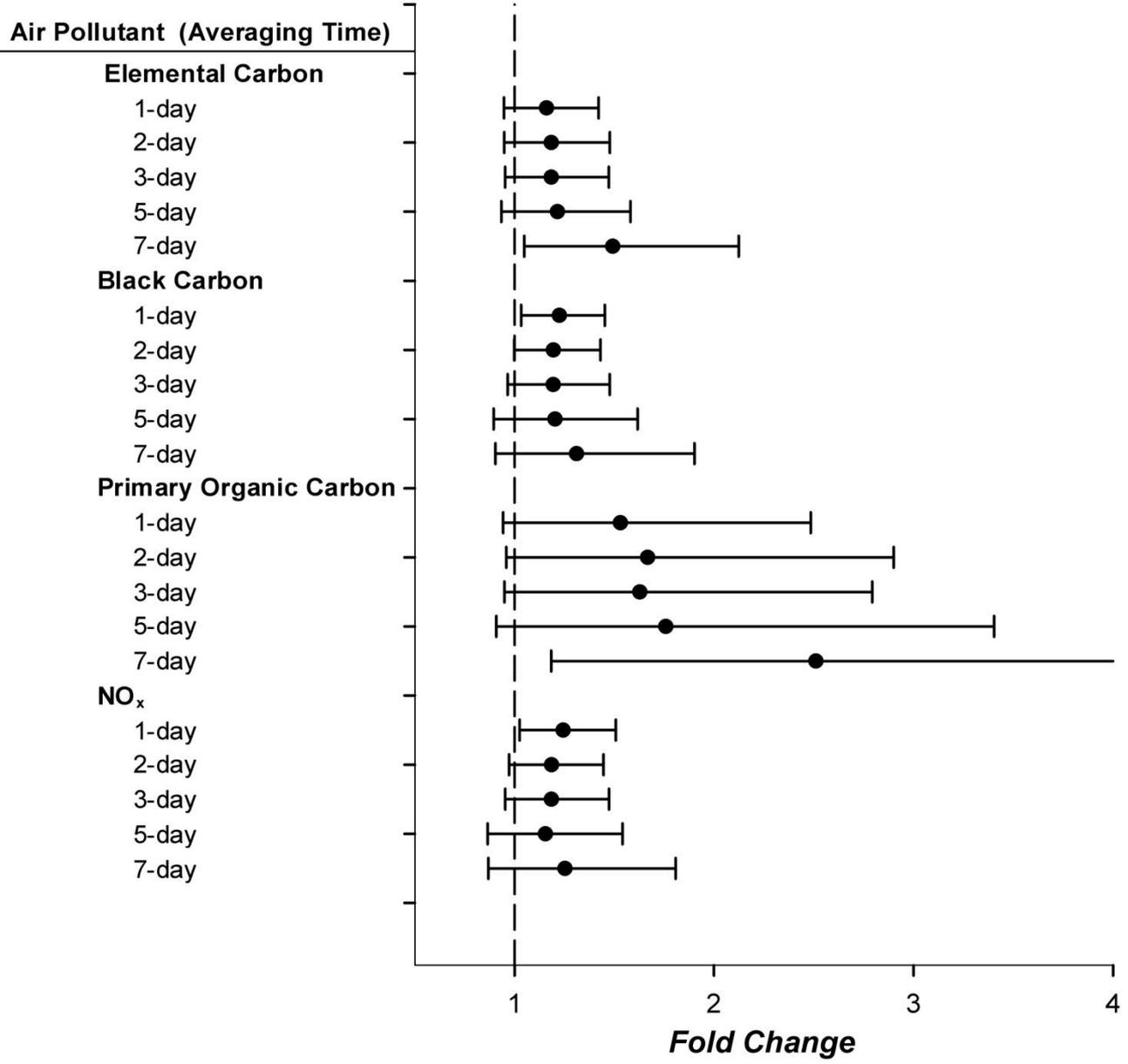
Analysis

- Log transformed the Biogazelle-normalized molecule concentrations. Used autoscaling transformation for more highly variable data (Willems. Anal Biochem. 2008;379:127-9.)
- Linear mixed effects model:
 - Random subject intercept nested in group & phase,
 - AR1 covariance structure,
 - Adjusted for temperature same lag,
 - Excluded person-observations if any infection that week.
 - Exposures: mean centered by community and season.
- Associations are expressed at interquartile (25th – 75th percentile) ranges of each air pollutant averaged 1-7 days before blood draw.

Summary of results for continuous air pollutant measurements

- Primary pollutants (BC, EC, primary OC, CO, and NO_x) were associated increased expression of:
 - Nrf2 gene (*NFE2L2*)
 - Nrf2-mediated or linked genes (*HMOX1*, *NQO1*, and *SOD2*) (phase II / oxidant defense enzymes).
 - *IL1B* (inflammation),
 - *SELP* (platelet activation), and
 - *CYP1B1* (phase I enzyme, xenobiotic metabolism)
 - However, many confidence intervals were wide and included 1.0 (indoor more so than outdoor air pollutants).
Outdoor shown next:

NRF2 Gene Expression



HMOX1 Gene Expression

Air Pollutant (Averaging Time)

Elemental Carbon

1-day

2-day

3-day

5-day

7-day

Black Carbon

1-day

2-day

3-day

5-day

7-day

Primary Organic Carbon

1-day

2-day

3-day

5-day

7-day

NO_x

1-day

2-day

3-day

5-day

7-day

0.5

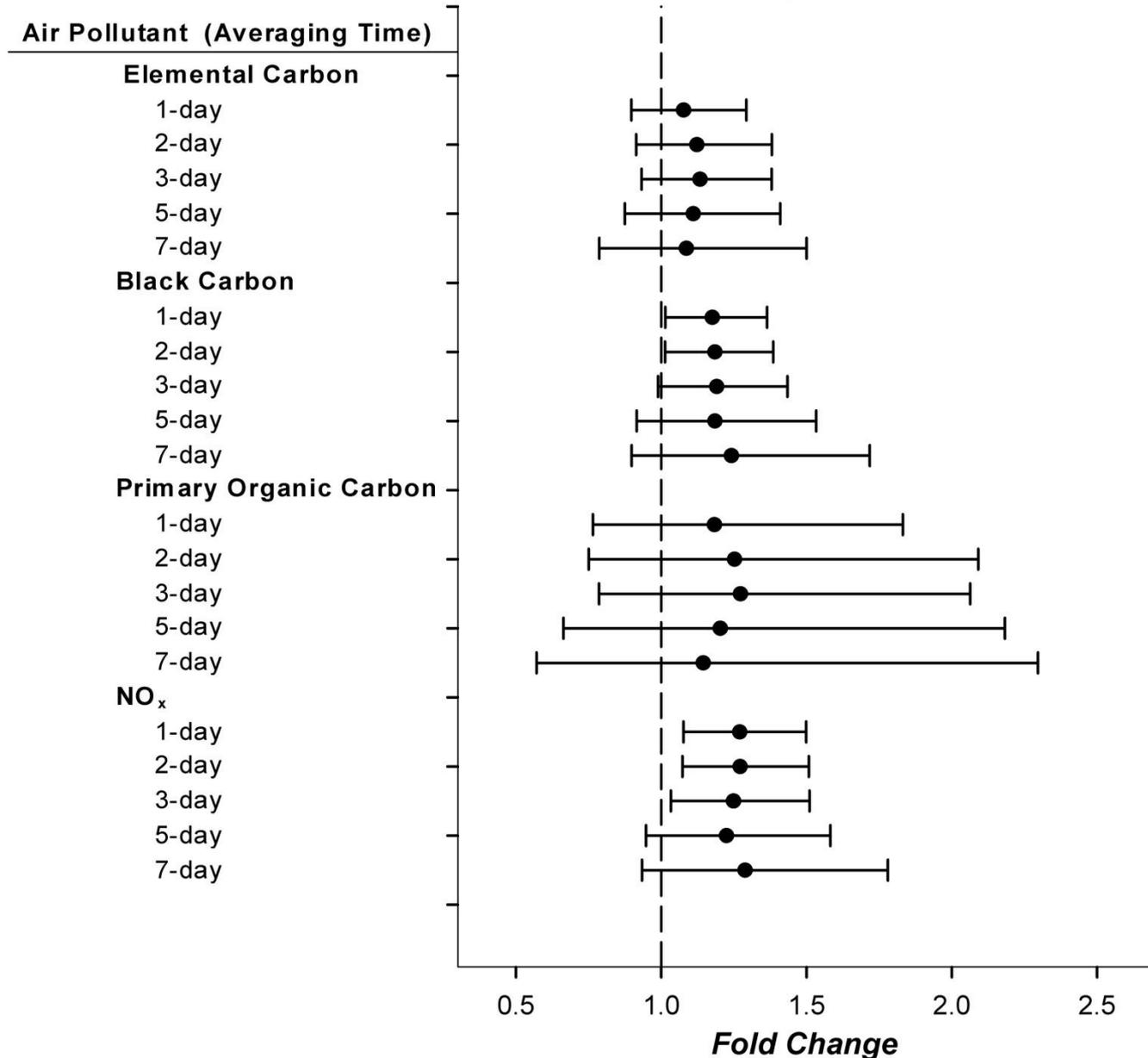
1.0

1.5

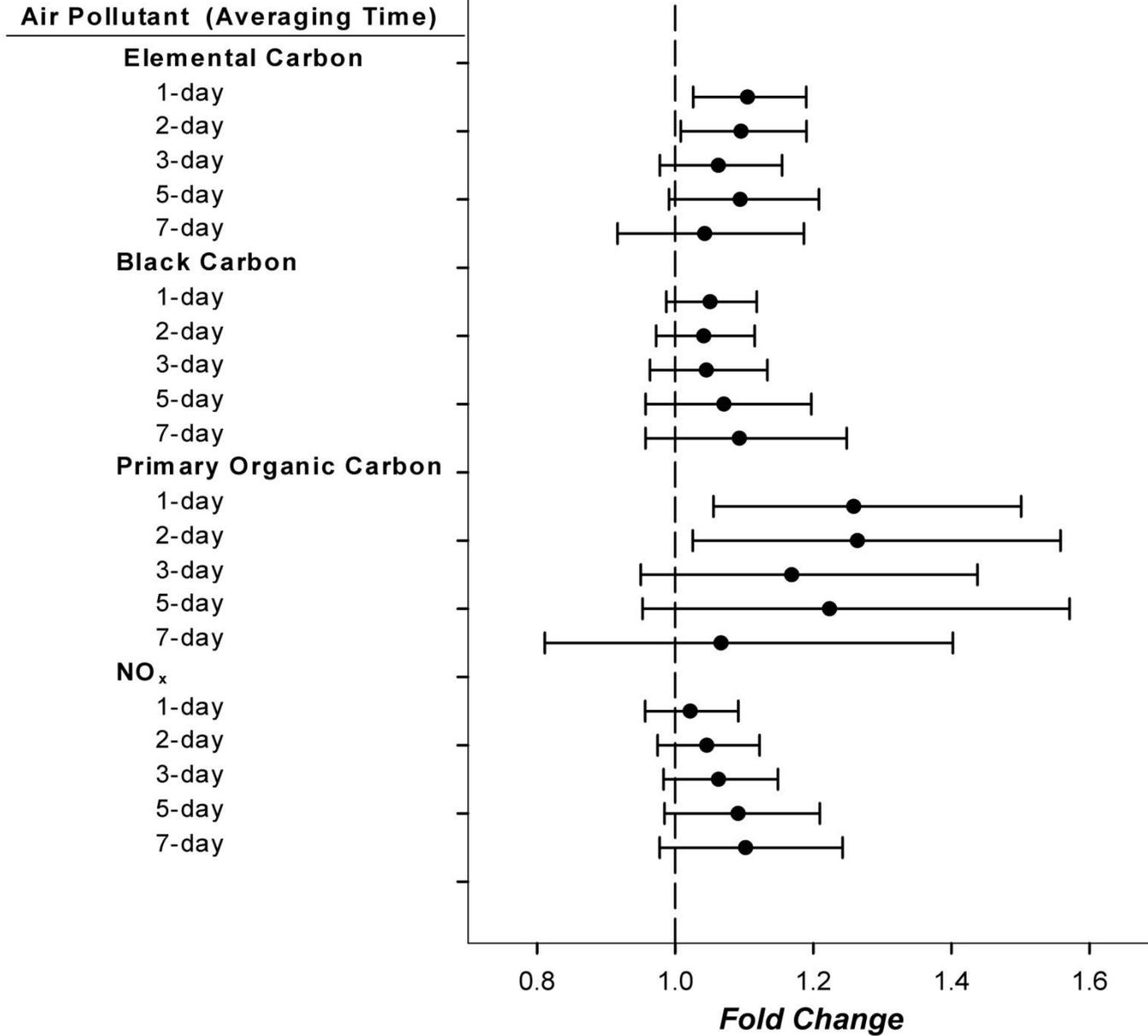
2.0

2.5

Fold Change



NQO1 Gene Expression



SOD2 Gene Expression

Air Pollutant (Averaging Time)

Elemental Carbon

- 1-day
- 2-day
- 3-day
- 5-day
- 7-day

Black Carbon

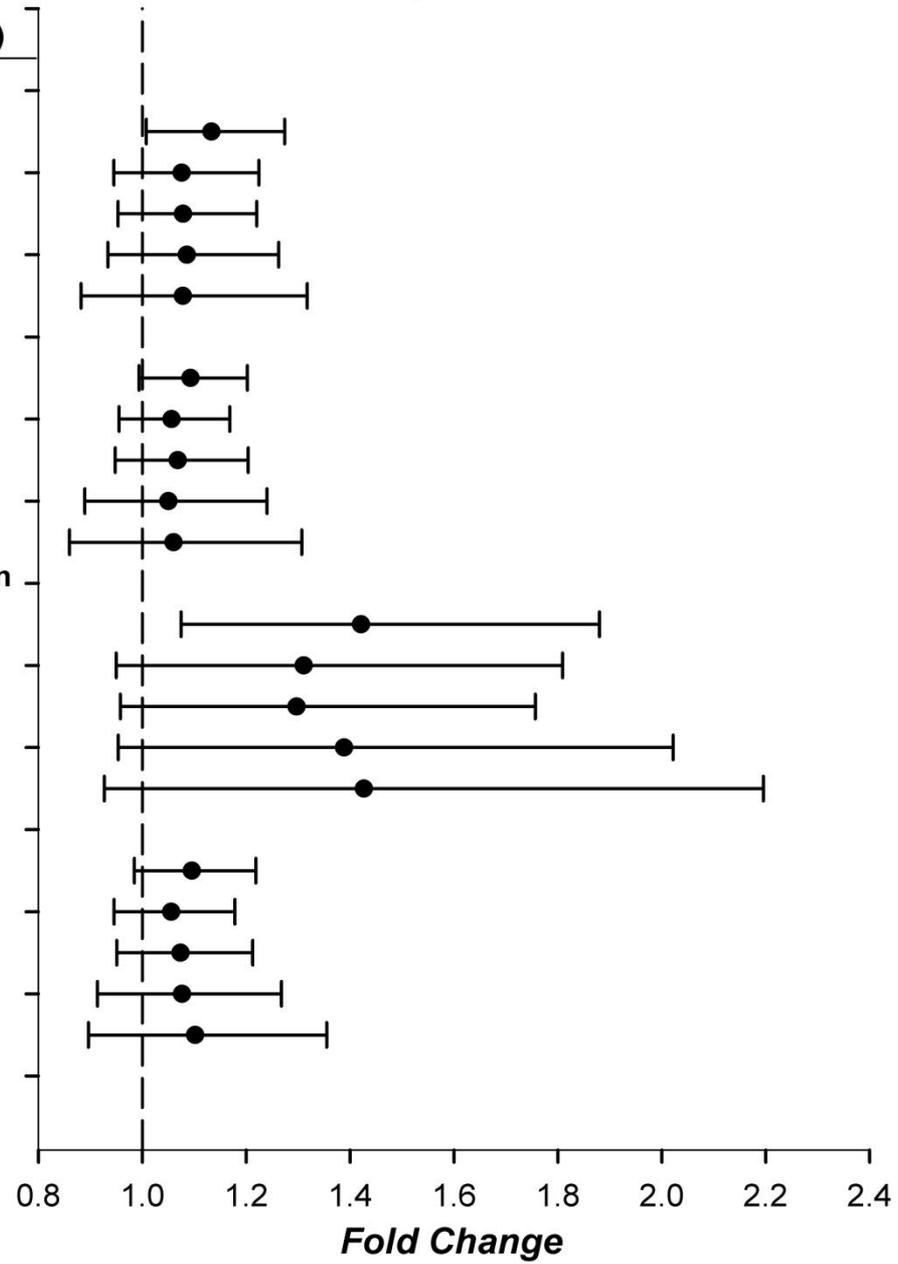
- 1-day
- 2-day
- 3-day
- 5-day
- 7-day

Primary Organic Carbon

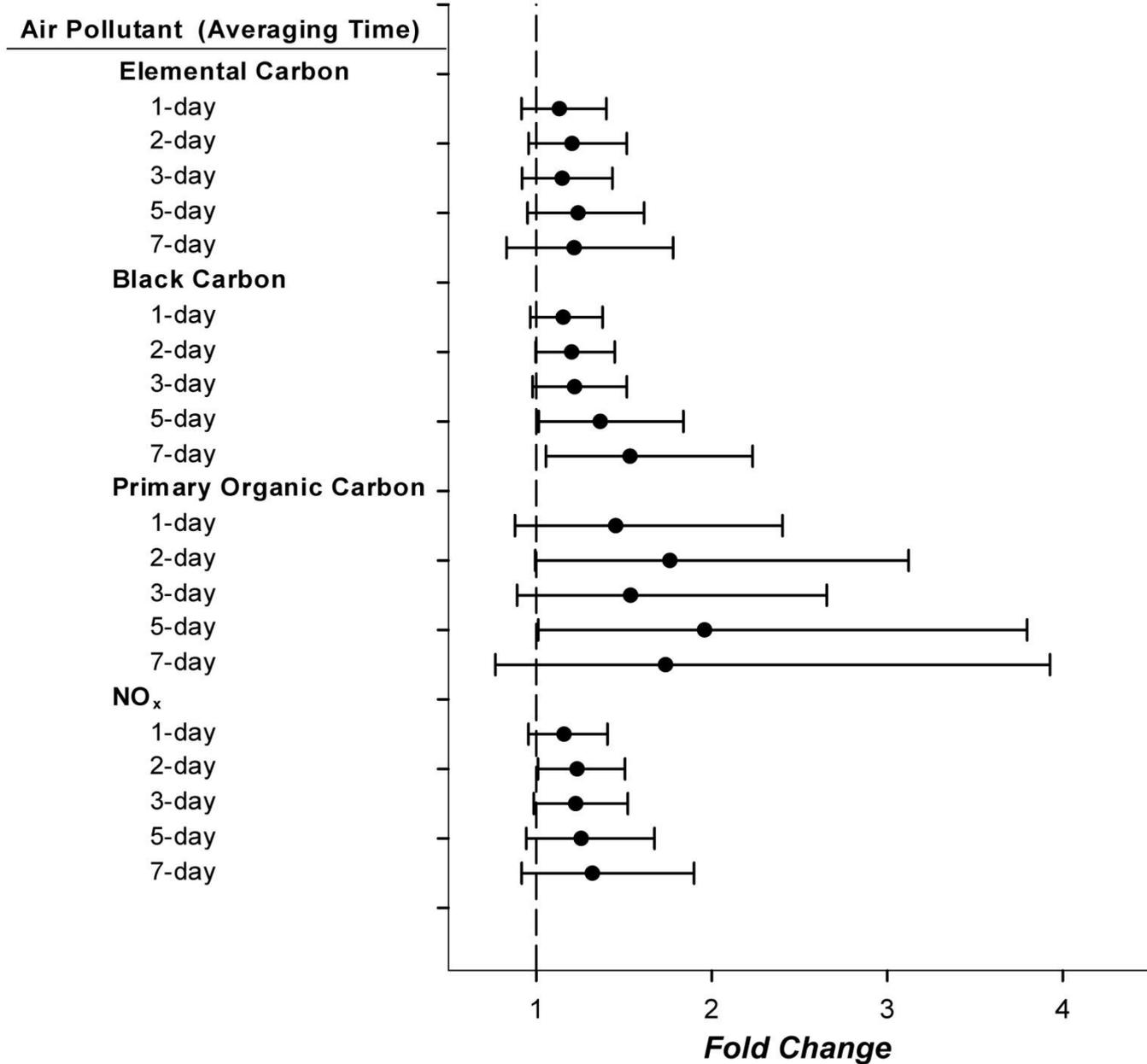
- 1-day
- 2-day
- 3-day
- 5-day
- 7-day

NO_x

- 1-day
- 2-day
- 3-day
- 5-day
- 7-day



CYP1B1 Gene Expression



IL1B Gene Expression

Air Pollutant (Averaging Time)

Elemental Carbon

1-day

2-day

3-day

5-day

7-day

Black Carbon

1-day

2-day

3-day

5-day

7-day

Primary Organic Carbon

1-day

2-day

3-day

5-day

7-day

NO_x

1-day

2-day

3-day

5-day

7-day

0.5

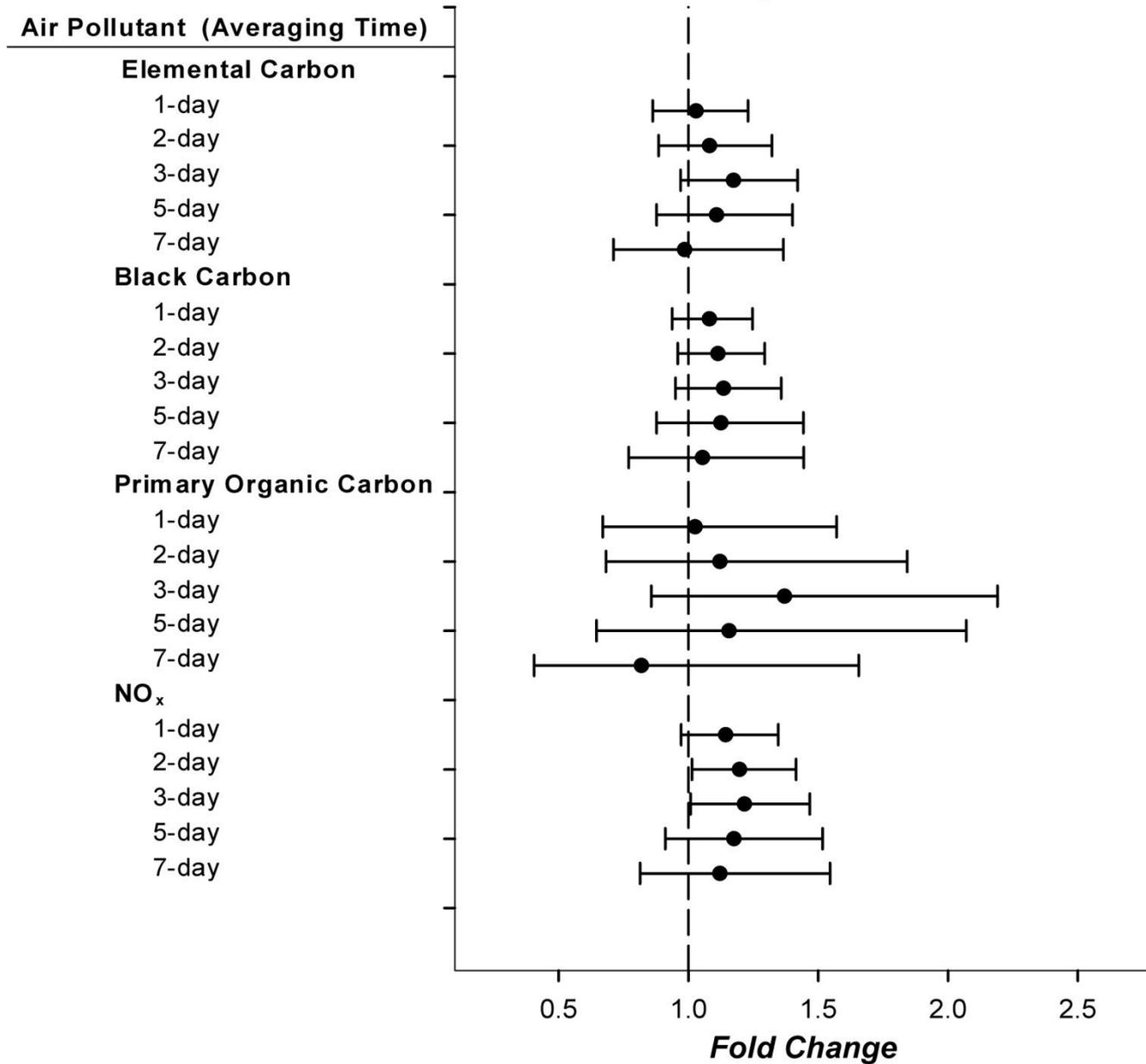
Fold Change

1.0

1.5

2.0

2.5



SELP Gene Expression

Air Pollutant (Averaging Time)

Elemental Carbon

1-day

2-day

3-day

5-day

7-day

Black Carbon

1-day

2-day

3-day

5-day

7-day

Primary Organic Carbon

1-day

2-day

3-day

5-day

7-day

NO_x

1-day

2-day

3-day

5-day

7-day

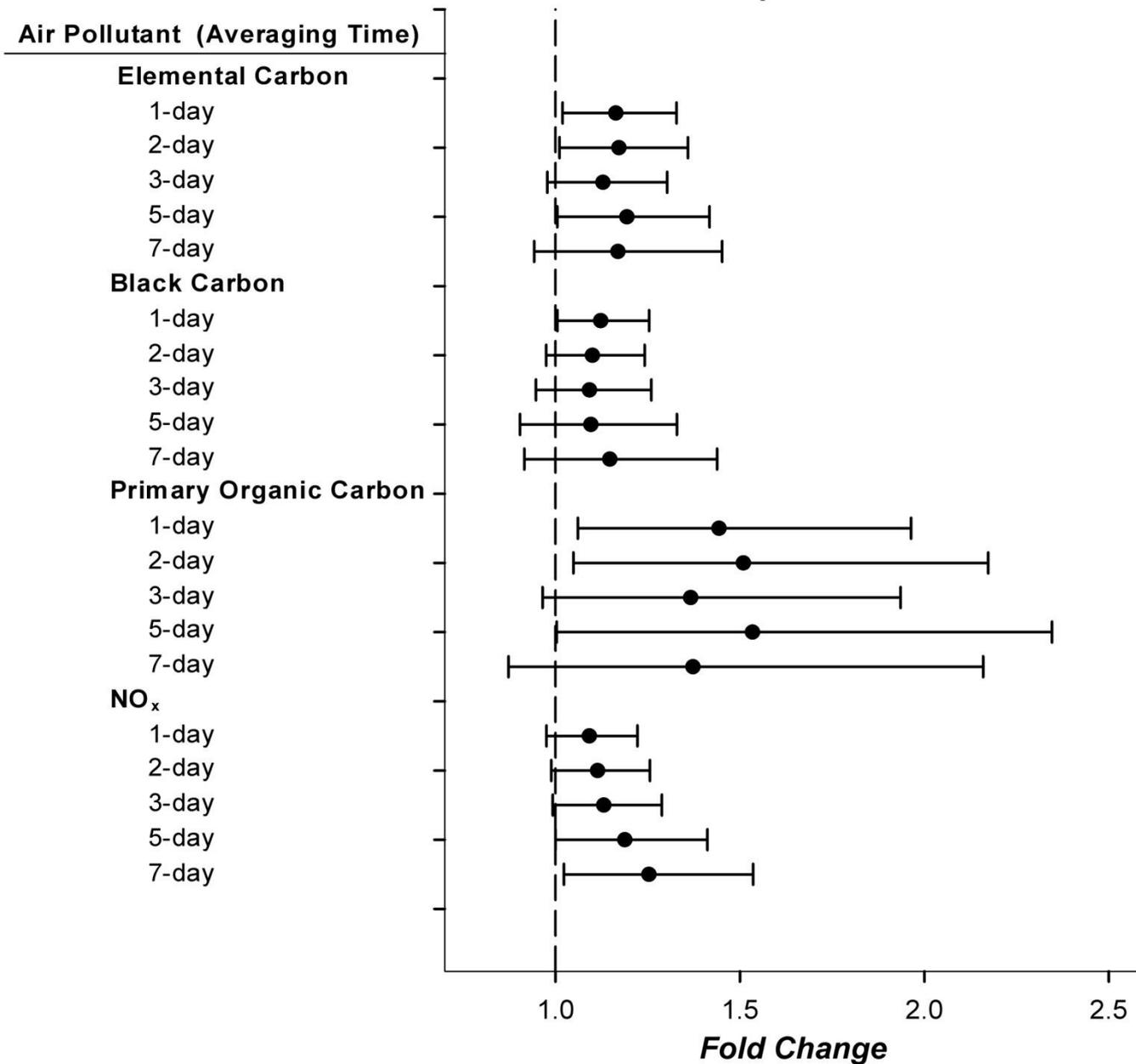
1.0

Fold Change

1.5

2.0

2.5



Summary of results for PM mass and composition (5-day composites)

- PM_{0.25} *in vitro* ROS was positively associated with expression of *NFE2L2*, *NQO1* and *CYP1B1*
- PM_{0.25-2.5} ROS was only associated with *CYP1B1*.
- Overall, stronger associations for PM_{0.25} PAH and/or ROS than for PM_{0.25-2.5} PAH and ROS.
- PM from biomass burning was positively and significantly associated with *HMOX1*, and was positively, but not significantly, associated with expression of 7 other genes (*ATF4*, *ATF6*, *GCLM*, *IL1B*, *KLF2*, *MPO* and *XBP1*) (not shown).

Nrf2 Gene Expression

Size-fractionated PM mass

PM_{0.25}

PM_{0.25-2.5}

PM_{2.5-10}

PM_{0.25} PAH

PAH total

PAH low molecular weight

PAH medium molecular weight

PAH high molecular weight

PM_{0.25} Macrophage ROS

PM_{0.25-2.5} PAH

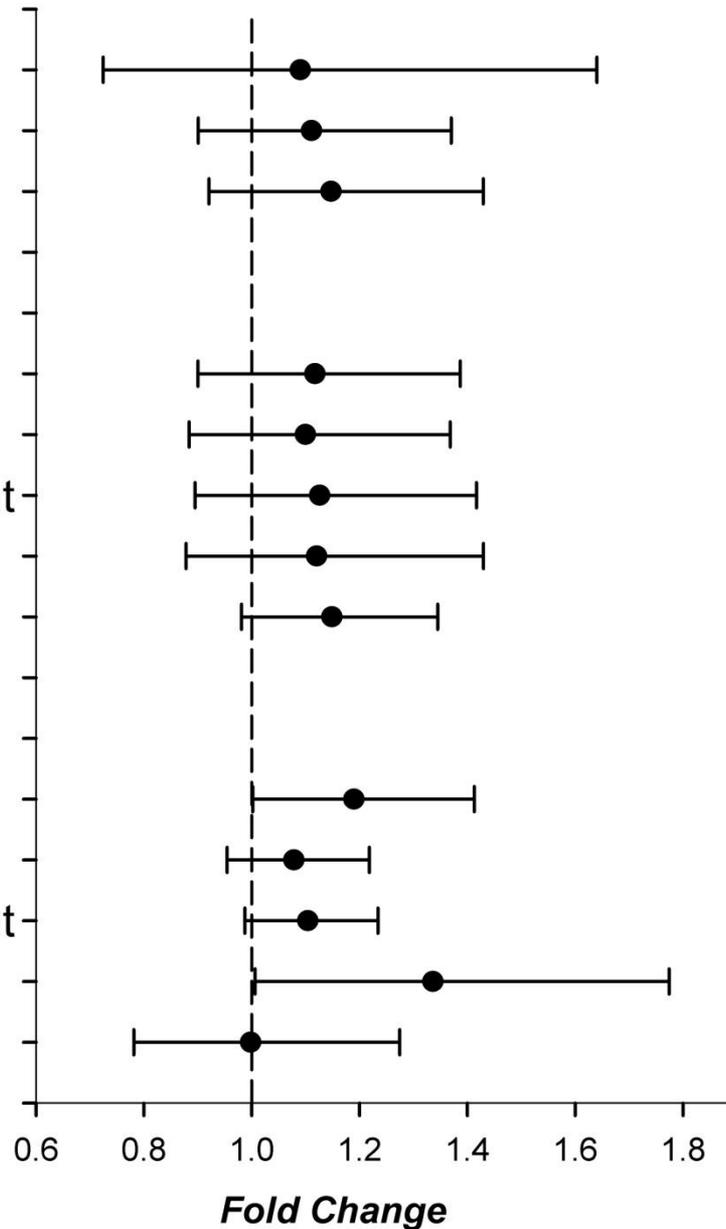
PAH total

PAH low molecular weight

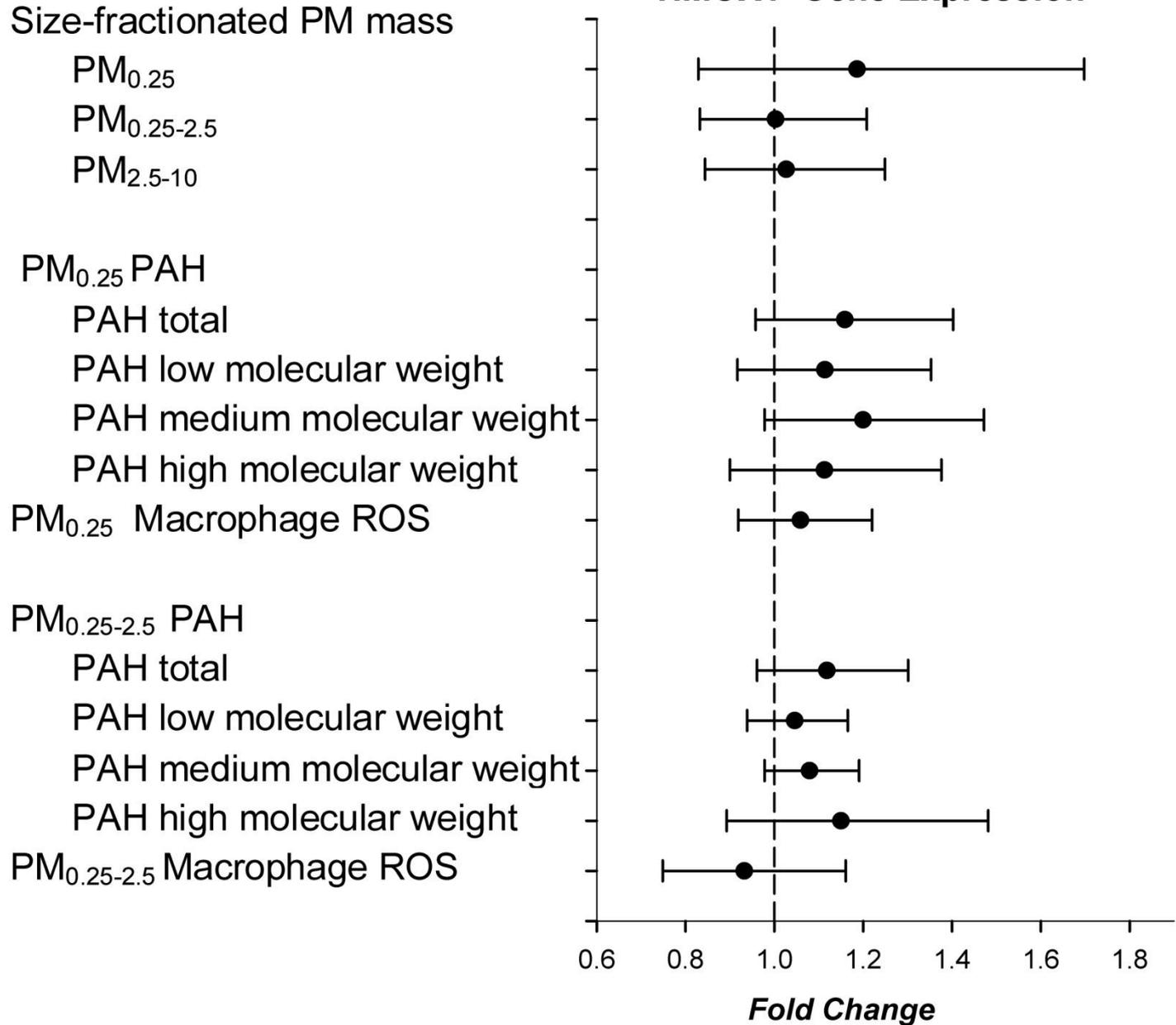
PAH medium molecular weight

PAH high molecular weight

PM_{0.25-2.5} Macrophage ROS



HMOX1 Gene Expression



NQO1 Gene Expression

Size-fractionated PM mass

PM_{0.25}

PM_{0.25-2.5}

PM_{2.5-10}

PM_{0.25} PAH

PAH total

PAH low molecular weight

PAH medium molecular weight

PAH high molecular weight

PM_{0.25} Macrophage ROS

PM_{0.25-2.5} PAH

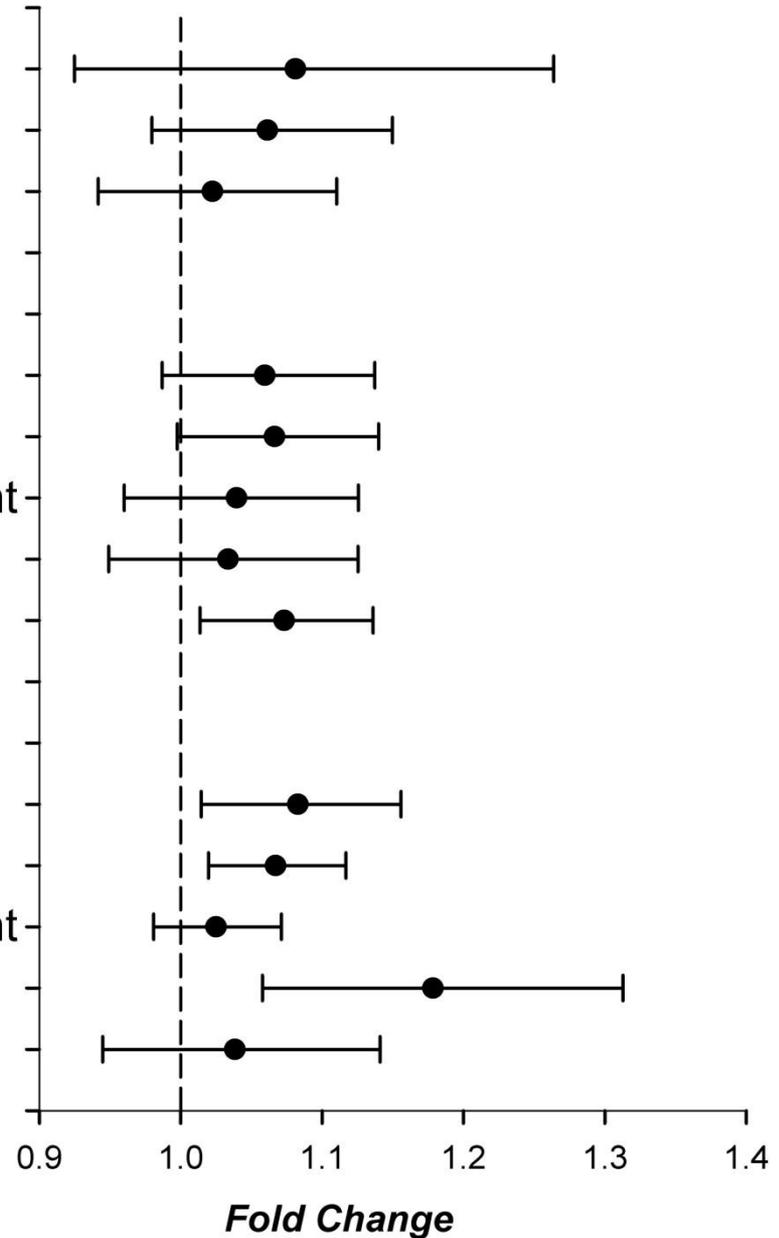
PAH total

PAH low molecular weight

PAH medium molecular weight

PAH high molecular weight

PM_{0.25-2.5} Macrophage ROS



SOD2 Gene Expression

Size-fractionated PM mass

PM_{0.25}

PM_{0.25-2.5}

PM_{2.5-10}

PM_{0.25} PAH

PAH total

PAH low molecular weight

PAH medium molecular weight

PAH high molecular weight

PM_{0.25} Macrophage ROS

PM_{0.25-2.5} PAH

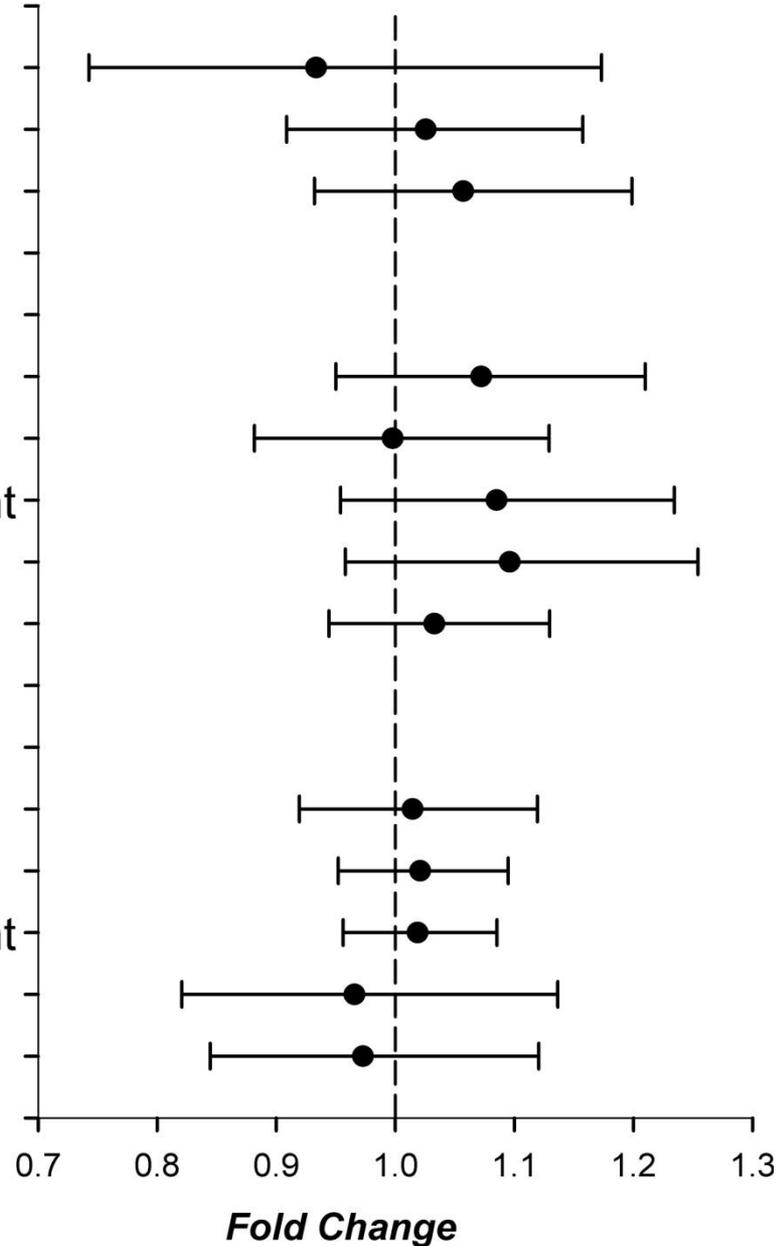
PAH total

PAH low molecular weight

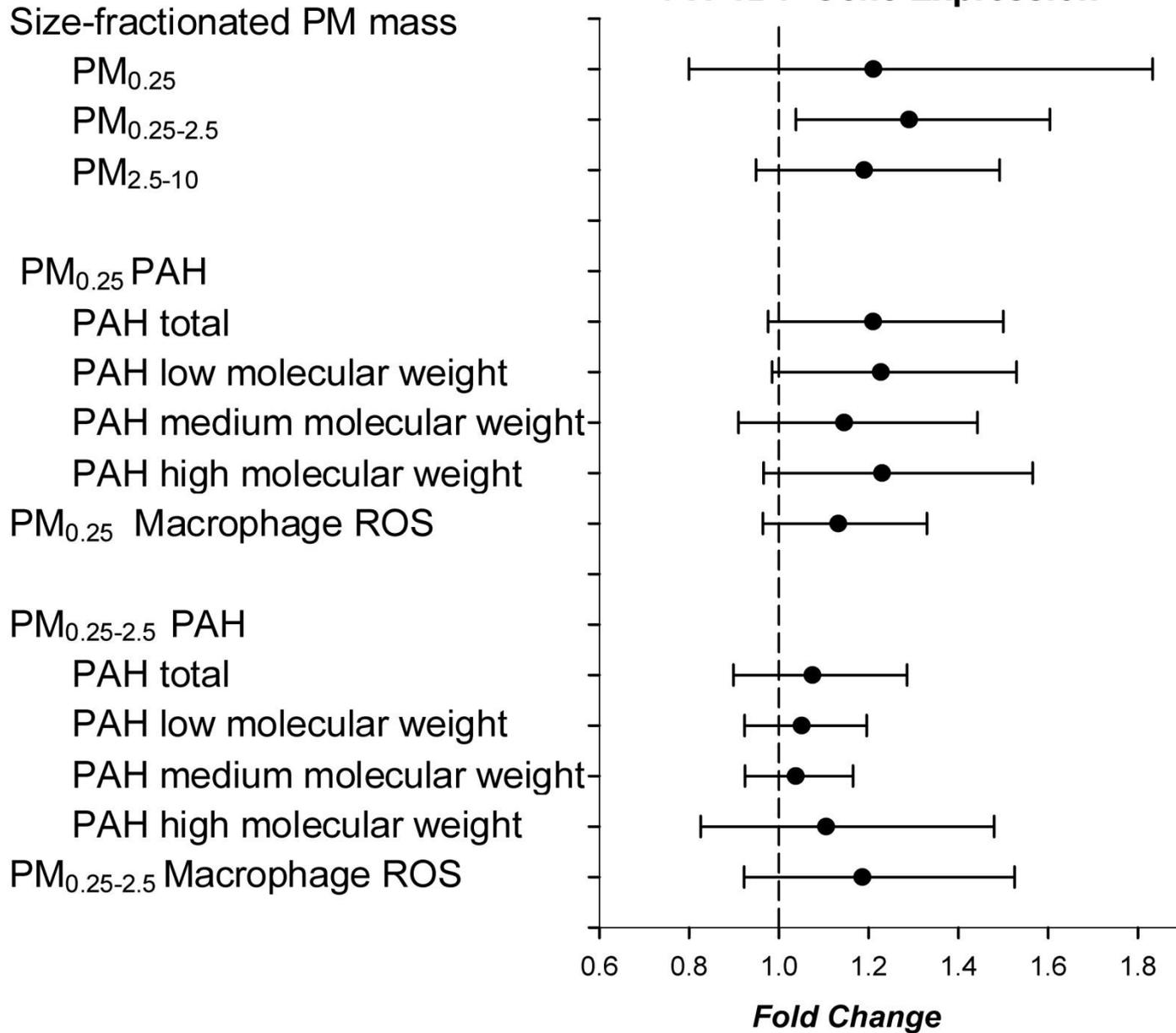
PAH medium molecular weight

PAH high molecular weight

PM_{0.25-2.5} Macrophage ROS



CYP1B1 Gene Expression



IL1B Gene Expression

Size-fractionated PM mass

PM_{0.25}

PM_{0.25-2.5}

PM_{2.5-10}

PM_{0.25} PAH

PAH total

PAH low molecular weight

PAH medium molecular weight

PAH high molecular weight

PM_{0.25} Macrophage ROS

PM_{0.25-2.5} PAH

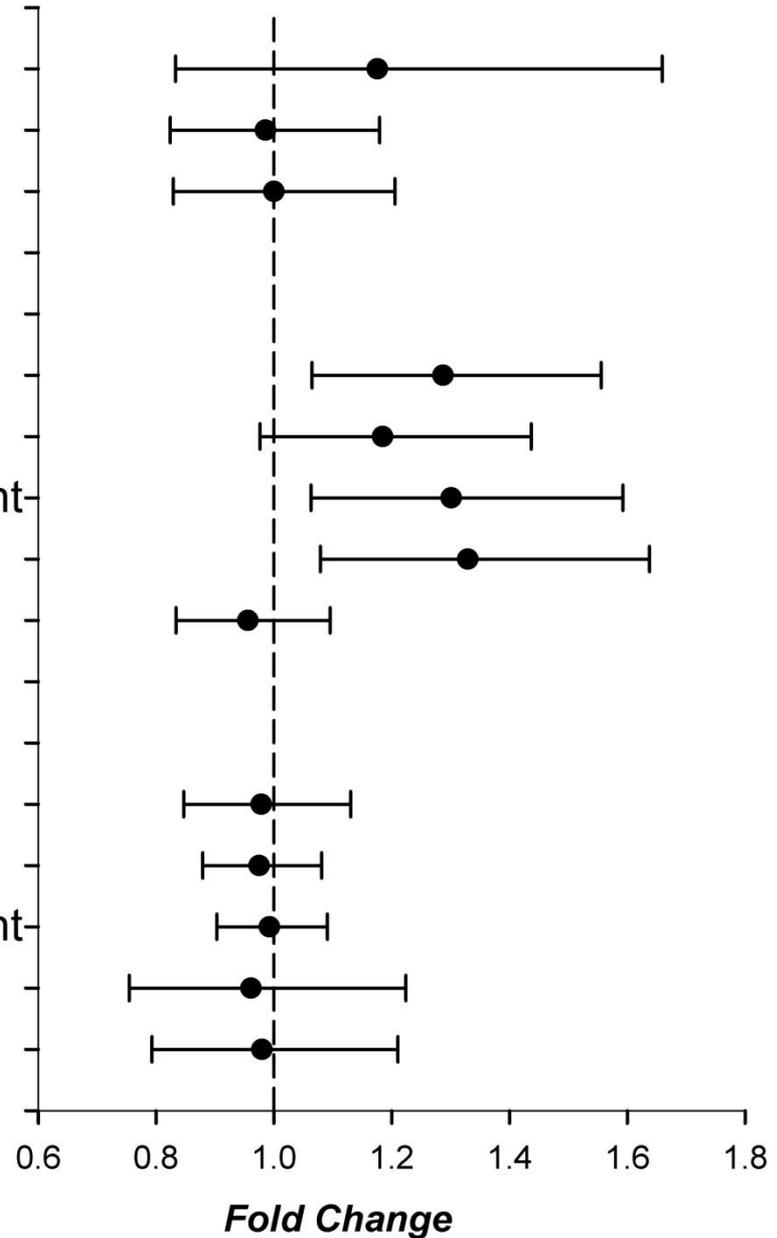
PAH total

PAH low molecular weight

PAH medium molecular weight

PAH high molecular weight

PM_{0.25-2.5} Macrophage ROS



SELP Gene Expression

Size-fractionated PM mass

PM_{0.25}

PM_{0.25-2.5}

PM_{2.5-10}

PM_{0.25} PAH

PAH total

PAH low molecular weight

PAH medium molecular weight

PAH high molecular weight

PM_{0.25} Macrophage ROS

PM_{0.25-2.5} PAH

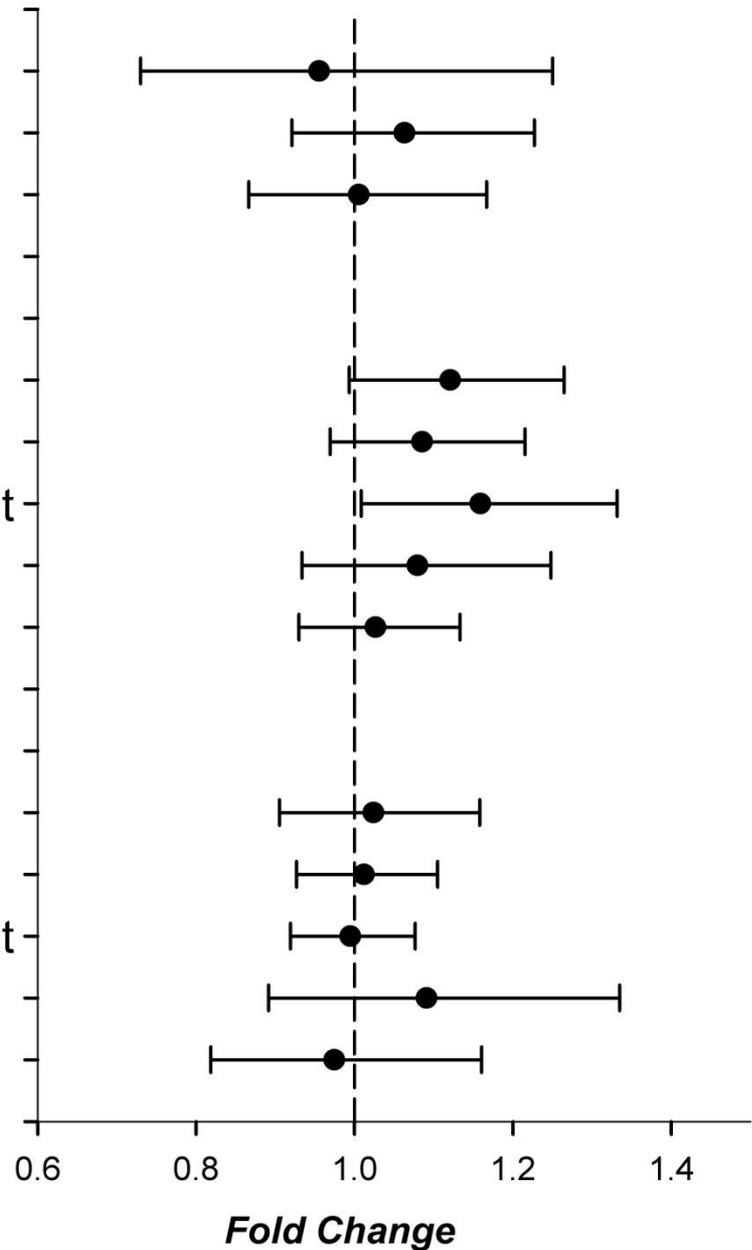
PAH total

PAH low molecular weight

PAH medium molecular weight

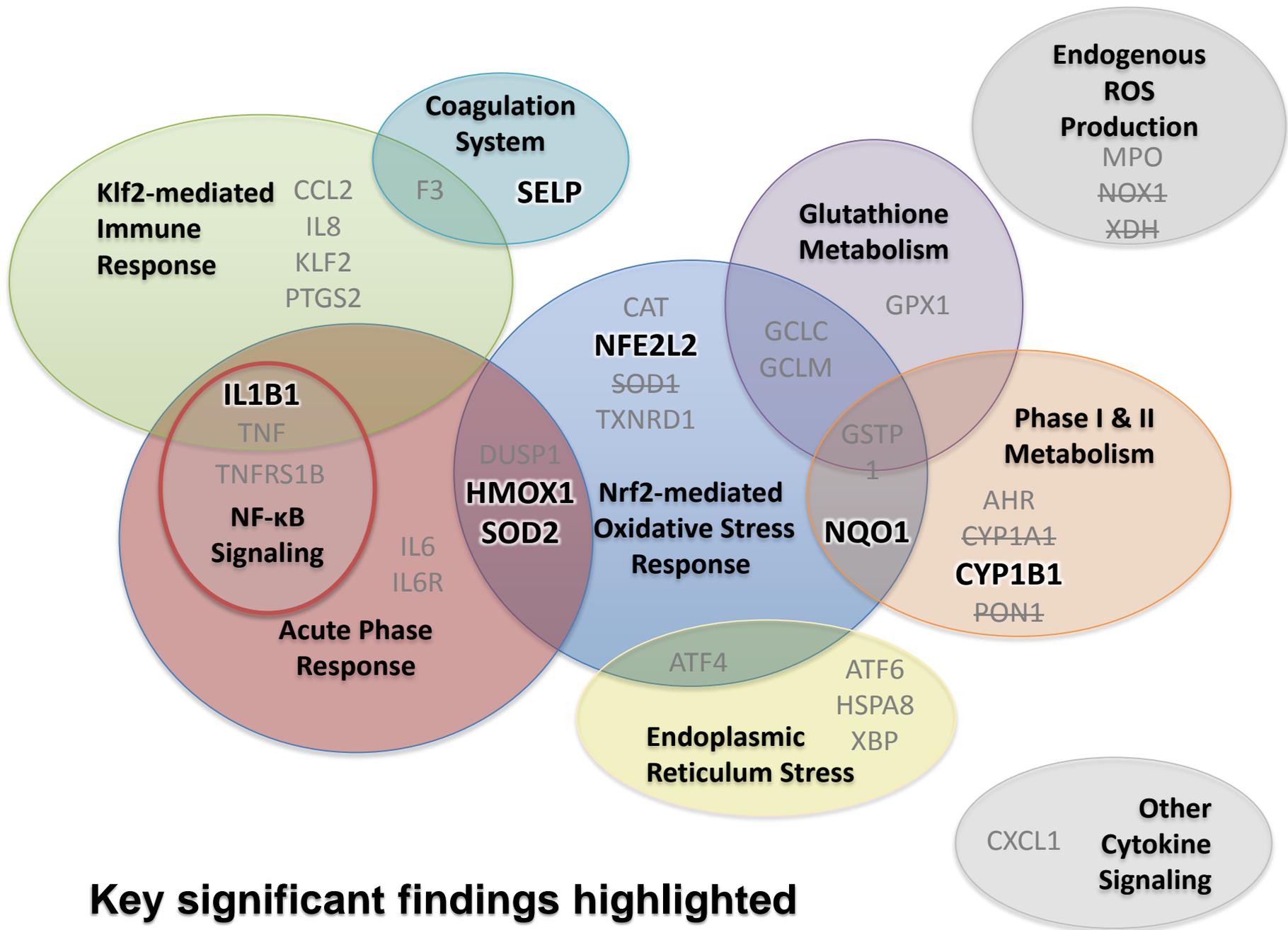
PAH high molecular weight

PM_{0.25-2.5} Macrophage ROS



Summary of results not shown

- Models with indoor air pollutant data showed largely consistent associations with models for outdoor primary air pollutants.
- Few to no associations for CMB-estimated SOA, PM_{2.5} SOC, O₃, or particle number.
- No associations for metals, total OC and CO.
- Few to no associations for the following 15 genes:
 - *AHR, CCL2, CXCL1, DUSP1, F3, GCLC, GPX-1, GSTP1, HSPA8, IL6, IL6R, IL8, PTGS2, TNF, TNFRS1B.*
 - These genes were in all of the studied biopathways except endogenous ROS production (MPO and biomass burning).



Outline

- Background and Rationale
- Tasks
- Parent Study / Methods and Measurements
- Tasks 1-2 Methods and Results
- Task 3 Methods and Results
- **Discussion**

Strengths

- Chemical speciation of organic components, and exposure analysis and source apportionment for indoor and outdoor particle size fractions that make up $PM_{2.5}$.
- Gene expression data provided clues regarding the molecular mechanisms responsible for consistent and significant associations of air pollutants with protein biomarkers observed in the CHAPS1 population, including associations with:
 - $PM_{0.25}$ mass concentration and $PM_{0.25}$ *in vitro* ROS .
 - markers of primary organic aerosols but not secondary organic aerosols in both $PM_{0.25}$ and $PM_{0.25-2.5}$
- Findings are supported by biological plausibility and experimental evidence.

Limitations

- Type I errors due to multiple comparisons: Observed Wald statistics were compared to critical values corresponding to family-wise level .05 test from a simulated distribution: p-values were no longer significant.
- Many 95% CIs included 1, but overall consistency of many positive associations across averaging times for nearly all primary air pollutants. This supports overall exposure-response relationship.
- Small subject number (N=43), but many repeated measures (≤ 12) allowing detection of associations at low fold-change level.
- Limited generalizability.
- Unmeasured time-varying factors (e.g. diet).

Conclusions

- Traffic-related air pollutants are associated with increased whole blood gene expression for genes involved in inflammatory (IL1B), coagulation (SELP), and Nrf2-mediated oxidative stress responses (NRF2, HMOX-1, NQ01, SOD2).
- Linked associations of air pollutants with phase I and phase II enzyme genes because their regulation is linked: XRE in the promoter region of NRF2 and xenobiotic-metabolizing enzyme genes (e.g., CYP1B1) are activated by aryl hydrocarbon receptor inducers like PAH.
- Future funded research in CHAPS2: larger sample size (~ 100 subjects) and repeated microarrays (~800).

Supplemental Findings

- Novel finding: Subjects with mitochondrial haplogroup U showed lower susceptibility to adverse effects of traffic-related air pollution compared to haplogroup H (more tightly coupled respiratory chain = higher ROS).
 - Wittkopp S, Staimer N, Tjoa T, Gillen D, Daher N, Schauer JJ, Sioutas C, Delfino RJ. Mitochondrial genetic background modifies the relationship between traffic-related air pollution exposure and systemic biomarkers of inflammation. *PLoS One*, 2013;8:e64444.
- Gene-environment interactions for SNPs in SELP, NRF2, SOD2, NQO1, CAT, MPO, GSTP1 plus GSTM1 and GSTT1 null genotypes.
 - Manuscript in preparation

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Questions?