Repeated measurements of PAH metabolites in women - initial results from a sub-study for Women's Health and the Environment (WHE)

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WHE Study Goals

- Long-term goal to understand how toxicants cause ovarian dysfunction.

- Pilot study to demonstrate feasibility of a larger study testing the hypothesis that genetic variations in biotransformation enzymes involved in metabolizing PAHs modulate the ovarian toxicity of PAHs in women.

  - Feasibility of using a microelectronic dipstick monitor to measure urinary reproductive hormone concentrations and home collection of urine samples for exposure biomarkers over multiple menstrual cycles.
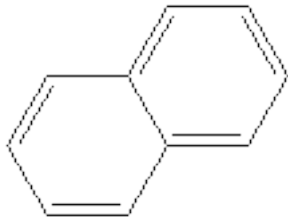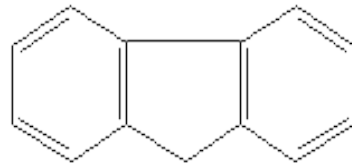
Sources of PAHs
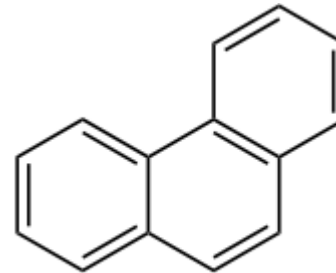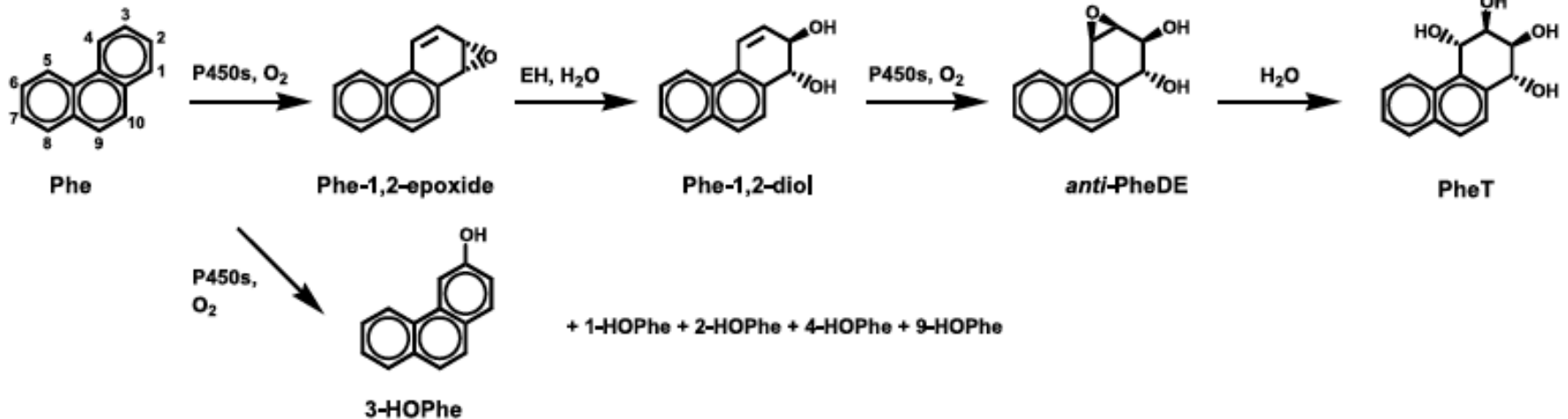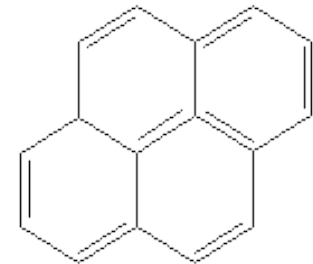
PAH structure and metabolism

Naphthalene

Fluorene

Phenanthrene

Pyrene

Phe → (P450s, O_2) → Phe-1,2-epoxide → (EH, H_2O) → Phe-1,2-diol → (P450s, O_2) → anti-PheDE → (H_2O) → PheT

Phe → (P450s, O_2) → 3-HOPhe + 1-HOPhe + 2-HOPhe + 4-HOPhe + 9-HOPhe

Ovarian toxicity of PAHs

- PAHs dose-dependently destroy primordial and primary ovarian follicles and cause premature ovarian failure (studies by Mattison and coworkers; Borman et al, 2000)

- In utero exposure to BaP decreases fertility and depletes germ cells (MacKenzie and Angevine, 1981; Lim et al, 2013)

- Women who smoke have earlier onset of menopause and decreased fecundity (Harlow, 2000; Alderete et al, 1995; Hassan and Killick, 2004)

- Daughters of women who smoked during pregnancy have decreased fecundity (Weinberg et al, 1989; Jensen et al, 1998) and earlier age at menopause (Strohsnitter et al, 2008)

WHE Study Overview

- <u>Participants:</u> Women 18-44 years old, residing in Orange County, CA, not using hormonal contraception, and with no history of surgical sterilization, infertility, treatment with radiation or chemotherapy

- <u>Study design:</u>
  - Baseline visit: questionnaire, blood sample, home urinary hormone monitor and urine collection kit
  - Daily urinary reproductive hormone monitoring; urine samples for OH-PAHs on cycle day 10 for up to 6 menstrual cycles
  - Daily diary included questions on menstrual bleeding, smoking, medications, and alcohol use

WHE Study Overview (*cont.*)

- Final study population consisted of 51 women, who completed sample and data collection for at least 2 menstrual cycles
  - 2 women had 2 cycles each and the remaining 49 women had 3 cycles each of urine samples available for analysis of OH-PAHs
  - 9 hydroxylated metabolites of naphthalene, pyrene, fluorene, and phenanthrene were measured by the CDPH EHL using isotope dilution gas chromatography/ high resolution mass spectrometry (GC-HR-MS)

WHE OH-PAH detection frequencies

| PAH metabolite	Frequency (%)
1-NAP	100
2-NAP	100
1-PHEN	99.3
3-PHEN	99.3
2-FLUO	99.3
9-FLUO	99.3
1-PYR	96.0
3-FLUO	93.7
2-PHEN	92.1

Geometric means for WHE OH-PAHs compared to NHANES 2011-12
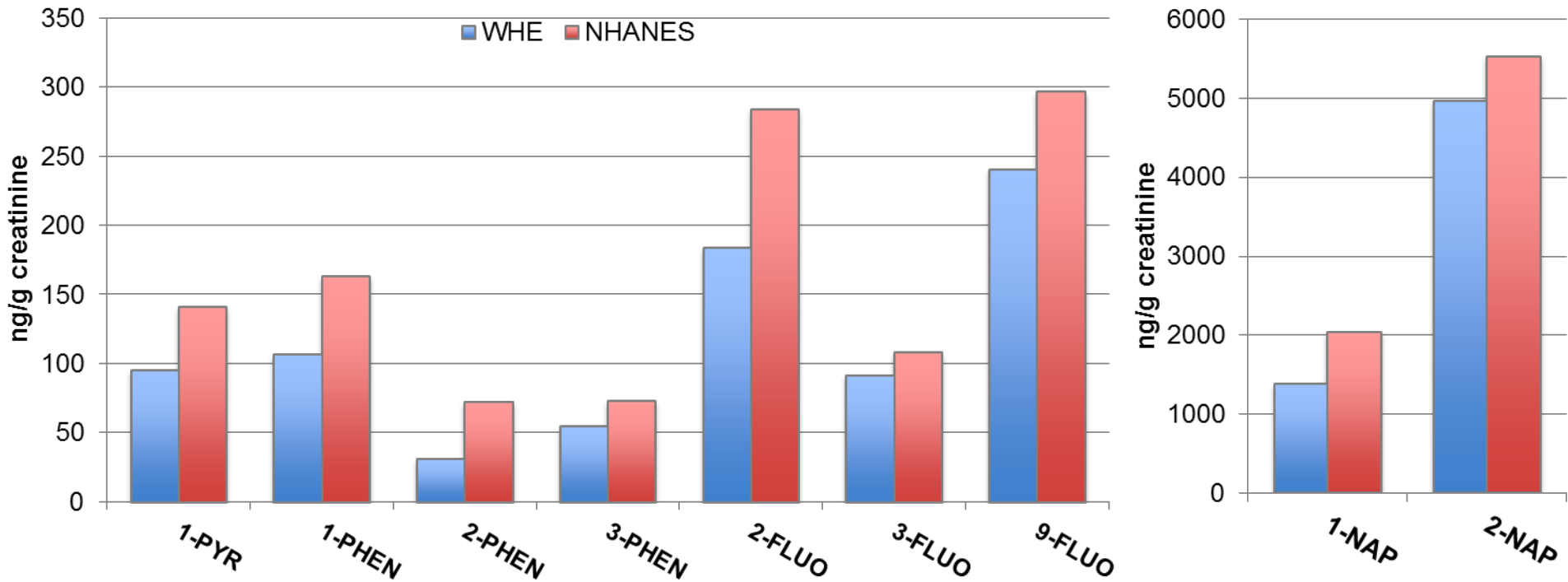
Correlations between PAH metabolites

	1-NAP	2-NAP	1-PYR	1-PHEN	2-PHEN	3-PHEN	2-FLUO	3-FLUO	9-FLUO
1-NAP	1	0.105	0.234	0.429	0.285	0.352	0.374	0.446	0.424
2-NAP		1	0.163	-0.038	0.030	-0.011	0.072	0.067	-0.113
1-PYR			1	0.597	0.574	0.654	0.385	0.465	0.379
1-PHEN				1	0.738	0.738	0.635	0.565	0.655
2-PHEN					1	0.764	0.573	0.536	0.615
3-PHEN						1	0.638	0.683	0.631
2-FLUO							1	0.887	0.470
3-FLUO								1	0.459
9-FLUO									1

Within person variability of OH-PAHs

Intraclass Correlation Coefficients*
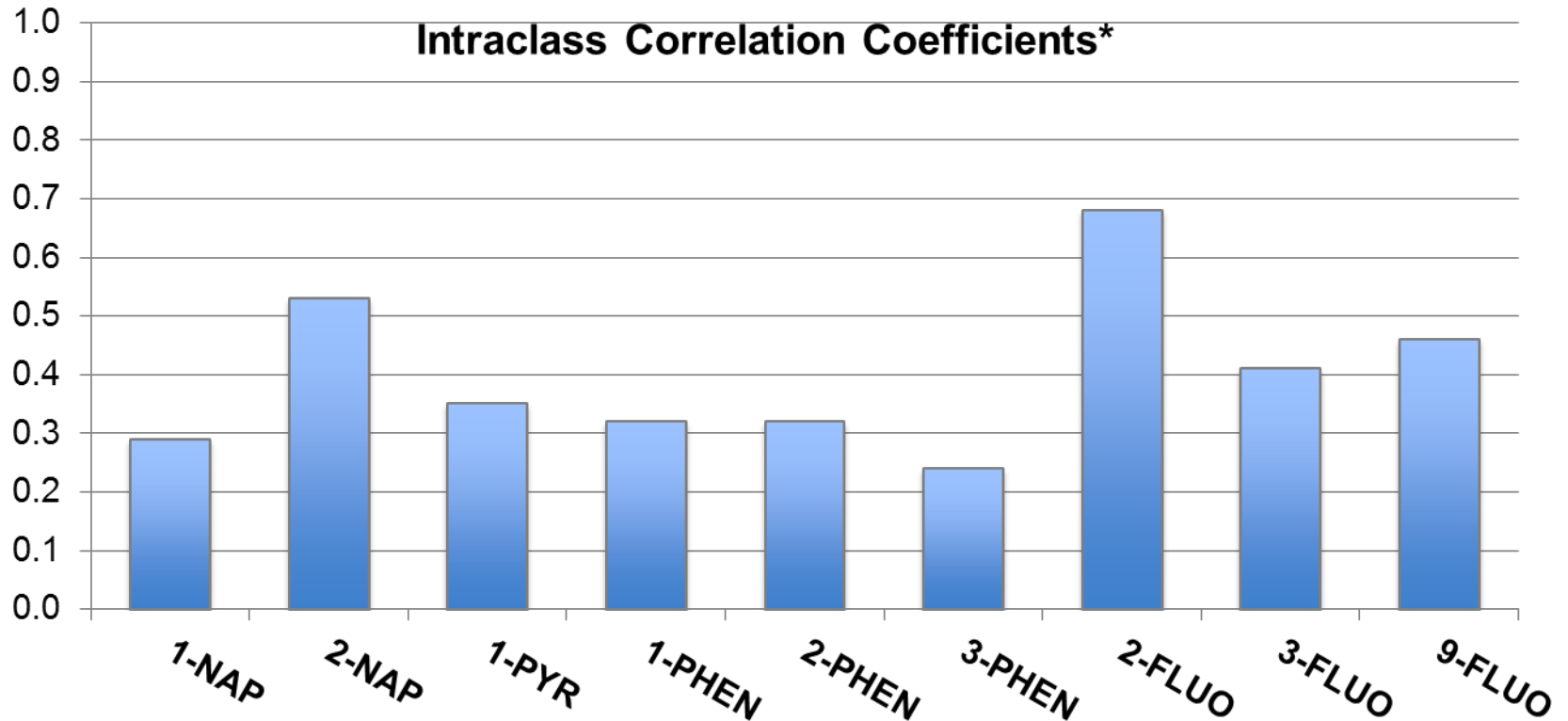
*3 samples for 49 women and 2 samples for 2 women

What may be sources of PAH exposure in this population?

- FLUO, PHEN, NAP metabolites: indoor air exposures

- 1-, 2-NAP: smoking

- 2-NAP: traffic exposure and residence in an industrial area

- 1-PYR: barbecued/grilled meat consumption

(Nethery et al, 2012; Li et al, 2010)

Summary

- Nearly all of these Orange County women had detectable concentrations of the nine hydroxylated PAH metabolites, which is consistent with NHANES findings.
- The geometric mean concentrations in these participants were lower than geometric means for females in NHANES.
- 8 of the 9 metabolites were highly correlated with each other, while 2-NAP was minimally correlated with the other OH-PAHs.
- The metabolite concentrations were not highly correlated across menstrual cycles within participants.

Ongoing work and future directions

- Currently finalizing analyses of associations between OH-PAHs and reproductive endocrine endpoints.

- Ongoing analyses of predictors of OH-PAH concentration.

- Measure 1-NP metabolites in these samples?

- Measure other exposure biomarkers of interest?

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