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Environmental Exposure Assessment among Infant: A Challenge of Methodology for Detecting Urinary OH-PAHs

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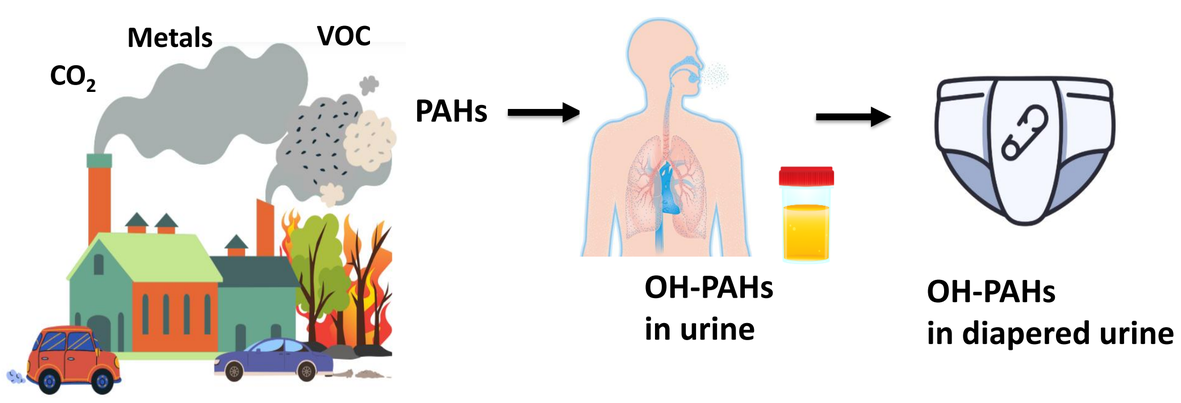
INTRODUCTION & AIM

Polycyclic aromatic hydrocarbons (PAHs) are common pollutants caused by incomplete combustion of fuels, industrial processes, and organic materials. They can enter the human body via inhalation, ingestion, or skin contact and cause major health problems such as cancer, genetic damage, and developmental harm. Inside the body, PAHs are converted into hydroxylated metabolites (OH-PAHs), which are routinely detected in urine as biomarkers of exposure. Urinary analysis of PAHs exposure is commonly employed, but infants mostly wear diapers. Therefore, extraction of PAHs metabolites from urinated diapers.

This study aims to develop and validate a method for extracting detecting ten hydroxylated polycyclic aromatic hydrocarbons (OH-PAHs) in diapered urine.



METHOD

- **Urine extraction of disposable diapers**

Thaw the diaper at room temp, cut the urine-soaked pad into a beaker using sterile scissors.

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Add 50 g/L $CaCl_2$ solution, stir, leave it, then filter the released urine from the gel.

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Centrifuge the remaining diaper, filter again to fully extracted (approx. 70–80 mL).

- **OH-PAHs measurement by using HPLC-FLD**

Acidify 10 mL of the extracted urine sample to pH 5.0

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Hydrolyzed with β-glucuronidase, and clean up the extract using solid phase extraction (C18 OH)

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Ten OH-PAH metabolites were measured using HPLC-FLD with deuterated internal standards

OH-PAHs analyzed

1-Hydroxynaphthalene (1-OHNap)
2-Hydroxynaphthalene (2-OHNap)
2-Hydroxyfluorene (2-OHFlu)
3-Hydroxyfluorene (3-OHFlu)
2-Hydroxyphenanthrene (2-OHPhe)
3-Hydroxyphenanthrene (3-OHPhe)
9-Hydroxyphenanthrene (9-OHPhe)
1-Hydroxyphenanthrene (1-OHPhe)
4-Hydroxyphenanthrene (4-OHPhe)
1-Hydroxypyrene (1-OHPyr)

RESULTS & DISCUSSION

Table 1. Median (IQR) concentrations of measured OH-PAHs (ng/mL)

Name	Normal Urine (U) Median (IQR) (ng/mL)	Diapered Urine (DU) Median (IQR) (ng/mL)	% Recovery
2-OHNap	1.89 (1.5-2.6)	0.85 (0.6-1.44)	45%
1-OHNap	0.13 (0.19-0.11)	0.06 (0.02-0.12)	46%
2+3-OHFlu	0.06 (0.1-0.04)	0.03 (0.03-0.05)	57%
2-OHPhe	0.04 (0.07-0.03)	0.03 (0.02-0.04)	75%
3-OHPhe	0.02 (0.05-0.02)	0.01 (0.01-0.02)	50%
9-OHPhe	0.03 (0.08-0.02)	0.03 (0.02-0.05)	76%
1-OHPhe	0.07 (0.08-0.07)	0.07 (0.07-0.08)	**95%**
4-OHPhe	0.04 (0.04-0.03)	0.04 (0.04-0.05)	**101%**
1-OHP	0.02 (0.03-0.02)	0.02 (0.02-0.03)	**99%**

Table 2. Validation results: Precision and Recovery

Name	(STD Lv4) Mean (ng/mL)	Median (ng/mL)	Nominal (ng/mL)	Precision (%RSD)	% Recovery
2-OHNap	0.29	0.27	0.25	**27%**	115%
1-OHNap	0.26	0.28	0.25	**37%**	105%
2+3-OHFlu	0.25	0.25	0.25	13%	100%
2-OHPhe	0.23	0.23	0.25	14%	93%
3-OHPhe	0.26	0.27	0.25	**16%**	103%
9-OHPhe	0.33	0.33	0.25	15%	**131%**
1-OHPhe	0.19	0.19	0.25	10%	**75%**
4-OHPhe	0.27	0.26	0.25	11%	107%
1-OHP	0.30	0.30	0.25	12%	118%

- DU from twenty individuals recovered OH-PAH metabolites from 45 - 101%, with 4-OHPhe (101%), and 1-OHP (99%) recovering the highest. These metabolites may have more constant background levels across populations.
- The variability indicated by the interquartile ranges (IQRs) is wider for 1-OHNap and 2+3-OHFlu, possibly reflecting heterogeneous exposure or metabolic rates.
- Lower recoveries for some metabolites result from diaper extraction losses and high calcium levels in diaper urine interfering with enzymatic hydrolysis.

CONCLUSION

- OH-PAH metabolites in infant diaper urine, recovery rates ranging from 45% to 101% by enzymatic hydrolysis from conjugated forms.
- This data highlights the importance of considering specific metabolites and population characteristics when assessing OH-PAH biomonitoring results

FUTURE WORK / REFERENCES

- Further steps will be used in non-enzymatic hydrolysis such as acid hydrolysis and then identify representative urinary OH-PAHs for exposure assessment among infants.

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