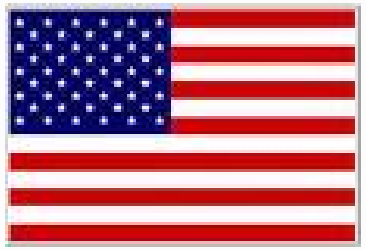
Urinary metabolites of 1-nitropyrene in

U.S.-Mexico border residents



who frequently cross the San Ysidro Port of Entry

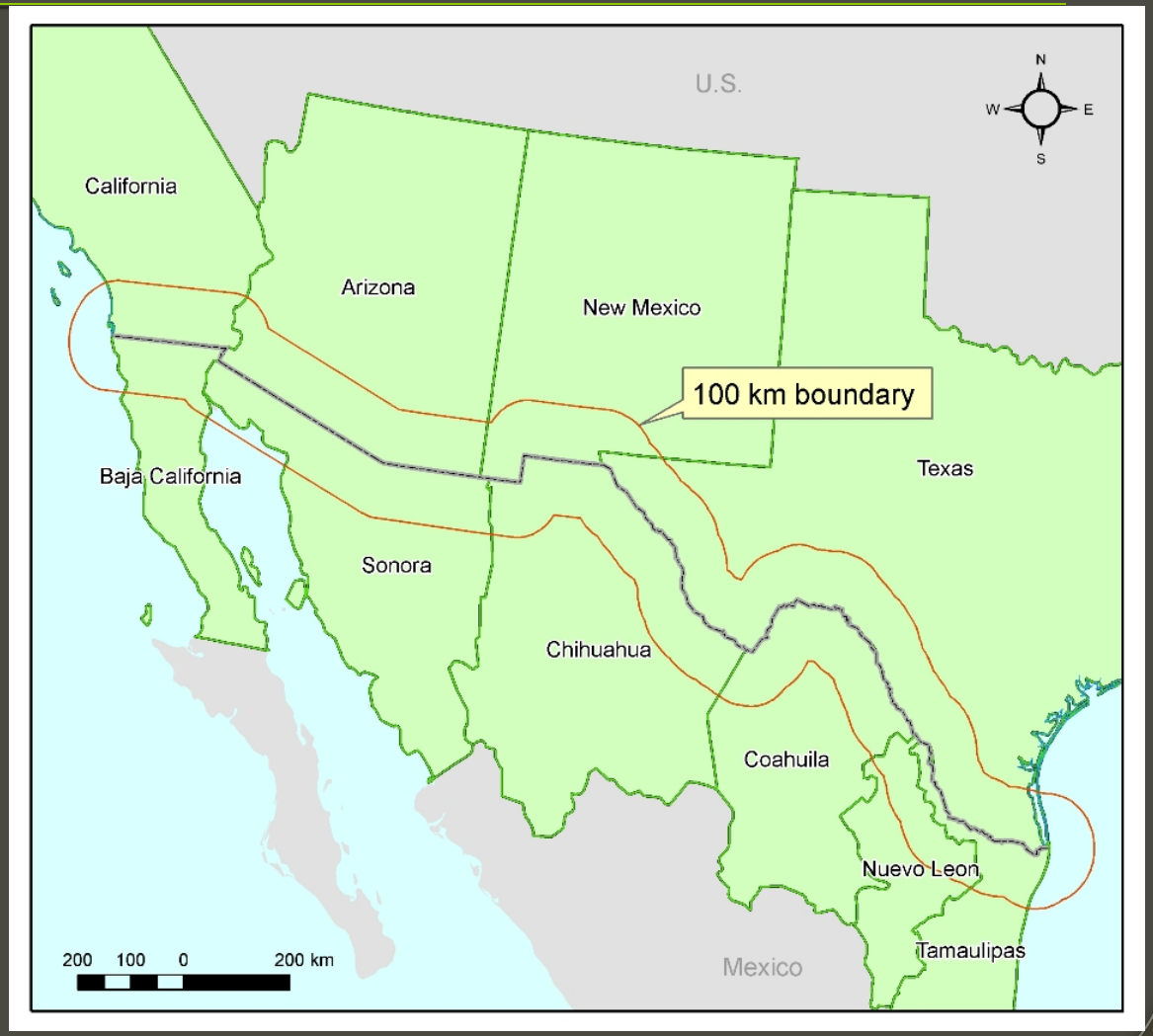
Vanessa E. Galaviz, PhD, MPH

Office of the Secretary

California Environmental Protection Agency

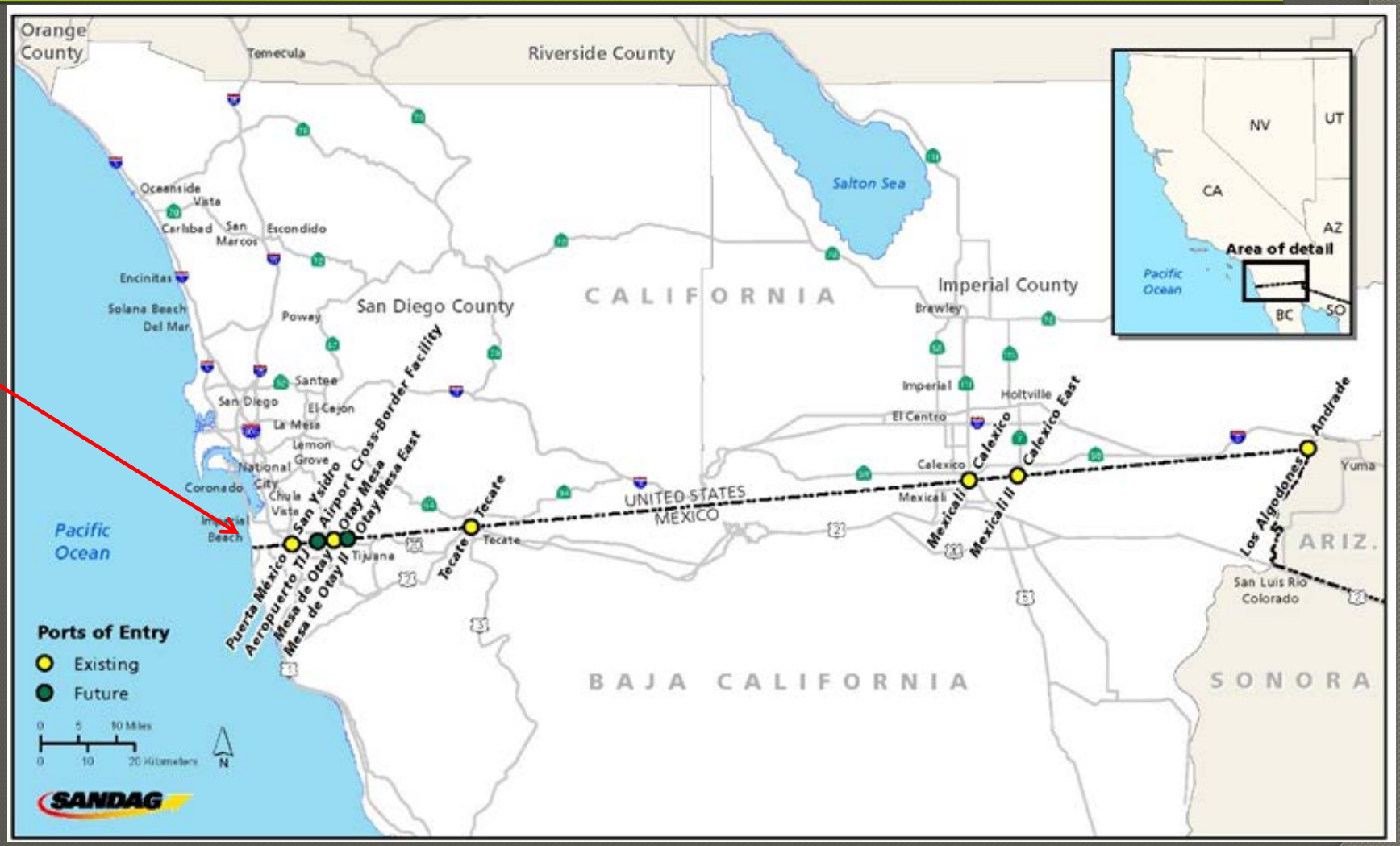
U.S.-Mexico Border

- U.S. EPA defines the U.S. Mexico Border Region as 62 miles north and south of the U.S.-Mexico Border and extends into the sea boundaries to the east and west



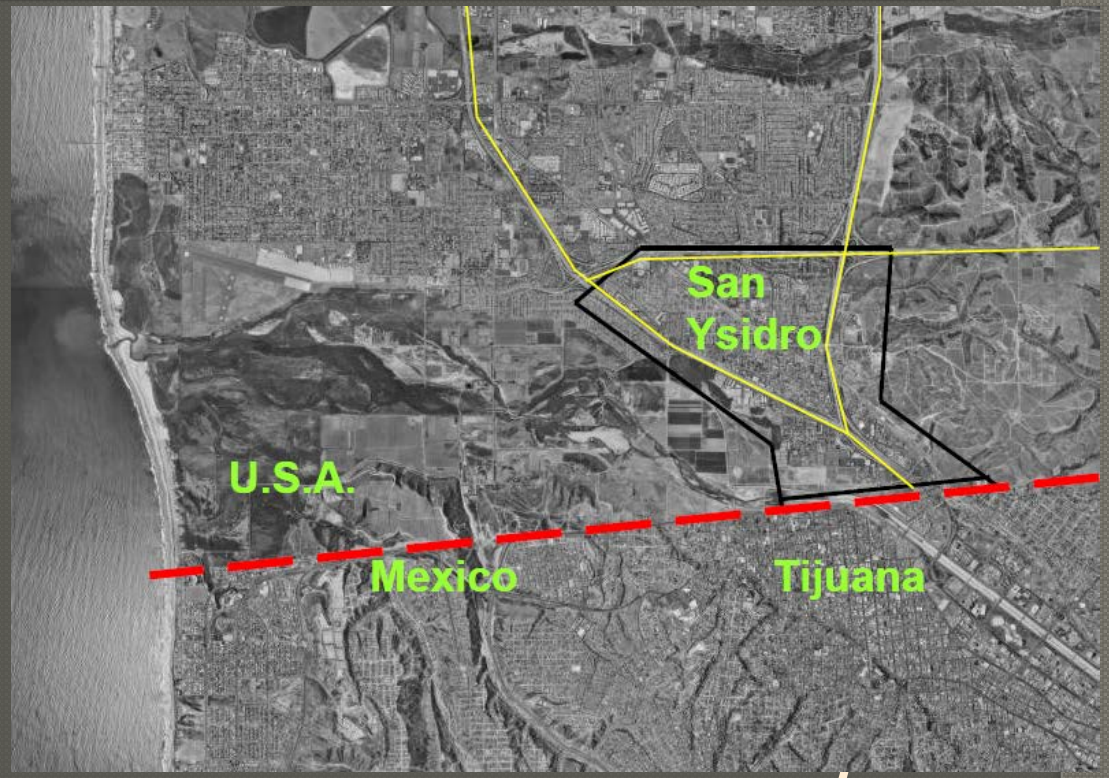
California-Baja California Land Ports of Entry

San Ysidro Port of Entry



San Ysidro Port of Entry

- Busiest land border crossing in the western hemisphere, according to U.S. General Services Administration[1].



	Personal Vehicles	Personal Vehicle Passengers	Pedestrians	Buses	Bus Passengers
2010 northbound[2]	13,348,364	23,600,605	6,439,952	70,548	550,301

San Ysidro Port of Entry

[1]*http://www.gsa.gov/portal/content/104872*
[2]*http://transborder.bts.gov/programs/international/transborder/TBDR_BC/TBDR_BC_Index.htm*

San Ysidro Port of Entry Pedestrian Pathway

Previous work

- Occupational studies have shown differences in DPM exposure between high exposure groups and low exposure groups using 1-NP **and/or** its urinary metabolites[1-3]
- Human studies have shown urinary metabolites of 1-NP are higher in participants with exposure to elevated levels of DPM[3,4-7]

Data Gap

- To date there has been no community study that has detected differences in 1-NP **and** its urinary metabolites in a lower exposure range

[1]*Simpson CD, Miller-Schulze J, Paulsen M, Kameda T, Cassidy B, Aguilar-Villalobos M, et al. 1-nitropyrene exposures in air and biomarker levels in urine amongst workers exposed to traffic-related air pollution in Trujillo, Peru. Epidemiol 2008; 19(6): 1161-1180.*

[2]*Seidel A, Dahmann D, Krekeler H, Jacob J. Biomonitoring of polycyclic aromatic compounds in the urine of mining workers occupationally exposed to diesel exhaust. Int J Hyg Environ Health 2002; 204(5-6): 333-338.*

[3]*Miller-Schulze JP, Paulsen M, Kameda T, Toriba A, Tang N, Tamura K, et al. Evaluation of urinary metabolites of 1-nitropyrene as biomarkers for exposure to diesel exhaust in taxi drivers of Shenyang, China. J Expo Sci Environ Epidemiol 2013; 23(2): 170-175.*

[4]*Toriba A, Kitaoka H, Dills RL, Mizukami S, Tanabe K, Takeuchi N, et al. Identification and quantification of 1-nitropyrene metabolites in human urine as a proposed biomarker for exposure to diesel exhaust. Chem Res Toxicol 2007; 20(7): 999-1007.*

[5]*Scheepers PTJ, Fijneman PHS, Beenakkers MFM, de Lepper AJGM, Thuis HJTM, Stevens D, et al. Immunochemical detection of metabolites of parent and nitro polycyclic aromatic hydrocarbons in urine samples from persons occupationally exposed to diesel exhaust. Fresenius J Anal Chem 1995; 351(7): 660-669.*

[6]*Laumbach R, Tong J, Zhang L, Ohman-Strickland P, Stern A, Fiedler N et al. Quantification of 1-aminopyrene in human urine after a controlled exposure to diesel exhaust. J Environ Monitor 2008; 11(1): 153-159.*

[7]*Huyck S, Ohman-Strickland P, Zhang L, Tong J, Xu X, Zhang J. Determining times to maximum urine excretion of 1-aminopyrene after diesel exhaust exposure. J Exp Sci Environ Epi 2010; 20(7): 650-655.*

Study Purpose and Specific Aims

Compare 1-NP in personal air samples with its urinary metabolites in same individual and assess ability to detect non-occupational diesel exposure concentrations at the US-Mexico border.

1) Comparison of urinary concentrations between border commuters and non-border commuters

2) Multilevel linear regression model to assess association between personal exposure to 1-NP and urinary metabolites

Eligibility Criteria

- ≥ 18 yrs of age
- Non-smokers in a non-smoking home
- Free of any chronic conditions
- Not occupationally exposed to DE
- IRB consent
- Border Commuters: Cross the border 2x a week or more as a pedestrian
- Non-Border Commuters: Did not cross any border crossings in the prior 4 months

Samples Collected

24-hr personal samples	
1-NP on a $PM_{2.5}$ filter and RH/Temp	Subset
Questionnaire	All
24-hr Time Activity Diary	All
Spot Urine Sample	All

Challenges

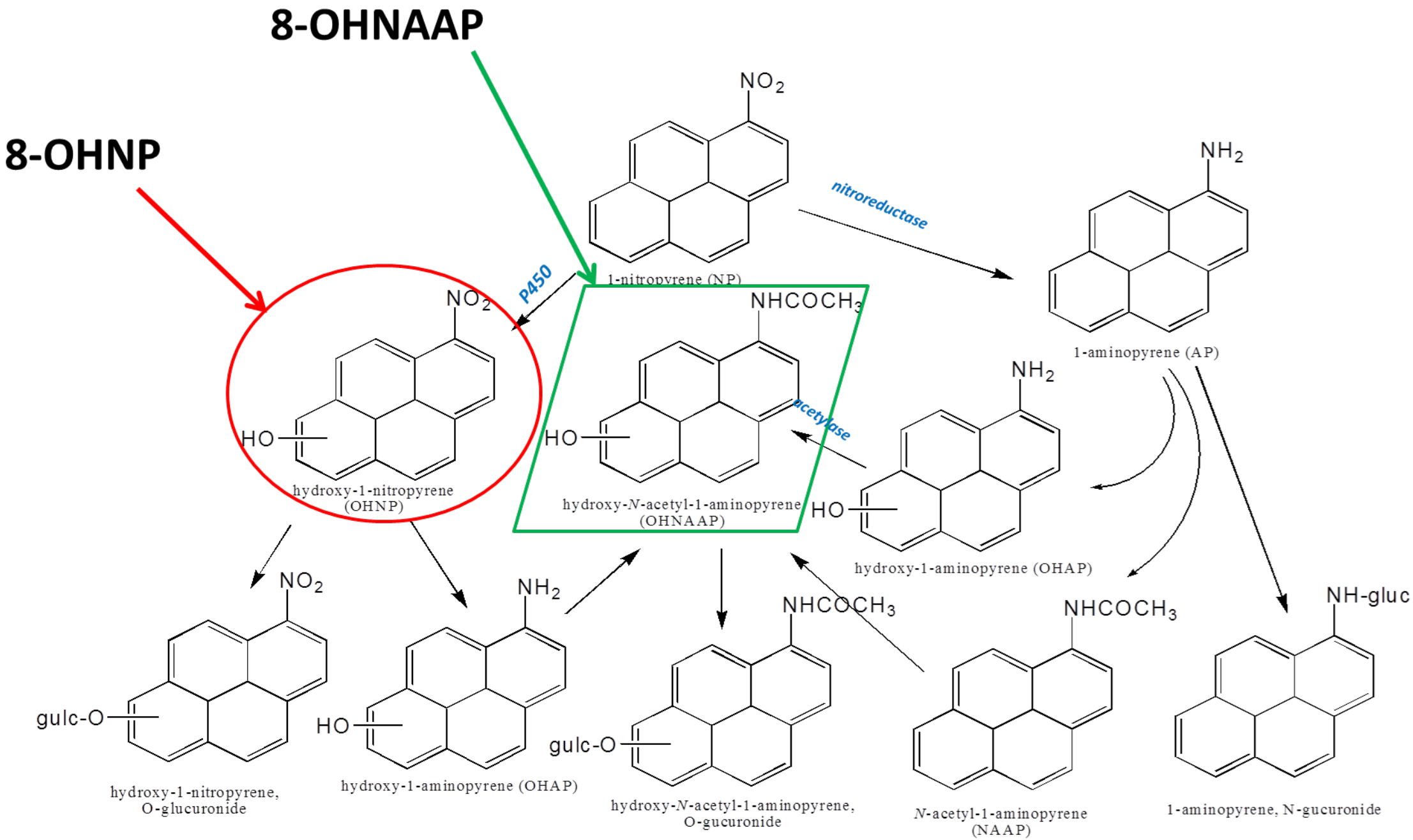
- High Security Area
- International Border
- Concerns for Participant Safety
- Timing of getting urine samples

Participants and Sampling Events

- Repeat participations, 73 border commuter sampling events and 18 non-border commuter sampling events
- Criterion that three weeks had passed from their last participation
- 27 border commuters and 17 non-border commuters total
- All border commuters who participated lived in Tijuana
- All non-commuters who participated lived in South San Diego
- All participants self-classified as Hispanic
- Reported mean northbound vehicle delay time (an indicator of the amount of idling traffic near pedestrian pathway) was 83min (range 32-137min)
- Border Commuters spent an average of 60min waiting in the northbound pedestrian lane (range 20-200min)

1-NP and Urine Extraction and Analysis

- Extracts quantified using 2D-HPLC MS/MS
- LOQ: (blank+2SD)/√2
- Urine: creatinine measured to adjust for excretion rate
  - in a subset of participants
  - not known if needed for these metabolites
  - measured by the University of Washington Hospital's clinical laboratory using a colorimetric assay


8-OHNAAP
8-OHNP
NO2
1-nitropyrene (NP)
nitroreductase
NH2
1-aminopyrene (AP)
P450
NO2
HO
hydroxy-1-nitropyrene
(OHNP)
NHCOCH3
HO
hydroxy-N-acetyl-1-aminopyrene
(OHNAAP)
acetylase
NH2
HO
hydroxy-1-aminopyrene (OHAP)
NO2
gulc-O
hydroxy-1-nitropyrene,
O-glucuronide
NH2
HO
hydroxy-1-aminopyrene (OHAP)
NHCOCH3
gulc-O
hydroxy-N-acetyl-1-aminopyrene,
O-gucuronide
NHCOCH3
N-acetyl-1-aminopyrene
(NAAP)
NH-gluc
1-aminopyrene, N-gucuronide

Results: Comparison of urinary concentrations between border commuters and non-border commuters

		n[a]	GM (GSD)	Range	P-value[b]	n (% < LOQ)[c]
8-OHNP						
Unadjusted (pg/mL)	Border commuter events	68	0.058 (0.059)	0.011 – 0.37	**< 0.01**	1 (1.5)
	Non-border commuter events	18	0.027 (0.020)	0.011 – 0.088		2 (11)
Creatinine Adjusted (pg/mg creatinine)	Border commuter events	50	0.057 (0.098)	0.010 – 0.71	0.07	1 (2)
	Non-border commuter events	17	0.035 (0.029)	0.016 – 0.13		2 (12)
8-OHNAAP						
Unadjusted (pg/mL)	Border commuter events	68	0.032 (0.095)	0.014 – 0.56	0.07	37 (54)
	Non-border commuter events	18	0.019 (0.013)	0.014 – 0.060		12 (66)
Creatinine Adjusted (pg/mg creatinine)	Border commuter events	50	0.035 (0.078)	0.0047 – 0.57	0.15	26 (52)
	Non-border commuter events	17	0.026 (0.024)	0.087 – 0.10		11 (67)
8-OHNP + 8-OHNAAP						
Unadjusted (pg/mL)	Border commuter events	68	0.11 (0.11)	0.025 – 0.66	**< 0.01**	1 (1.5)
	Non-border commuter events	18	0.048 (0.025)	0.025 – 0.10		2 (11)
Creatinine Adjusted (pg/mg creatinine)	Border commuter events	50	0.11 (0.12)	0.024 – 0.76	**0.024**	1 (2)
	Non-border commuter events	17	0.063 (0.047)	0.031 – 0.23		2 (12)

Abbreviations: GM, geometric mean; GSD, geometric standard deviation; LOQ, limit of quantification; 8-OHNP, 8-hydroxy-1-nitropyrene; 8-OHNAAP, 8-hydroxy-N-acetyl-1-aminopyrene. [a]Number of participant events. [b]Bold indicates value was P<0.05. [c]n<LOQ, number of urine samples below LOQ of 0.011 pg/mL for 8-OHNP and 0.014 pg/mL for 8-OHNAAP. Samples below and above LOQ were included in the analysis

Results: Multilevel linear regression model to assess association between personal exposure to 1-NP and urinary metabolites

$$\text{Ln(Personal 1-NP)}_{ij} = \beta_0 + \beta_1 \text{Ln(metabolite)}_{ij} + \mu_i + e_{ij}$$

Where:

- $_i$ represents border commuters and non-border commuters
- $_j$ represents a specific urine and filter sample pair
- β_0 is the intercept parameter
- β_1 is the slope estimate for the corresponding predictor variable (8-OHNP, 8-OHNAAP or 8-OHNP+8-OHNAAP)
- $\mu_i + e_{ij}$ is the random part of the model with the following distributions: $\mu_i \sim N(0,\gamma^2)$, $e_{ij} \sim N(0,\sigma^2)$

Results for unadjusted metabolites above and below LOQ

- 14% increase in 1-NP for each 10% increase in 8-OHNP (P=0.3)
- 20% increase in 1-NP for each 10% increase in 8-OHNAAP (P=0.02)
- 16% increase in 1*NP for each 10% increase in summed metabolites (P=0.01)
- Effect estimates similar for creatinine-adjusted models
- Effect estimates were modestly attenuated when data below LOQ were excluded

Conclusions

- Border Commuters had higher urinary concentrations of 1-NP metabolites as compared to Non-border commuters
- Detect differences in 1-NP and its urinary metabolites in a lower exposure range than has been previously demonstrated (sum of metabolites most robust)
- Higher urinary 1-NP metabolite concentrations were associated with higher personal 1-NP exposures. However, the regression models explained only a small proportion of the variability between 1-NP measured in the prior 24-hours and the urinary metabolites

Published in: Galaviz VE, Quintana PJ, Yost MG, Sheppard L, Paulsen MH, Camp JE, Simpson CD. Urinary metabolites of 1-nitropyrene in US-Mexico border residents who frequently cross the San Ysidro Port of Entry. J Expo Sci Environ Epidemiol. 2015 Dec 16.

Questions?
¿Preguntas?