Biomarkers of Effect in Air Pollution Intervention Studies: Study Design Issues

Maggie L. Clark

Associate Professor, Environmental Epidemiology

Colorado State University

Disclosure

- I have no actual or potential conflict of interest to declare

Outline

CHOOSING APPROPRIATE BIOMARKERS - GENERAL

HAPIN STUDY EXAMPLE

STUDY DESIGN CONSIDERATIONS

Choosing appropriate biomarkers

- For biomarkers of effect, how informative is the marker regarding disease prediction (often insight into mechanism is secondary)?
- How stable is the marker (i.e., low within-person variability) as it relates to the hypothesized time course of the modifiable effect (i.e., ability to observe changes in the marker over the relevant time period)?
- Is it feasible to measure the marker in the relevant setting?

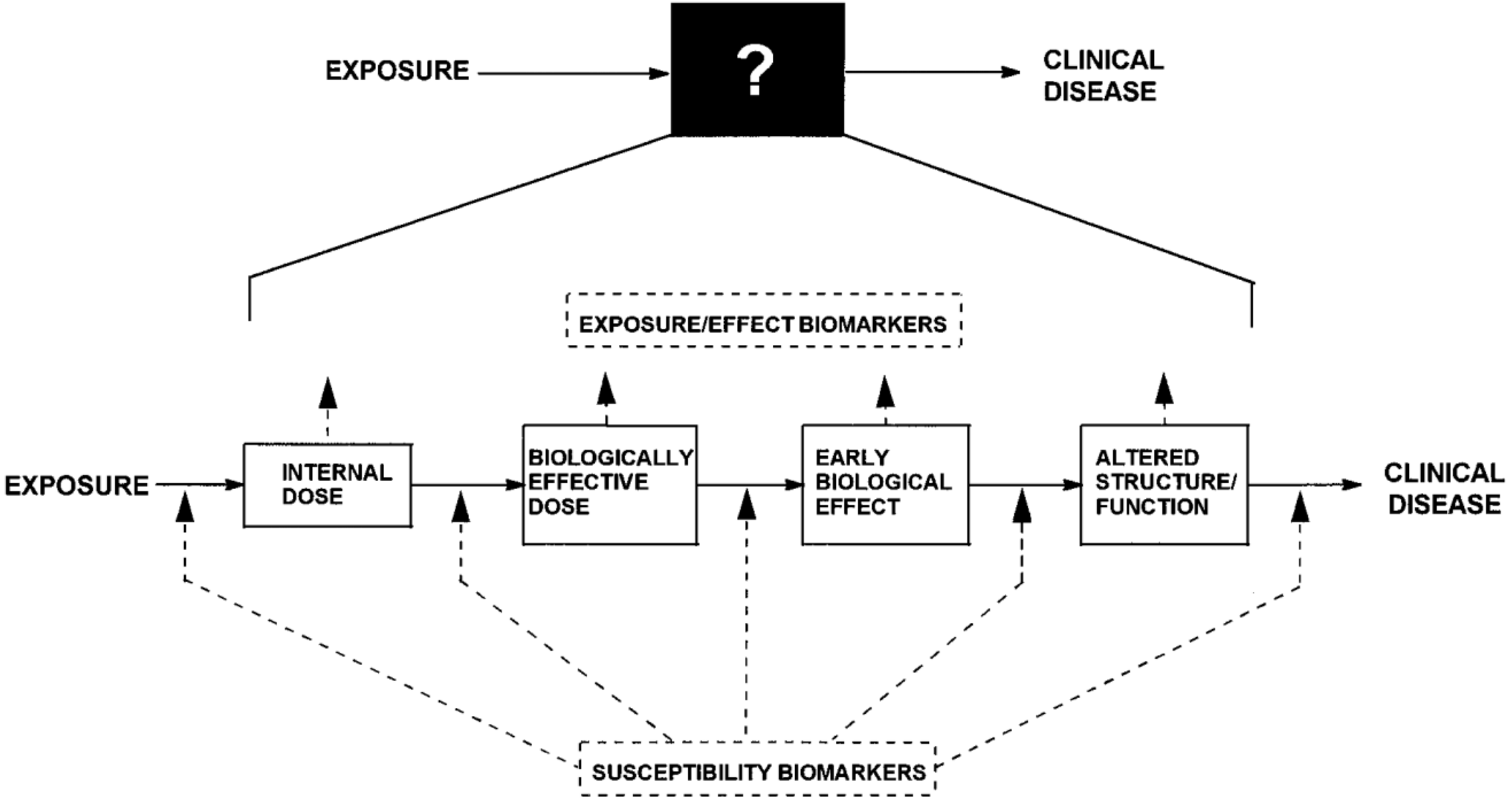


Figure. Schematic depicting the mechanism linking exposure to disease and multiple options to consider when choosing which biomarkers along the continuum to measure. Schematic from *DeCaprio (Environ Sci Technol) 1997.*

Household Air Pollution Intervention Network (HAPIN)

HAPIN

Publications: Study design

Research

A Section 508–conformant HTML version of this article is available at https://doi.org/10.1289/EHP6407.

Design and Rationale of the HAPIN Study: A Multicountry Randomized Controlled Trial to Assess the Effect of Liquefied Petroleum Gas Stove and Continuous Fuel Distribution

Thomas Clasen,[1] William Checkley,[2] Jennifer L. Peel,[3] Kalpana Balakrishnan,[4] John P. McCracken,[5] Ghislaine Rosa,[6] Lisa M. Thompson,[7] Dana Boyd Barr,[1] Maggie L. Clark,[3] Michael A. Johnson,[8] Lance A. Waller,[9] Lindsay M. Jaacks,[10] Kyle Steenland,[1] J. Jaime Miranda,[11] Howard H. Chang,[9] Dong-Yun Kim,[12] Eric D. McCollum,[13] Victor G. Davila-Roman,[14] Aris Papageorghiou,[15] Joshua P. Rosenthal,[16] and HAPIN Investigators

[1]Department of Environmental Health, Rollins School of Public
[2]Division of Pulmonary and Critical Care, School of Medicine,
[3]Department of Environmental and Radiological Health Scienc
[4]Department of Environmental Health Engineering, ICMR Cen
Higher Education and Research (Deemed University), Chennai,
[5]Center for Health Studies, Universidad del Valle de Guatemal
[6]Department of Disease Control, Faculty of Infections and Trop
[7]Nell Hodgson Woodruff School of Nursing, Emory University
[8]Berkeley Air Monitoring Group, Berkeley, California, USA

Research

A Section 508–conformant HTML version of this article is available at https://doi.org/10.1289/EHP6422.

Air Pollutant Exposure and Stove Use Assessment Methods for the Household Air Pollution Intervention Network (HAPIN) Trial

Michael A. Johnson,[1] Kyle Steenland,[2] Ricardo Piedrahita,[1] Maggie L. Clark,[3] Ajay Pillarisetti,[2] Kalpana Balakrishnan,[4] Jennifer L. Peel,[3] Luke P. Naeher,[5] Jiawen Liao,[2] Daniel Wilson,[6] Jeremy Sarnat,[2] Lindsay J. Underhill,[7] Vanessa Burrowes,[7] John P. McCracken,[8] Ghislaine Rosa,[9] Joshua Rosenthal,[10] Sankar Sambandam,[4] Oscar de Leon,[7] Miles A. Kirby,[2] Katherine Kearns,[4] William Checkley,[7] Thomas Clasen,[2] and HAPIN Investigators

[1]Berkeley Air Monitoring Group, Berkeley, California, USA
[2]Gangarosa Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, Georgia, USA
[3]Department of Environmental and Radiological Health Sci
[4]Department of Environmental Health Engineering, ICMR (
Higher Education and Research (Deemed University), Chen
[5]Department of Environmental Health Science, College of F
[6]Geocene, Vallejo, California, USA
[7]Division of Pulmonary and Critical Care, School of Medici
[8]Center for Health Studies, Universidad del Valle de Guater
[9]Department of Infectious and Tropical Diseases, London S

Research

A Section 508–conformant HTML version of this article is available at https://doi.org/10.1289/EHP5751.

Design and Rationale of the Biomarker Center of the Household Air Pollution Intervention Network (HAPIN) Trial

Dana Boyd Barr,[1] Naveen Puttaswamy,[2] Lindsay M. Jaacks,[3] Kyle Steenland,[1] Sarah Rajkumar,[2] Savannah Gupton,[1] P. Barry Ryan,[1] Kalpana Balakrishnan,[2] Jennifer L. Peel,[4] William Checkley,[5,6] Thomas Clasen,[1] and Maggie L. Clark[4] (HAPIN Investigative Team)

[1]Gangarosa Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, Georgia, USA
[2]Department of Environmental Health Engineering, ICMR Center for Advanced Research on Air Quality, Climate and Health, Sri Ramachandra Institute for Higher Education and Research (Deemed University), Chennai, India
[3]Department of Global Health and Population, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA
[4]Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, Colorado, USA
[5]Division of Pulmonary and Critical Care, School of Medicine, Johns Hopkins University, Baltimore, Maryland, USA
[6]Center for Global Non-Communicable Disease Research and Training, School of Medicine, Johns Hopkins University, Baltimore, Maryland, USA

HAPIN

The Intervention

- High-quality, locally available liquified petroleum gas (LPG) stove having at least two burners
- Continuous free supply of LPG fuel for 18 months; no COVID interruptions
- Promotion of safe and exclusive stove use

HAPIN

HAPIN: Specific Aims

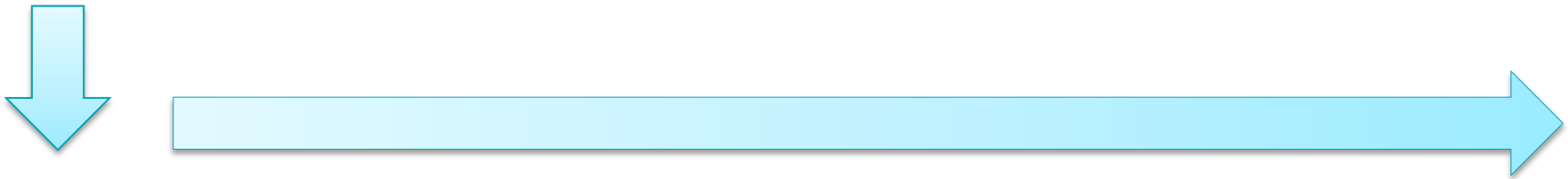
- Determine the effect of a randomized LPG stove and fuel intervention on health in four diverse low- and middle-income country (LMIC) populations.
- Establish an exposure-response curve for all primary and secondary outcomes.
- Determine relationships between LPG intervention and biomarkers of exposure and health.

Study Design: Multi-country Randomized Controlled Trial (RCT)

Pregnant Women (PW)
N=800 Rwanda
N=800 India
N=800 Peru
N=800 Guatemala

Older Adult Women (OAW)
≅ 15% of pregnant women

Index Child (C)
N=800 Rwanda
N=800 India
N=800 Peru
N=800 Guatemala



1. Non-smoking pregnant women screened for eligibility (including ultrasound for gestational age >9 to <20 weeks)
2. Enrollment and baseline assessment
3. Households randomized to intervention or control 1:1 (stratified by country)
4. Followed until child's first birthday (≈18 months)

HAPIN

Table 1. Schedule of exposure and outcome assessment.

	<20 wk gestation (baseline)			24–28 wk gestation (1–3 months postrandomization)			32–36 wk gestation/birth (3–5 months postrandomization)			~3 months old (~9 months postrandomization)			~6 months old (~12 months postrandomization)			~9 months old (~15 months postrandomization)			~12 months old (~18 months postrandomization)		
Child age (study time point)	PW	C	O	PW	C	O	PW	C	O	NM	C	O	NM	C	O	NM	C	O	NM	C	O
Personal exposure																					
24-h $PM_{2.5}$, CO, BC	X		X	X		X	X		X	X	X	X	X	X	X				X	X	X
Urinary biomarkers	X		X	X		X	X		X		X	X		X	X					X	X
Primary outcomes																					
Birth weight								X[a]													
Severe pneumonia[b]																					
Stunting																				X	
Blood pressure			X			X			X			X			X						X
Secondary outcomes																					
Maternal blood pressure	X			X			X			X			X						X		
Fetal growth		X			X			X													
Child linear growth (continuous)								X[a]			X			X			X			X	
Preterm birth								X[a]													
Child development											X			X			X			X	
WHO severe pneumonia[a]																					
BART			X																		X
CIMT			X																		X
SGRQ			X																		X
SF-36			X																		X
Expenditures/time use	X		X																		X
Chronic disease biomarkers			X			X			X			X			X						X
Metabolomics/microRNA[c]														X	X						
Hemoglobin (anemia)	X			X			X							X						X[a]	
Covariates																					
Sociodemographics	X		X																		X
Clinical history	X		X	X		X	X		X	X	X	X		X	X		X	X		X	X
Weight/height/BMI	X		X	X		X	X		X			X			X						X
Diet/food security	X		X																X		X
IYCF											X			X			X			X	
Biospecimens collected																					
Urine	X		X	X		X	X		X		X	X		X	X					X	X
Dried blood spots	X		X	X		X	X	X[a]	X		X	X		X	X					X	X

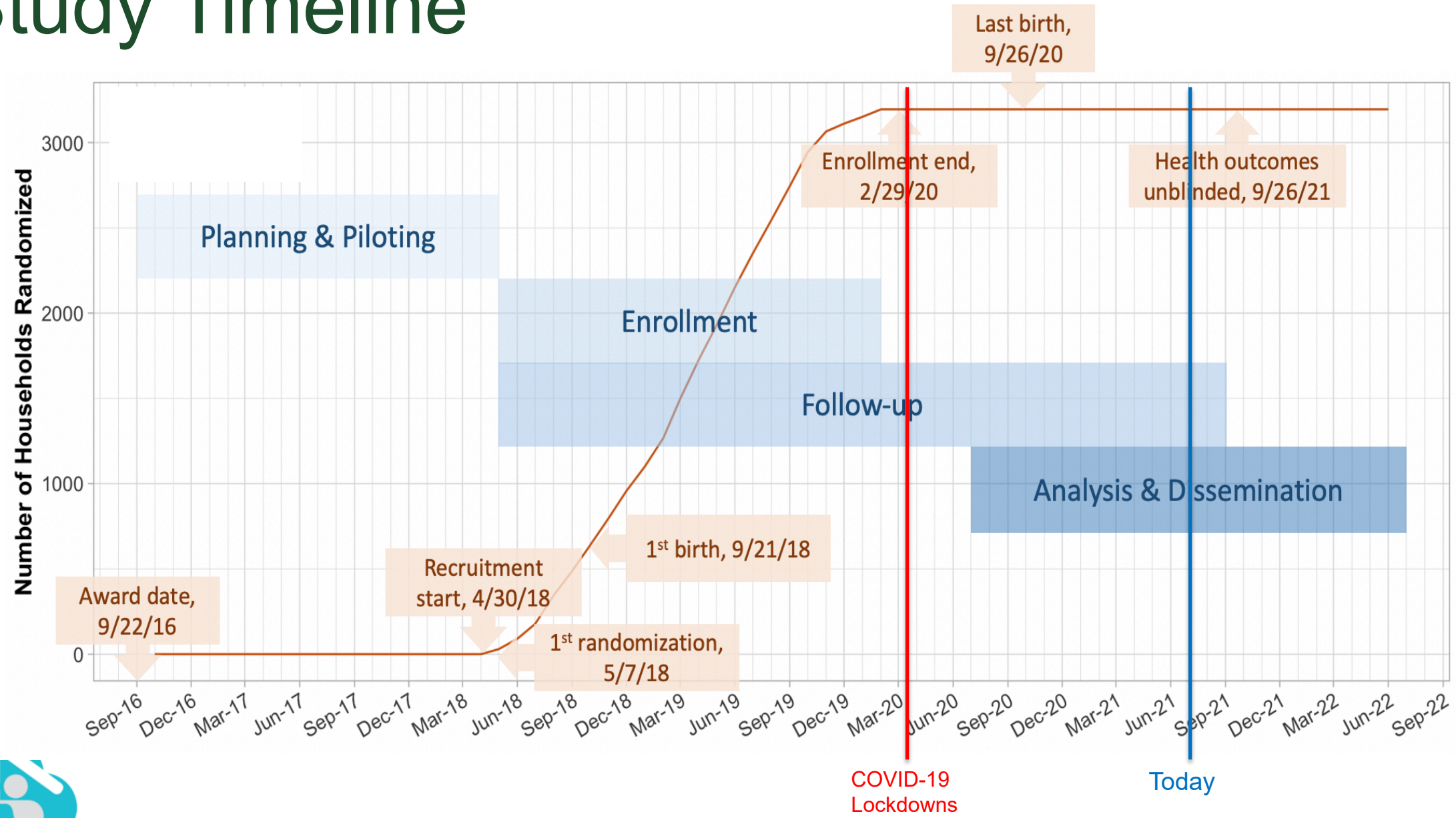
Note: BART, brachial artery reactivity testing; BC, black carbon; BMI, body mass index; C, child; CIMT, carotid intima-media thickness; CO, carbon monoxide; IYCF, infant and young child feeding practices; NM, new mother; O, older adult woman in household; $PM_{2.5}$, fine particulate matter with aerodynamic diameter ≤2.5 μm; PW, pregnant woman; SF-36, Short Form 36 survey; SGRQ, St. George Respiratory Questionnaire.

[a]Measured at birth.

[b]Recorded whenever children present to HAPIN health facilities with respiratory symptoms.

[c]Metabolomics/microRNA biomarker discovery in 100/site for the older adult woman and child.

Study Timeline



HAPIN

HAPIN Team

EMORY
ROLLINS SCHOOL OF PUBLIC HEALTH

Colorado State University

JOHNS HOPKINS UNIVERSITY

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

1966
UNIVERSIDAD DEL VALLE DE GUATEMALA

PRISMA

EAGLE RESEARCH CENTER

SRI RAMACHANDRA
INSTITUTE OF HIGHER EDUCATION AND RESEARCH
(Category - I Deemed to be University) Porur, Chennai

BERKELEY AIR MONITORING GROUP

1785
The University of Georgia

HARVARD T.H. CHAN
SCHOOL OF PUBLIC HEALTH
Department of Global Health and Population

THE GLOBAL LPG PARTNERSHIP

SPIRITUS UBI VULT SPIRAT
UNIVERSIDAD PERUANA CAYETANO HEREDIA

WASHINGTON UNIVERSITY IN ST. LOUIS
Per Veritatem Vis
1853

Biomarker Core: Objectives

To provide high-capacity, high-quality, and high-throughput analysis of a wide range of biomarkers in samples collected from each participant group (pregnant woman, older adult woman, child)

- To provide training and monitoring compliance of sample collection, handling, and storage including developing collection and aliquoting protocols, ensuring sample integrity throughout the process and developing short- and long-term archival system
- To develop local laboratory capacity and harmonize biomarker measures across study locations
- **To identify, prioritize, and measure specific biomarkers of household air pollution (HAP) exposure and effect in urine (first morning void) and dried blood spots (DBS)**
- To develop and validate novel biomarkers that will provide insight into the broader mechanistic questions linking HAP exposure to disease development

HAPIN




- The HAPIN field teams are in diverse settings across 4 countries
- The Biomarker Core is supported by two analytical biomarker laboratories:
  - Laboratory for Exposure Analysis and Development in Environmental Research (LEADER) at Rollins School of Public Health, Emory University, Atlanta, USA
  - Laboratory at Sri Ramachandra Institute of Higher Education and Research (SRIHER), Chennai, India

HAPIN

Biomarker Collection Design

Table 1. Biosample collection timeline.

Child age (study time point)	<20 weeks gestation (baseline)			24–28 weeks gestation (1–3 months post-randomization)			32–36 weeks gestation/birth (3–5 months post-randomization)			~3–7 months of age (~9 months post-randomization)			~6 months of age (~12 months post-randomization)			~12 months of age (~18 months post-randomization)		
	PW	C	OAW	PW	C	OAW	PW	C	OAW	NM	C	OAW	NM	C	OAW	NM	C	OAW
Urine	X	—	X	X	—	X	X	—	X	—	X	X	—	X	X	—	X	X
Dried blood spots	X	—	X	X	—	X	X	X birth	X	—	X	X	—	X	X	—	X	X
Oral rinse[a]	—	—	X	—	—	—	—	—	—	—	—	—	—	—	X	—	—	—
Nasal turbinate sample[a]	—	—	X	—	—	—	—	—	—	—	—	—	—	—	X	—	—	—
Buccal scrape[a]	—	—	X	—	—	—	—	—	—	—	—	—	—	—	X	—	—	—
Venous blood (RBC, BC and plasma)[a]	—	—	X	—	—	—	—	—	—	—	—	—	—	—	X	—	—	—

Note: —, not applicable; BC, buffy coat; C, child; NM, new mother; OAW, older adult woman; PW, pregnant woman; RBC, red blood cells.
[a]Peru and Guatemala international research collaborating sites only.

HAPIN

”Working objective” for biomarker selection:

- Previous links to air pollution/HAP exposure
- Biomarkers of effect:
  - Capture pathways involving cardiovascular, metabolic, cancer, and respiratory effects
  - Major chronic diseases share common mechanisms (e.g., inflammation and oxidative stress)
- Biomarkers of exposure:
  - PAH metabolites
  - VOCs
  - Levoglucosan
  - Cotinine

Biomarker Prioritization Scheme

- Over 55,000 samples!
- Not logistically feasible to measure each analyte in every sample
- Relate health outcomes to participant subset (e.g., cardiovascular markers in older adult women); exposure biomarkers in all participants
- Working literature review (prioritize one analyte per "pathway")
- Validation results (confidence in DBS, intraclass correlation coefficient [ICC])
- Cross-sectional analysis of biomarkers with 24-hr $PM_{2.5}$ concentrations from baseline (inform intention-to-treat [ITT] and longitudinal exposure-response analyses)

Biomarker	Mother	OAW	Child
Cardiovascular/endothelial markers		X	
Oxidative stress markers		X	
Lipids		X	
HbA1c		X	
Hb	X		X
Exposure biomarkers	X	X	X
Inflammation markers		X	X
Metabolome (subset)	X	X	X
Microbiome (subset)		X	
miRNA/mRNA (subset)		X	
DNA methylation (subset)		X	

OAW=older adult woman; HbA1c=hemoglobin A1c; Hb=hemoglobin

HAPIN

Biomarker Prioritization Scheme – *Child example*

			Child age: 3-month	**Child age: 6-month**	**Child age: 12-month**
Sample	**Aliquot**	**Birth**	**Post-birth 1**	**Post-birth 2**	**Post-birth 4**
Urine	1 (3ml)		Exposure 1 (PAHs: 1.5ml)	Exposure 1	Exposure 1
	2 (3ml)		BioLINCC	BioLINCC	BioLINCC
	3 (7.5ml)		Exposure 2 (tobacco, levoglucosan, 8-OHdG, VOCs: 2ml)	Exposure 2	Exposure 2
	4 (7.5ml)				
DBS	Spot 1	*Telomere length (?)*			COVID-19 antibodies
	Spot 2	*Telomere length (?)*		CRP (*proposed*)	CRP (*proposed*)
	Spot 3			100/country: "*discovery*"	Ancillary: Kirby (Malaria), Rwanda, n=800
	Spot 4				
	Spot 5	BioLINCC	BioLINCC	BioLINCC	BioLINCC
Capillary blood	Hb			Sinharoy (*ancillary*)	Sinharoy (*ancillary*)

Biomarker Prioritization Scheme – *Older adult woman example*

			Gestation: 24-28 weeks	**Gestation: 32-36 weeks**	**Child age: 3-month**	**Child age: 6-month**	**Child age: 12-month**
Sample	**Aliquot**	**Baseline**	**Pregnancy 1**	**Pregnancy 2**	**Post-birth 1**	**Post-birth 2**	**Post-birth 4**
Urine	1 (3ml)	Exposure 1	Exposure 1	Exposure 1	Exposure 1	Exposure 1	Exposure 1
	2 (3ml)	BioLINCC	BioLINCC	BioLINCC	BioLINCC	BioLINCC	BioLINCC
	3 (7.5ml)	Exposure 2	Exposure 2	Exposure 2	Exposure 2	Exposure 2	Exposure 2
	4 (7.5ml)						
DBS	Spot 1	CRP	*Prioritization*	*Prioritization*	*Prioritization*	CRP	COVID-19 antibodies
	Spot 2	*Prioritization*	*Prioritization*	*Prioritization*	*Prioritization*	*Prioritization*	*Prioritization*
	Spot 3	*Prioritization*	*Prioritization*	*Prioritization*	*Prioritization*	100/country: "*discovery*"	*Prioritization*
	Spot 4	*Prioritization*	*Prioritization*	*Prioritization*	*Prioritization*	*Prioritization*	*Prioritization*
	Spot 5	BioLINCC	BioLINCC	BioLINCC	BioLINCC	BioLINCC	BioLINCC
Capillary blood	HbA1c	POC (point-of-care)	POC	POC	POC	POC	POC

Study Design Considerations

- Comparison groups
  - What we really want to know: *the frequency of the "outcome" in a group of exposed people vs. the frequency of the "outcome" in the same group of people if they were not exposed*
  - Reality (study design)
- "Speed" of the exposure-response mechanism being evaluated
  - Reasonable expectation that the biomarker can change within the time-period of the study
- Anticipated variability in the biomarker measures
  - Between- and within-person variability (informs sample size and number of repeated measures necessary)
  - Diurnal variability (needs to be incorporated in the study design)
- Susceptible populations

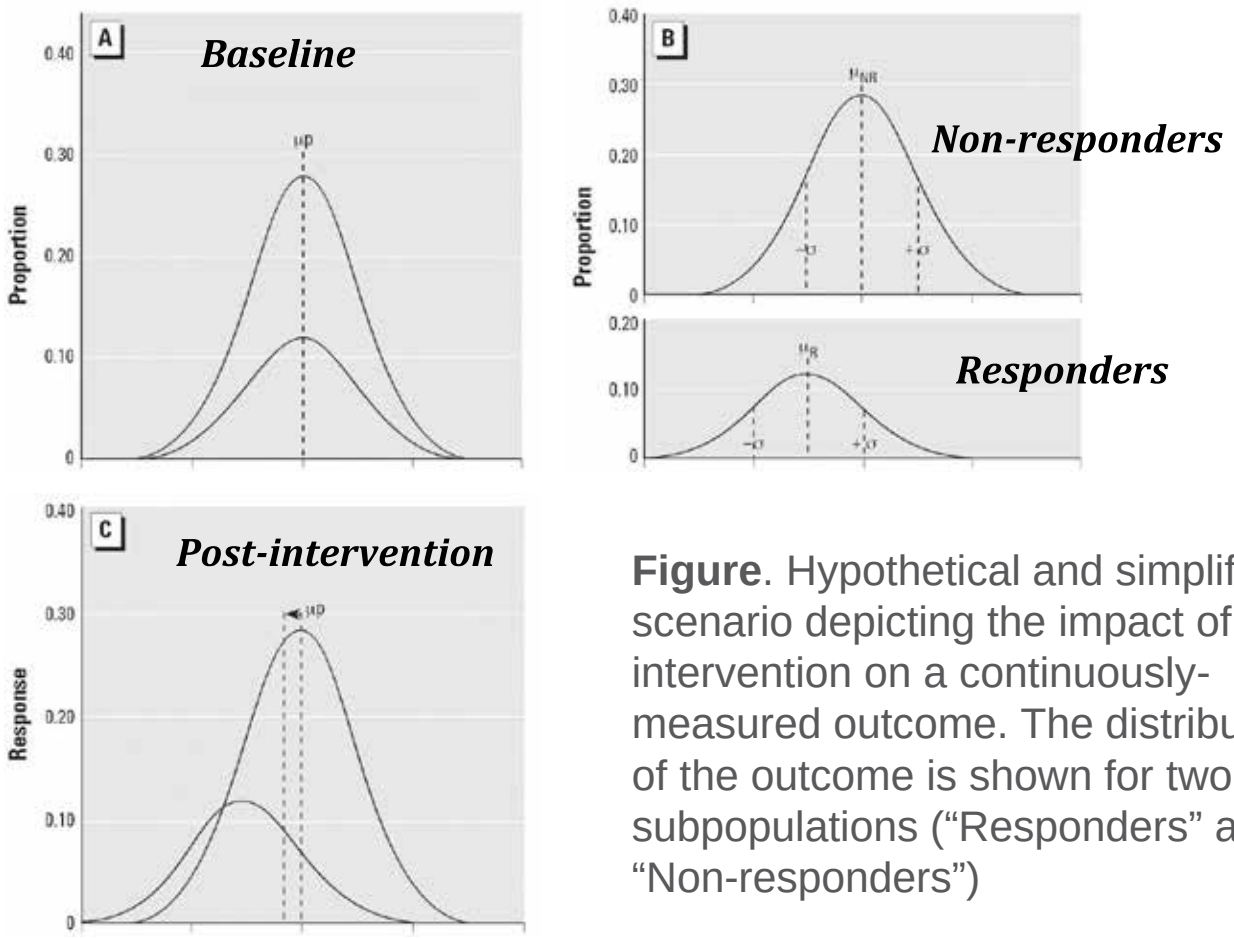


Figure. Hypothetical and simplified scenario depicting the impact of an intervention on a continuously-measured outcome. The distribution of the outcome is shown for two subpopulations ("Responders" and "Non-responders")

Clark and Peel (Curr Environ Health Rpt) 2014

References

- Barr D, Puttaswamy N, Jaacks L, Steenland K, Rajkumar S, Gupton S, Ryan P, Balakrishnan K, Peel J, Checkley W, Clasen T and Clark M (2020) *Design and Rationale of the Biomarker Center of the Household Air Pollution Intervention Network (HAPIN) Trial*, Environmental Health Perspectives, 128:4, Online publication date: 1-Apr-2020.
- Clark ML, Peel JL (2014) *Perspectives in household air pollution research: who will benefit from interventions? ,* Current Environmental Health Reports, 1:3, 250-257.
- Clasen T, Checkley W, Peel J, Balakrishnan K, McCracken J, Rosa G, Thompson L, Barr D, Clark M, Johnson M, Waller L, Jaacks L, Steenland K, Miranda J, Chang H, Kim D, McCollum E, Davila-Roman V, Papageorghiou A and Rosenthal J (2020) *Design and Rationale of the HAPIN Study: A Multicountry Randomized Controlled Trial to Assess the Effect of Liquefied Petroleum Gas Stove and Continuous Fuel Distribution*, Environmental Health Perspectives, 128:4, Online publication date: 1-Apr-2020.
- DeCaprio AP (1997) *Biomarkers: Coming of Age for Environmental Health and Risk Assessment*, Environmental Science & Technology, 31:7, 1837–1848.
- Johnson M, Steenland K, Piedrahita R, Clark M, Pillarisetti A, Balakrishnan K, Peel J, Naeher L, Liao J, Wilson D, Sarnat J, Underhill L, Burrowes V, McCracken J, Rosa G, Rosenthal J, Sambandam S, de Leon O, Kirby M, Kearns K, Checkley W and Clasen T (2020) *Air Pollutant Exposure and Stove Use Assessment Methods for the Household Air Pollution Intervention Network (HAPIN) Trial*, Environmental Health Perspectives, 128:4, Online publication date: 1-Apr-2020.

Thank you

Colorado State University