

PROTECT

Puerto Rico Testsite for Exploring Contamination Threats

Puerto Rico Testsite for
Exploring Contamination
Threats (PROTECT)
NIEHS SRP P42 Research Program

Co-Directors

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Agenda

1. Welcome & Introductions
2. Program Overview
3. Research Projects and Support
Cores Overview
4. Future Meetings

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Primary Institutions

- Primary Institutions:
 - Northeastern University (NU)
 - UPR, Medical Sciences Campus (UPR-MSC)
 - UPR, Mayaguez (UPRM)
 - University of Michigan (UM)

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PROTECT Team



Tom Ferdi Akram José Raul John Sangchul

Rita Braulio Roger Ingrid Mariah Kristin Michael Dave

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Advisory Committee Members

Welcome to the Scientific Advisory Committee Members

- Alan R. Fleischman, March of Dimes Foundation
- Philip Landrigan, Mount Sinai Medical Center
- Jeff Murray, University of Iowa
- David A. Savitz, Mount Sinai Medical Center

- Malcolm Field, EPA Office Of Research & Development
- Tissa Illangasekare, Colorado School of Mines
- Susan T. Weintraub U. of Texas Health Sciences Center
- William B. White, Penn State

- Mitchell K. Beard, EarthSoft, Inc.
- Carl-Axel P. Soderberg, EPA Caribbean Division

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Advisory Committee Role

- Provide Scientific Advice to the Program
- Attend Annual Program meeting and participate in web conference
- Prepare an annual report with the assistance of the program staff

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Advisory Committee Planned Events

- June 23 Web-conference
 - Objective: Introduce the program to the Scientific Advisory Committee (SAC) and answer questions
- September 21 Meeting in Boston
 - Objective: Program description, evaluation and feedback from SAC
- Retreat in Puerto Rico
 - Jan/Feb 2011
 - Jan/Feb 2012
- Web-conferences in 2011 and 2012
 - dates to be determined

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Program Overview

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Grand Challenge

Preterm Births

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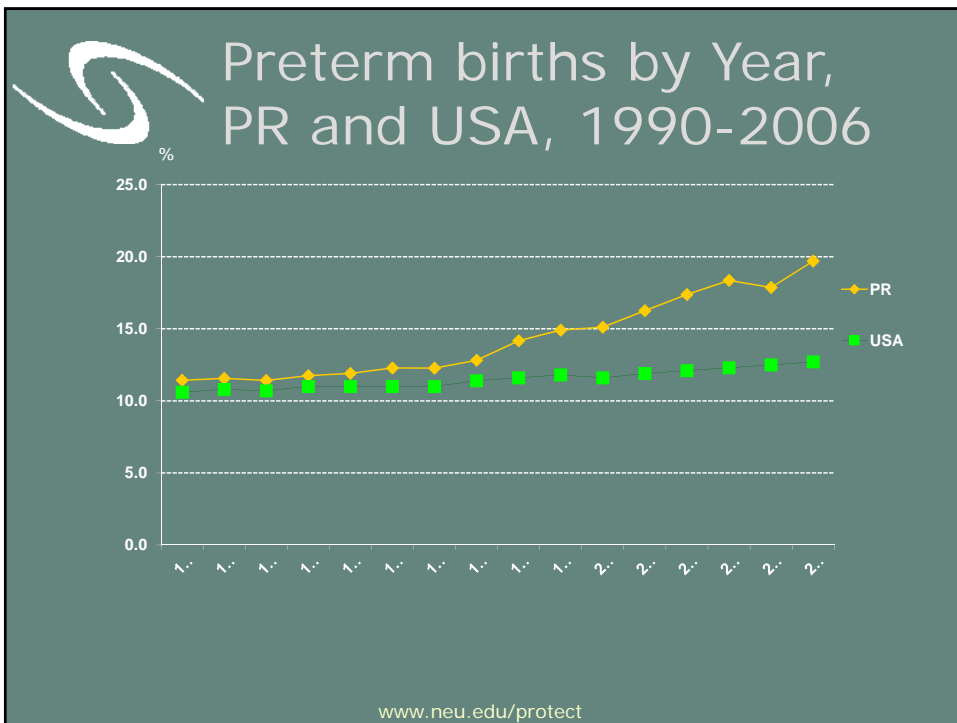
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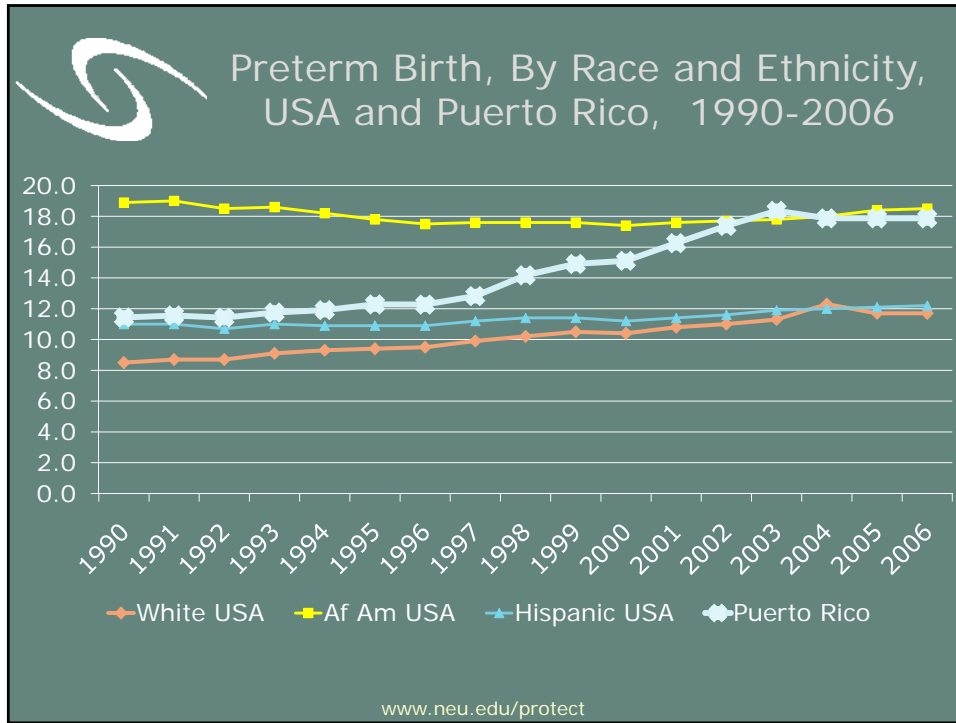


Preterm Births, United States and Puerto Rico

- Babies born before 37 completed weeks of gestation are considered preterm.
- Subgroups of preterm births include:
 - Late preterm (34-36 weeks)
 - Moderately Preterm (32-24 weeks)
 - Very Preterm (< 32 weeks)
- In 2007
 - 12.3% of babies born in the US were preterm
 - 19.9% of babies born in Puerto Rico were preterm
- Preterm birth is the leading cause of neonatal mortality in the US, and the leading cause of infant and neonatal mortality in Puerto Rico
- In the United States preterm births represent a major health and societal expenditure, estimated to be at least \$26 billion a year
- Preterm birth is a risk factor for neonatal complications that can lead to lifelong disability
- Known risk factors for preterm birth include lack of prenatal care, smoking, multiple gestations & Assisted Reproductive Technology (ART), and maternal complications.
- Those risk factors do not explain the marked increase in preterm births in Puerto Rico
- Other risk factors for preterm births, including environmental exposures need to be studied

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Potential Contribution of Environmental Contamination

Several studies¹ have related

<p>Contaminant Exposure to</p> <ul style="list-style-type: none"> • Phthalates • Chlorinated solvents (e.g. TCE) • Pesticides • Heavy Metals 	<p>Adverse Reproductive Outcomes</p> <ul style="list-style-type: none"> • Reproductive damage • Preterm birth • Low birth weight • Perinatal mortality • Spontaneous abortions
---	--

All have been found in groundwater in Puerto Rico

¹ATSDR, 2007; CERHR, 2006; Latini et al., 2003; Ha and Cho 2002; Khattak, 1999; Lipscomb and Fenster, 19991

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Justification

- High concentrations of phthalate metabolites are associated with premature breast development (premature thelarche) in Puerto Rico
- Recent studies support an association between phthalate exposure and decreased gestational age
- Pilot data show statistically significant elevations for most phthalate metabolite concentrations in urines of women who delivered preterm compared with women who delivered at term (Project 3)
- Studies of women exposed occupationally to TCE and other solvents have reported increased risk for spontaneous abortion and low birth weight
- Phthalates and chlorinated solvents (or their metabolites) stimulate cellular oxidative stress, apoptosis, DNA damage and inflammatory responses in nongestational tissues
- Oxidative stress, apoptosis, DNA damage and inflammatory responses in gestational tissues (placenta, extraplacental membranes) are associated with preterm birth and other adverse birth outcomes

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System of Interest

Puerto Rico

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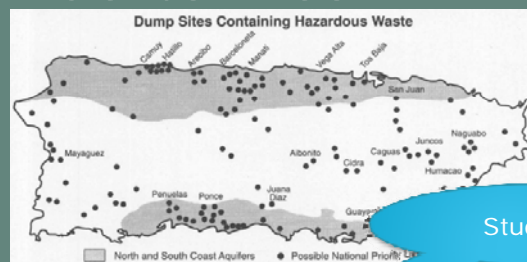
Contamination in Puerto Rico

- 188 Hazardous Waste Sites
- 14 Sites listed on the National Priority List (NPL)
- Many sites include unlined landfills above aquifer in karst geologic formations
- Aquifer is primarily limestone with highly permeable karst aquifers from which most of the wells draw water
- The aquifer discharges to a wetland area that supports a large aquatic and bird population, and primary grazing for dairy farms
- People swim and fish in some of the rivers within the sites and watershed

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Contamination in Puerto Rico



Study Site



Superfund sites in Puerto Rico (14 are on the final NPL list)

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Research Questions

- What is the contribution of environmental contaminants to preterm births in PR?
- How significant is drinking water as a mode of contaminant exposure?
- Can we develop better strategies for detection and green remediation to minimize or prevent exposure to environmental contamination?



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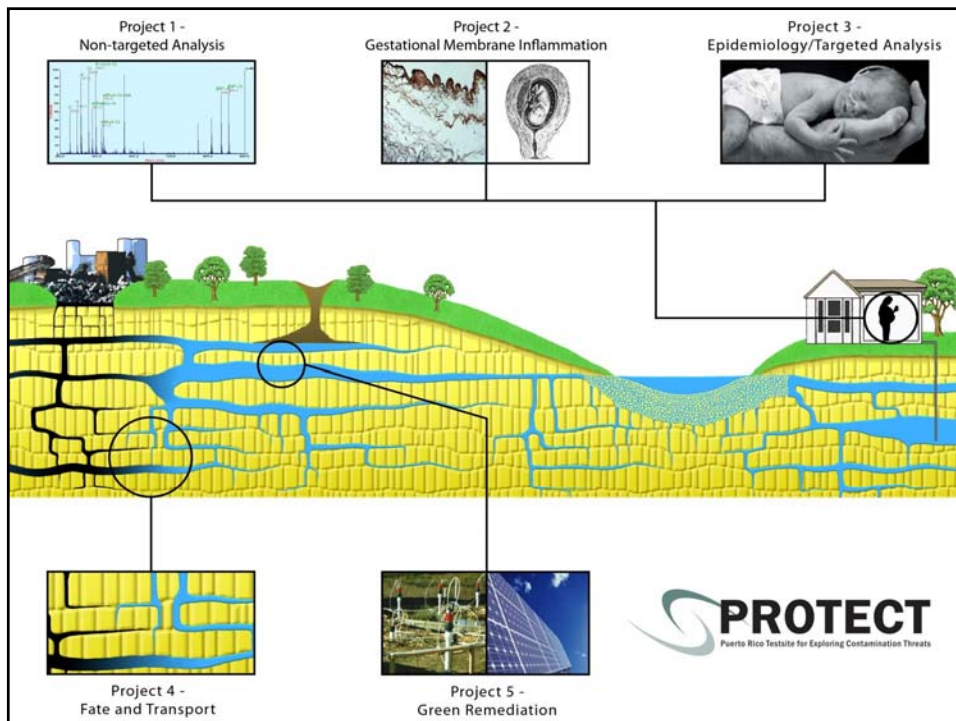
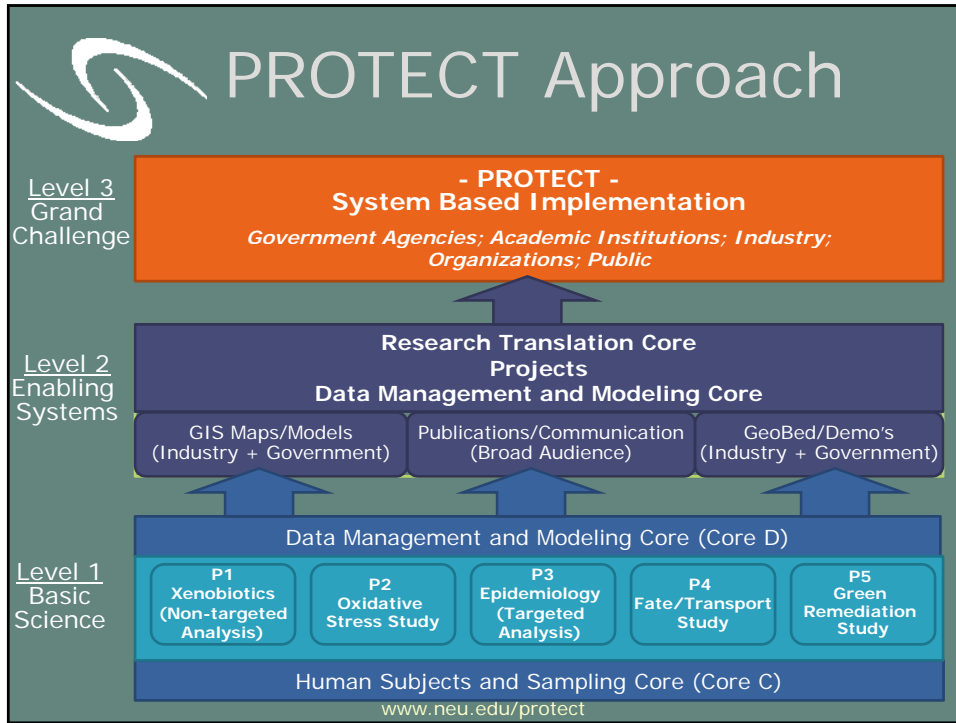
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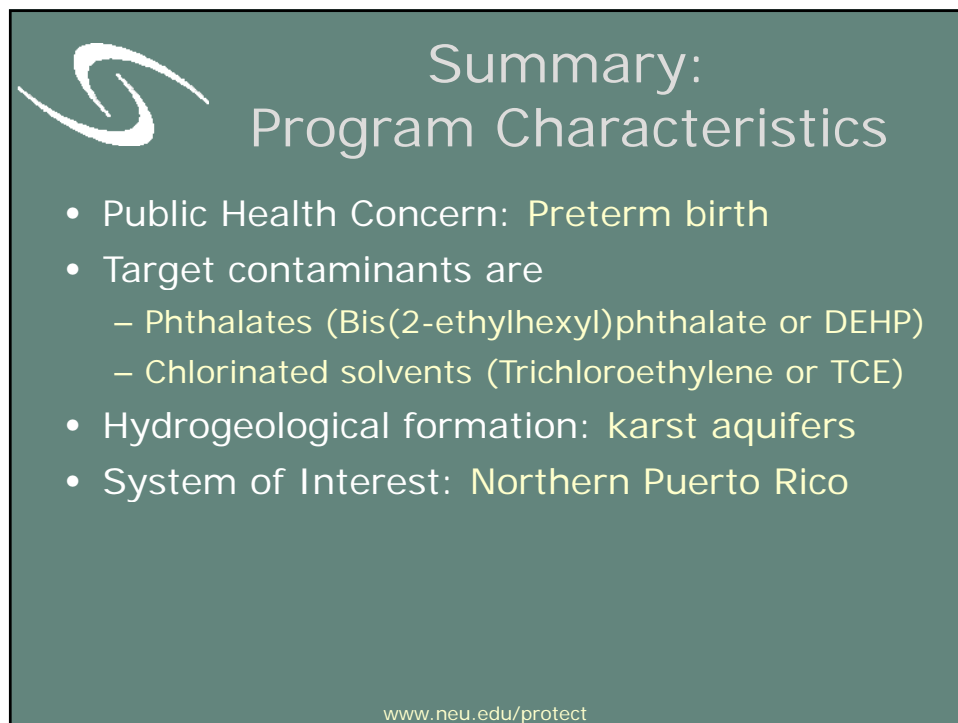
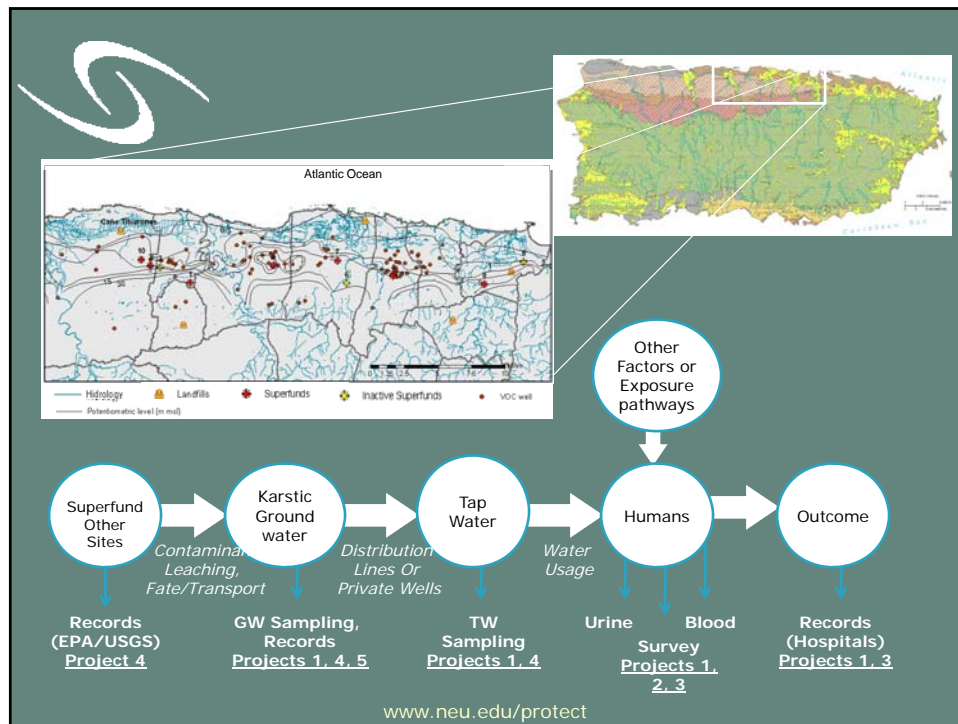



PROTECT Program Theme and Goals

- Theme
 - Puerto Rico as a test site with dynamic exposure pathways through karstic aquifers to study Superfund and related contaminants and their contribution to preterm birth.
- Goals:
 - Define the relationship between exposure to Superfund and related contaminants and preterm birth.
 - Develop new technology for discovery, transport characterization, and green remediation of Superfund and related contaminants in karstic aquifers.

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3. Research Projects and Support Cores

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Research Projects and Support Cores

- Core C: Human Subjects and Sampling Core
- Project 3: Targeted Epidemiologic Study
- Project 1: Non-Targeted Chemical Analysis
- Project 4: Fate and Transport Study
- Project 2: Oxidative Stress Mechanistic study
- Project 5: Groundwater Remediation Mechanistic Study
- Core D: Data Modeling and Management Core

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Core C: Human Subjects and Sampling (HSS) Core

José F. Cordero (UPR), Core Leader
Braulio Jimenez (UPR), Core Co-Investigator
John Meeker (UM), Core Co-Investigator

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Aims

Aim 1: Establish and maintain infrastructure for the recruitment and follow-up of pregnant women

- Developing a single infrastructure to serve biologic projects.
- Data collection focus on the Northern Coast of Puerto Rico

Aim 2: Collect information on demographics, medical, residential, and exposure history of the women.

- Women will be interviewed in the clinic and in their homes by a trained nurse interviewer using standardized questionnaires
- Same questions are used in other large-scale studies to allow us to compare our findings with those from studies such as NHIS, NCS, & NHANES.

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Aim 3: Collect biological samples from the women at 3 times:

1. at recruitment in second trimester (week 20),
 2. at a second prenatal visit early in third trimester (week 28),
 3. at delivery (among a subset of women)
- urine, blood, hair, toenails, cord blood, and placental tissue will be collected

Aim 4: Collect water samples in the study area (groundwater samples w/USGS) and in participants' homes (tap water).

- Add collection of air & dust samples from homes in the future?

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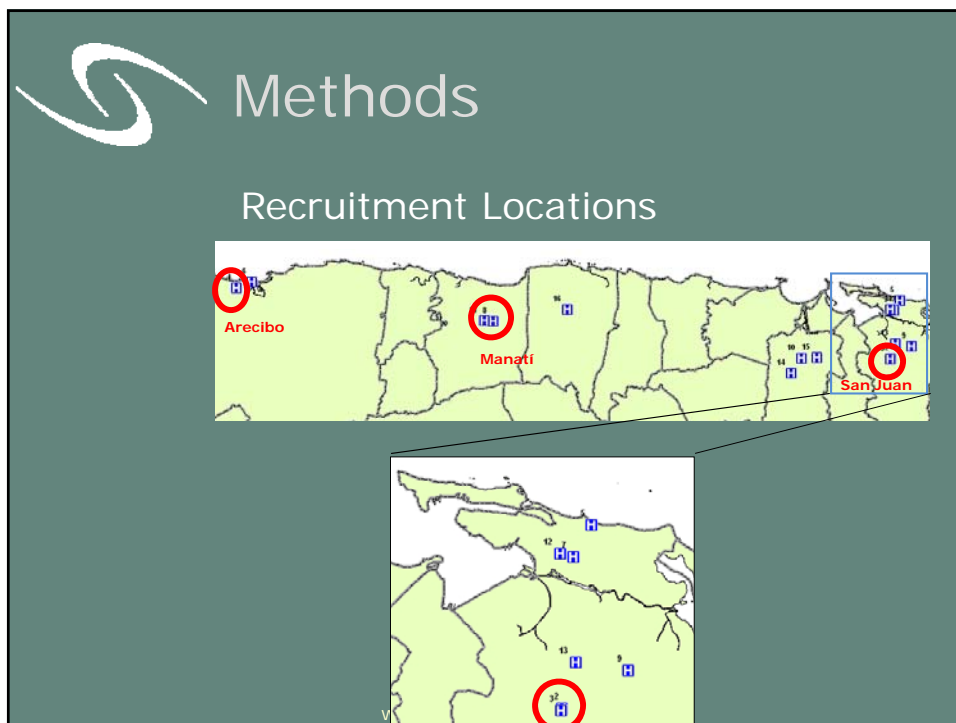
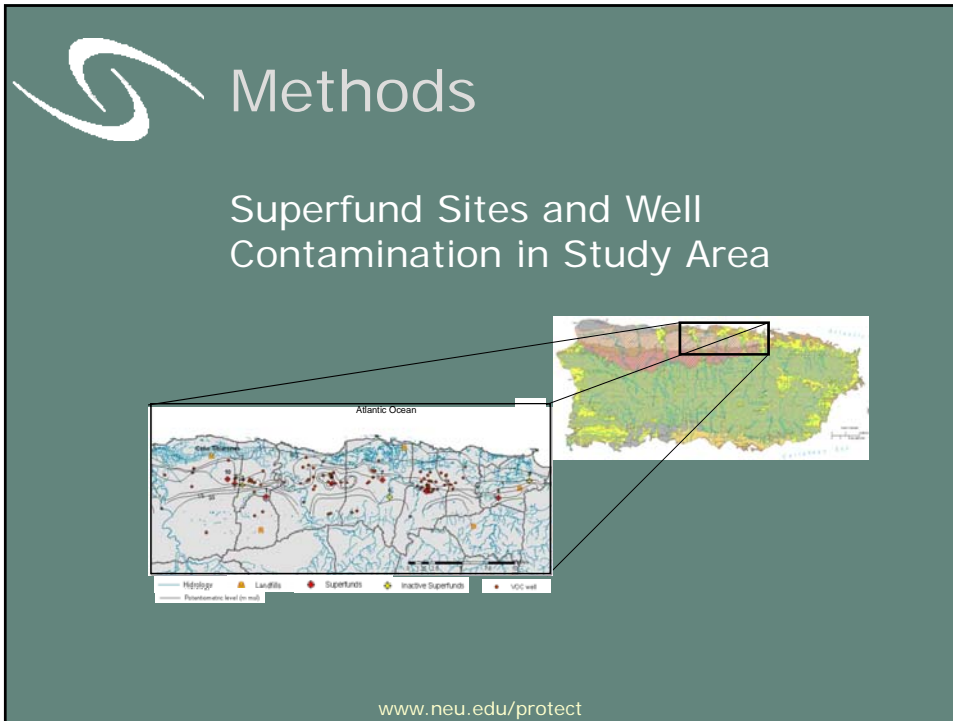
Aim 5: Process the collected samples according to the individual project needs

- e.g. separate sample fractions, pre-treat, aliquot, etc.
- Provide temporary and permanent sample storage
- Package and ship samples and tissues to various investigators and laboratories that are analyzing, utilizing or further archiving the samples /tissues

Aim 6: Provide central data and specimen support for biomedical and non-biomedical projects as needed.

- Core C will work with the Data Management Core that will provide the informatics support and data access to connect all projects

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Methods

Location, Deliveries and Preterm Births at Hospitals Selected for Participant Recruitment

Facility	Municipality	Deliveries (Percent Preterm)		
		2002	2003	2004
Hospital Universitario	San Juan	1589 (46.7)	1726 (46.5)	1687 (41.5)
Hospital Cayetano Coll y Toste	Arecibo	1612 (28.2)	1495 (27.2)	1587 (24.3)
Hospital Doctors Center Inc.	Manatí	1641 (13.7)	1647 (15.5)	1594 (17.0)

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Project 3: Phthalate Exposure and Molecular Epidemiologic Markers of Preterm Birth Among Women in Puerto Rico

John Meeker (UM), Project Leader
 Howard Hu (UM), Co-Investigator
 Bhramar Mukherjee (UM), Co-Investigator
 Antonia Calafat (CDC), Collaborator
 Robert Lyons (UM), Collaborator
 Daniel McConnell (UM), Collaborator

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Pilot Data: Mexico City

Higher Phthalate Metabolites in Preterm Birth Cases vs. Controls: Geometric mean and median 3rd trimester urinary phthalate metabolite concentrations in women with term or preterm births.

Phthalate Metabolite	Geometric Mean		Median (25 th , 75 th percentile)		p-value ^a
	Term (n=30)	Preterm (n=30)	Term (n=30)	Preterm (n=30)	
MBP	38.1	89.9	33.4 (21.3, 74.0)	97.1 (56.0, 139)	0.005
MCPP	1.1	2.4	1.3 (0.5, 2.0)	2.3 (1.1, 4.9)	0.002
MBzP	2.3	5.2	2.9 (1.0, 5.2)	5.4 (2.6, 9.5)	0.01
MEP	112	204	108 (47.1, 224)	171 (69.4, 437)	0.1
MEHP	2.3	3.7	3.0 (0.6, 4.4)	4.3 (2.2, 7.1)	0.06
MEHHP	13.6	24.0	17.1 (6.2, 28.4)	28.7 (18.1, 37.5)	0.04
MEOHP	10.4	18.9	13.6 (5.0, 24.5)	20.8 (14.4, 25.5)	0.04
MECPP	29.7	51.2	38.2 (14.3, 53.8)	55.2 (39.2, 73.3)	0.02
Sum DEHP metab.	57.8	99.3	71.5 (26.8, 113)	112 (69.8, 135)	0.03

Meeker et al. 2009, EHP

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Project 3 Aims

- A prospective cohort study of preterm birth among 900 pregnant women in Puerto Rico
- Will utilize state-of-the-art methods for estimating phthalate exposure and for assessing molecular epidemiologic markers
- Will provide much needed human data on demographic, environmental, and genetic predictors of preterm birth in Puerto Rico.
- The study also aims to identify conditions and activities contributing to high phthalate exposures for effective exposure reduction strategies.

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Specific Aims

Specific Aim #1

Define the distribution of urinary phthalate metabolites in pregnant women in Puerto Rico, and determine individual factors associated with elevated phthalate exposure.

Hypothesis #1: Pregnant women in Puerto Rico have higher urinary phthalate metabolite concentrations compared to pregnant women in the US population.

Hypothesis #2: Demographic variables (e.g. residence), phthalate levels in drinking (tap) water, and modifiable or preventable behaviors/activities (e.g. diet, consumer products, and fragrant use) are associated with phthalate exposure among pregnant women in Puerto Rico.

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Specific Aims

Specific Aim #2

Assess the relationship of maternal exposure to phthalates with gestation length and preterm birth among the women.

Hypothesis #3: Maternal exposure to phthalates is associated with shorter gestation length and increased risk of preterm birth (<37 weeks gestation), specifically late preterm birth (32 to 37 weeks).

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Specific Aims

Specific Aim #3

Explore mechanistic pathways between phthalate exposure and preterm birth. Specifically, markers of 1) endocrine disruption, 2) inflammation, and 3) oxidative stress/genotoxicity.

Hypothesis #4: Phthalate exposure is associated with 1) altered maternal hormone levels; and increases in markers of: 2) inflammation and 3) oxidative stress.

Hypothesis #5: In addition to being associated with phthalate exposure, markers of endocrine disruption, inflammation, and oxidative stress are also associated with shorter gestation length and increased risk of preterm birth.

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Specific Aims

Phthalate Metabolism

Preliminary data: large inter-individual variation in susceptibility-relevant phthalate metabolite ratios

Phthalate Metabolite Ratio	N	Selected Percentiles						
		Min	10 th	25 th	50 th	75 th	90 th	Max
MEHP%	208	0.2	3	6	10	17	31	61
Free:Total MECPP	82	16	26	31	40	52	61	78
Free:Total MEP	82	30	51	66	80	88	92	98

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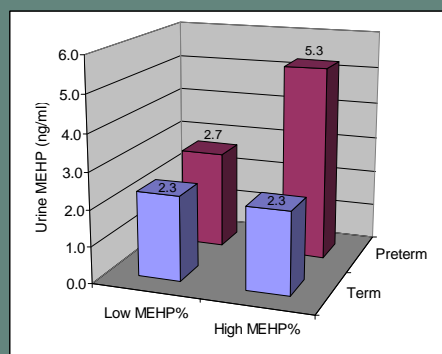


Specific Aims

Individual Susceptibility

Evidence for Interaction and Individual Susceptibility:

Geometric mean MEHP concentrations (ng/ml) in women delivering term or preterm when stratified by MEHP% (< or > median). Difference in MEHP between term and preterm births was statistically significant among women with high MEHP% ($p=0.03$).



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Specific Aims


Exploratory Aims

Explore evidence of individual susceptibility and gene-environment interactions

Hypothesis #6: Specific polymorphisms in phase 1 and phase 2 enzymes are associated with differential phthalate metabolism, manifest as differences in specific metabolite ratios, which may increase one's susceptibility to health effects

Hypothesis #7: polymorphisms in enzymes involved in phthalate metabolism or hypothesized mechanistic pathways are predictors of preterm birth risk *and* effect modifiers in the association between phthalate exposure and preterm birth

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Project 1
Discovery of Xenobiotics Associated
with Preterm Birth

Roger Giese, Core Leader
Poguang Wang, Collaborator
Gang Shao, Collaborator

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Hypothesis

- Hypothesis:** Xenobiotics contribute to preterm birth.
- Assumptions:**
 1. Xenobiotics of interest are organic and more or less nonpolar.
 2. Xenobiotics of interest are elevated in samples associated with preterm birth.
 3. Xenobiotics of interest may act as genotoxic agents, especially as oxidative stressors.
 4. Xenobiotics of interest may be multiple, and not the same for every affected individual.

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Aims

1. Develop a practical, remote extraction method for xenobiotics in water and urine.
2. Develop and apply a non-targeted analytical method based on MS for xenobiotics.
3. Develop and apply a "Nucleotide Exposure Assay" for discovery of xenobiotics.
4. Extend and apply "Mass Tag Profiling" to detect DNA adducts from oxidative stress.

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Methods I

1. Large samples: tea bag solid phase extraction
2. Sample fractionation mainly by chromatography
3. Mass spectrometry: GC-TOF-MS, LC-MALDI-TOF/TOF-MS, and LC-ESI-TOF-MS involving general and specific internal standards, quality control samples, and data conditioning
4. Mass tag profiling of DNA adducts
5. Nucleotide Exposure Assay
6. Large, stabilized sample extracts
7. Embedded targeted chemical analysis
8. Focus on analytical differences (cases vs controls)
9. Progressive analytical snapshots

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Methods II

Number of Samples from Puerto Rico^a

Sample	Test	PTB Cases (Minimum)	Controls (Minimum)
Gestational Membrane ^b Placenta ^b	DNA Adducts	10	10
	DNA Adducts	10	10
Urine ^c	Xenobiotics	30	30
Tap Water Ground Water	Xenobiotics	20	20
	Xenobiotics	20	20
Leukocytes ^b	DNA Adducts	10	10

^a Not including animal and cell culture samples from Project 2
^b Core C collects 40
^c Not including 20 Boston urines

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Dynamic Transport of Phthalates and TCE in Karst Groundwater Systems

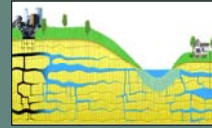
Ingrid Padilla
Sangchul Hwang
Raul Machiavelli

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Background



Contaminant Transport Processes in Karst Groundwater Systems

- Influenced by the flow mechanism predominating in the aquifer

- **Conduit-flow** conveys solutes rapidly through the system to a discharge point without much attenuation



- **Diffuse-flow** is associated with flow through the aquifer rock matrix and interconnected fracture zones



- The carbonate aquifer in northern PR is characterized by a mixture of both



- Preliminary results show that contaminants are stored for a long time in the northern karst aquifer of Puerto Rico

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P4 - Objective

- Determine the fate and transport mechanisms controlling the release, mobility, persistence, and potential path for exposure of organic contaminants **and/or remediation zones** in karst groundwater

- La Plata- Arecibo Hydrogeophysical System
- Chlorinated solvents (TCE) and phthalates (DEHP/DOP)
- Involves laboratory experiments and field sampling and analysis



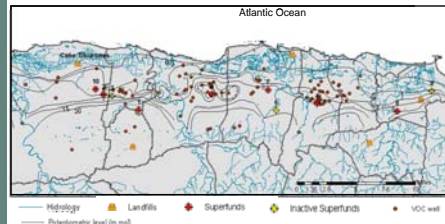
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Hypothesis 1, Specific Aim & Methods

- **Hypothesis 1:** Contaminants released into karst groundwater systems follow spatial paths at the hydrogeophysical system level that can be used to assess areas of potential exposure

Specific Aim 1.1 – Assess areas of potential groundwater exposure at the system level



Methods

- Baseline data will be collected and analyzed for contaminants in groundwater, drinking-water distribution systems, and tap water
- Years 1-4

-Data Collection

- Available (Historical and on-going)
- 96 GW samples
- 10 wells, 2 springs 2X/yr
- 800 Tap water samples

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Hypothesis 2 & Specific Aims

- **Hypothesis 2:** Contaminant transport in karst groundwater system is closely related to hydrologic events
 - Storm flow vs. baseflow fate and transport
 - Advection / Dispersion
 - Storage
 - Rate-limiting mass transfer
 - Retardation or attenuation
 - **Specific Aim 2.1** - Determine the main processes controlling contaminant fate and transport in karst ground-water systems characterized by variable conduit and/or diffusion-dominated flow
 - **Specific Aim 2.2** - Relate temporal contaminant concentrations in space with hydrologic flow conditions
 - **Specific Aim 2.3** – Determine the effect of flow conditions on contaminant distribution at the field scale

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Hypotheses 3 & Specific Aim

- **Hypothesis 3:** Phthalates fate and transport is influenced by the presence and transport of other contaminant phases.
 - Dissolve phthalate partition into Dense Non-Aqueous Phase Liquids (DNAPLs) present in the medium, and their transport behavior in karst groundwater systems is modified according to the transport behavior of the DNAPL
 - **Specific Aim 3.1** - Determine the effect of NAPL mixtures in the fate and transport of dissolved contaminants in karst systems.

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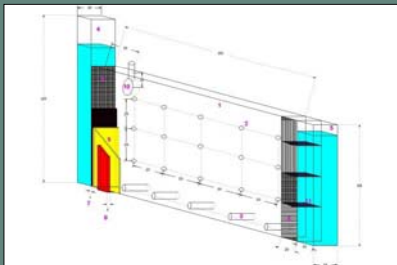


Methods



North Coast Limestone

- Specific Aims 2 and 3
 - Miscible and multi-phase elution experiments in physical models representing karst groundwater systems
 - Laboratory-scale experiments entail injecting single and multiple contaminants into the systems, and monitoring their concentration spatially and temporally from source to discharge areas
 - Multiple-contaminants experiments entail injecting dissolved phthalate (DEHP) in the presence of mobile or trapped DNAPLs (TCE)
 - The experiments will be conducted under high- and low-flow conditions



Karstified-Limestone Physical Models

Experiments	Activity	Year			
		V1	V2	V3	V4
Miscible Displacement	Model Development	x			
	Experiments	x	x		
Multiphase Fate and Transport	Model Development	x	x		
	Single Solute (NATCE)		x	x	x
	Mixture (TCE+DEHP)			x	x

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Methods

- Specific Aims 2.3 - Determine the effect of flow conditions on contaminant distribution at the field scale
 - Hydrologic analysis of historical water level, spring flow, and rainfall will be conducted to segregate data into temporal baseflow and high-flow conditions and assess temporal changes in point-based hydraulic gradients (Years 2-3)
 - Correlation statistics will be applied to water levels data, gradient estimates, and contaminant detection and concentrations under baseflow and high flow conditions (Years 3-4)

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Hypothesis 4 & Specific Aim

- **Hypothesis 4:** Dynamic biological activity and physico-chemical conditions within landfills enhance degradation of phthalate-containing waste materials (PWMs) and mobility of phthalates in landfills
 - **Specific Aim 4.1** - Assess enhanced degradation and leaching characteristics of PWMs in “typical” characteristic landfills
 - **Methods**
 - Bacteria Isolation and Enrichment (Year 1)
 - Leaching experiments with PWMs
 - Batch reactors (Years 1-2)
 - Column reactors (years 3-4)



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Project 2
Pollutant Activation of Cell
Pathways in Gestational Tissues

Rita Loch-Caruso, PhD, Project Leader
Craig Harris, PhD, Project Co-Investigator
Richard Lieberman, MD, PhD, Project Investigator
Laura Rozek, PhD, Project Investigator
Lauren Tetz, Predoctoral Candidate

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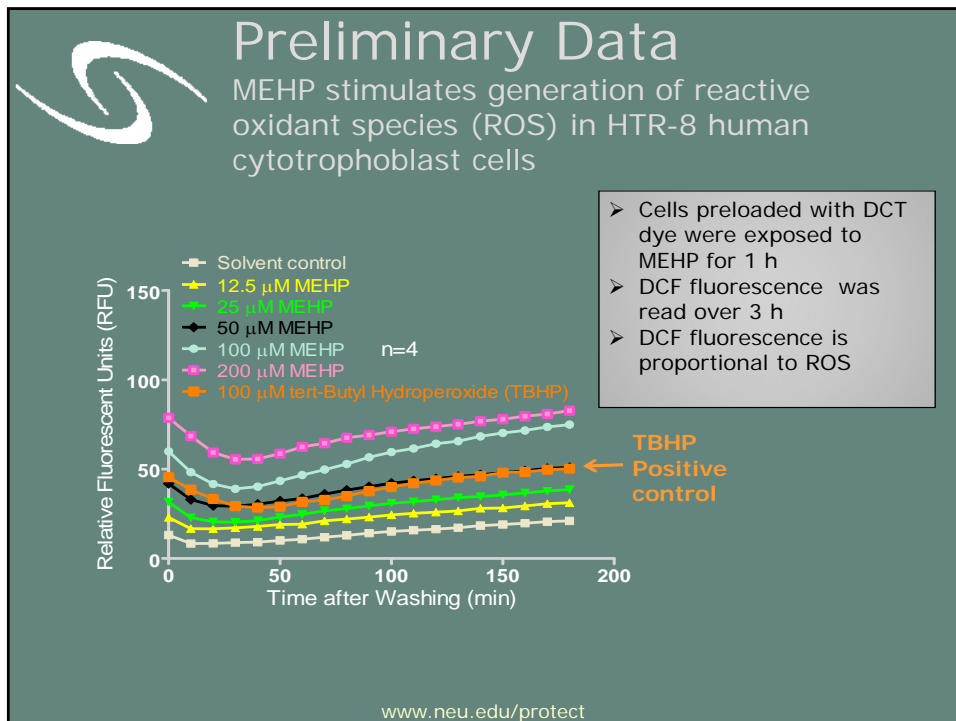
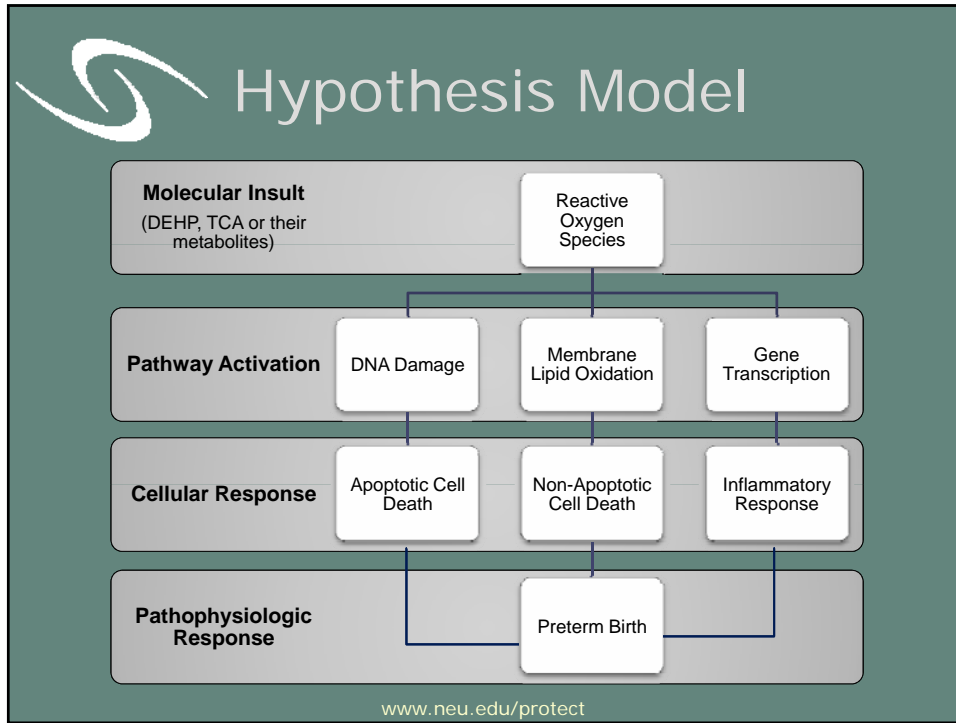
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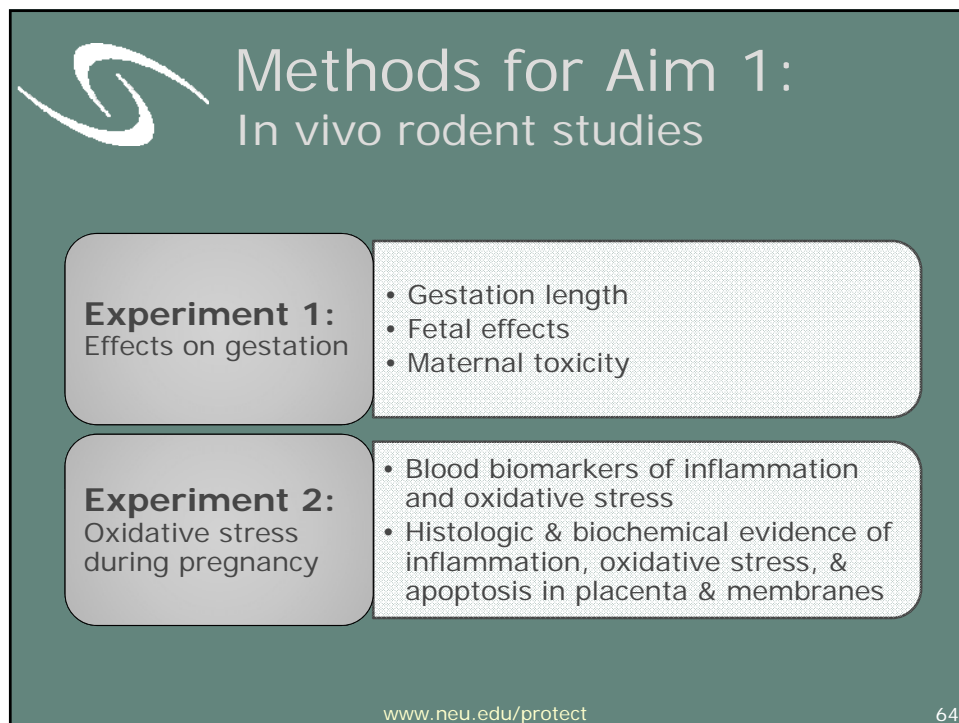
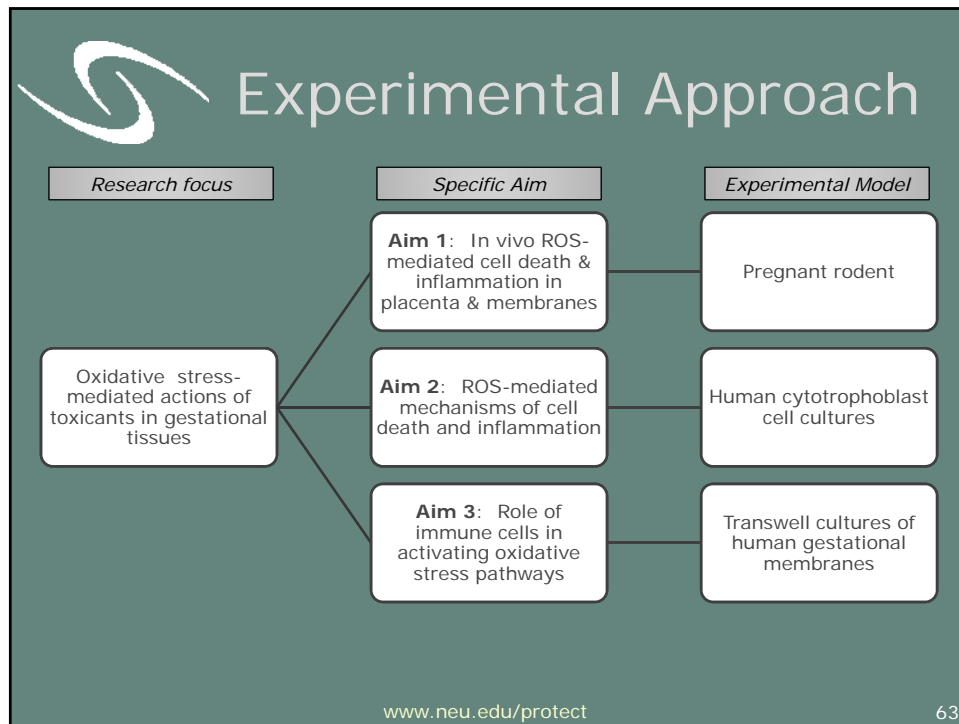


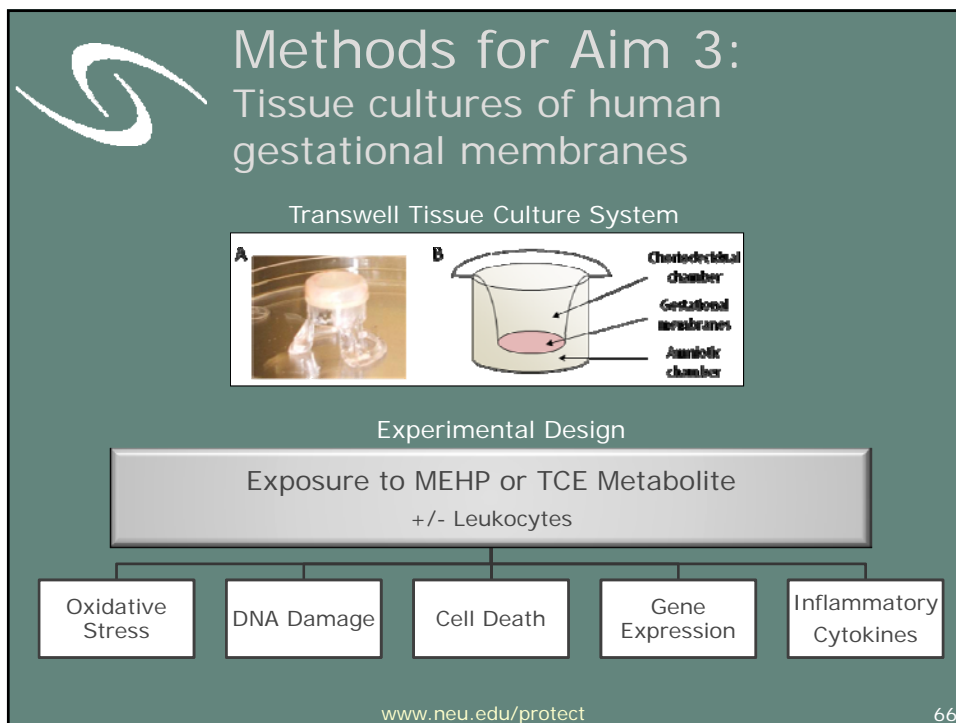
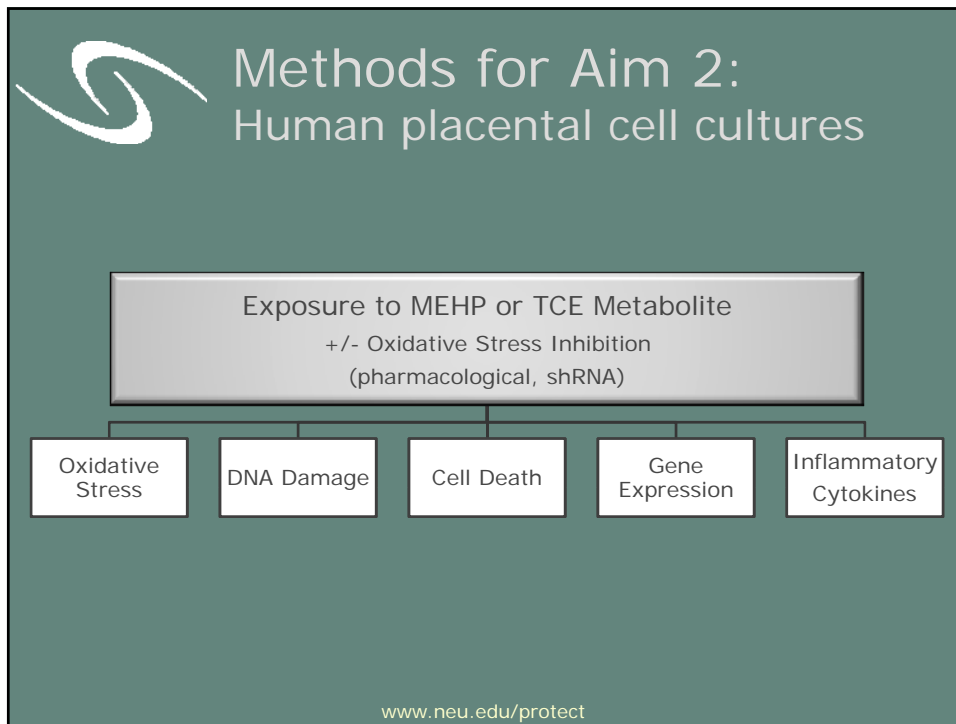
Hypothesis

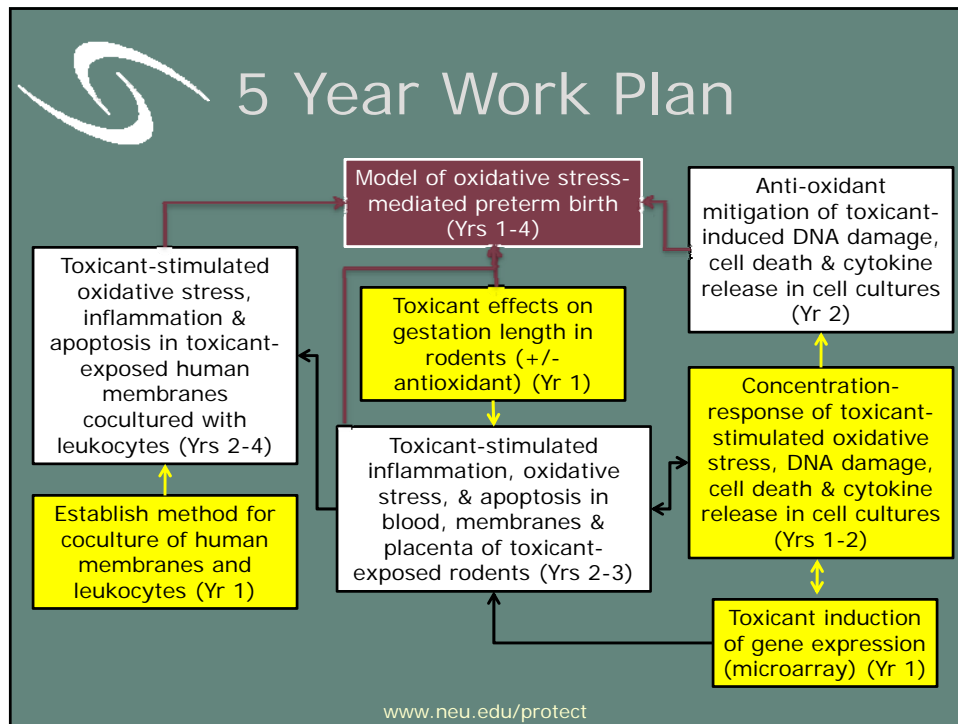
- Superfund-related contaminants activate reactive oxygen species-sensitive pathways in gestational tissues to promote onset of parturition and thereby increase risk for preterm birth
 - Focus on the phthalate DEHP, the solvent TCE, and their metabolites


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Project 5: Green Remediation by Solar Energy Conversion into Electrolysis in Groundwater

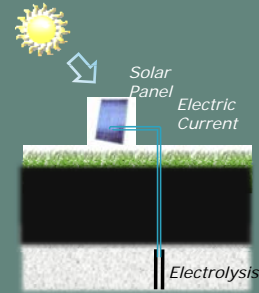
Akram N. Alshawabkeh, Project Leader
Elizabeth Podlaha-Murphy, Co-Investigator
Dorothy J. Vesper, Co-Investigator
Ingrid Padilla, Co-Investigator

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Long Term Goal

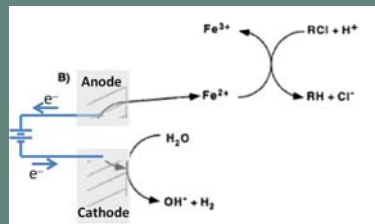
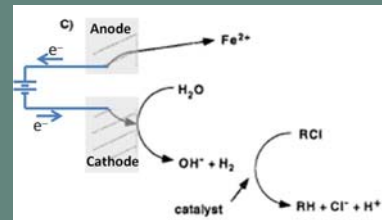
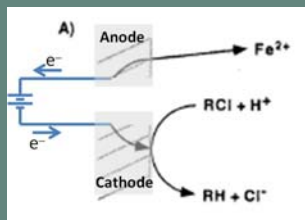
- Developing “Green” soil and groundwater remediation technologies based on conversion of solar energy into electrochemical redox in soil and groundwater



The process is green because it uses low-cost natural resources (solar energy and iron) without adverse impacts on the environment.

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Potential Transformation Mechanisms



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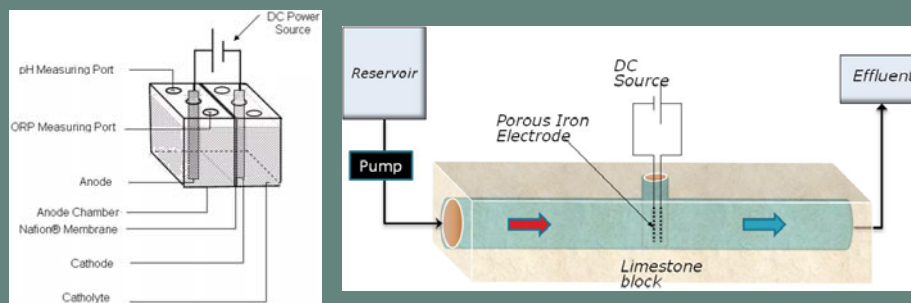
70

Project Aims

- **Aim 1:** Determine dynamic geochemical changes and controlled manipulation of redox conditions in karstic groundwater by iron electrolysis using direct, pulse, and polarity reversal electric currents
- **Aim 2:** Evaluate chemical reduction of contaminants by iron electrolysis in karstic groundwater.
- **Aim 3:** Determine xenobiotics, adverse effects and cytotoxicity of treated karstic groundwater.
- **Aim 4:** Implement a model for transformation kinetics by iron electrolysis in aquifers and develop the process as “green remediation” technology.

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Experimental Setups



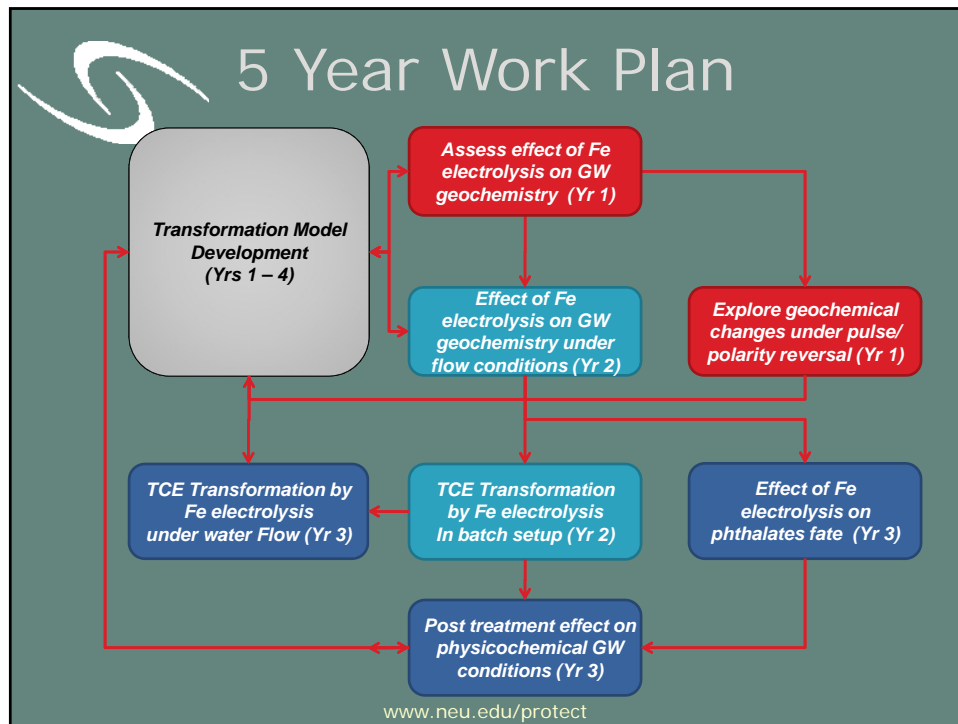
Controlled variables:


Concentrations, GW chemistry, flow rate, current type/density

Measured Variable:

Concentrations (space and time), Groundwater chemistry

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Core D: Data Management and Modeling Core (DMMC)

David Kaeli
Ferdi Hellweger
John Chen

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Overview

- Responsible for the storage and management of biological, survey data, and environmental data via a database system
- The DMMC will provide for the secure and reliable storage and access to this information
- The DMMC will provide for the ability to cross-index data (based on time and space) and data mine the information stored in the database system
 - Global XML-based data dictionary across the project
- GIS-based mapping of this information is provided via EarthSoft's EQUiS software and ArcView
- The DMMC is also responsible for
 - environment modeling
 - extensive data mining software

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Participants

- David Kaeli
 - Core D Leader
 - Responsible for the database system, EQUiS software, and data mining software
- Ferdi Hellweger
 - Responsible for environmental modeling and GIS data
- John Chen
 - Database design/administration
- Patrick McHallam
 - GIS/database consultant

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Core D: Aims

- **Aim 1:** Build a customized user interface to allow for the efficient cleaning and entry of the human subject data. The interface needs to be able to talk directly to the EQuIS system.
- **Aim 2:** Build a customized user interface to allow for the efficient entry and access of the environmental data.
- **Aim 3:** Develop an integrated database management system that can provide a repository that multi-disciplinary investigators can perform relational queries across both environmental and biomedical data.

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Core D: Aims

- **Aim 4:** Build a set of standardized graphical reports that produce layered maps utilizing GIS mapping software
- **Aim 5:** Provide data mining algorithms integrated into the database user interface to perform clustering analysis and pattern recognition
- **Aim 6:** Provide resources and support for adaptive data and implementation of water distribution, contaminant transport and exposure models.

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Core D: Aims

- **Aim 7:** Effectively use and expand upon the modeling tools (e.g., WaterCAD, MODFLOW, GMS and/or DYNYSYSTEM family of codes), for groundwater and contaminant fate and transport modeling capabilities.
- **Aim 8:** Provide a layer of user authentication to guard access to low-level (i.e., individual level) access to the data. While the system will be available to the general research community via the Internet, users requesting low-level data access (versus group-based statistics) will need to log into the system with an approved authentication process.

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Discussion & Questions

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Thank you

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