Environmental Exposures Enhance Severity of Respiratory Tract Infections in Children

Stephania A. Cormier, PhD

Department of Pediatrics
University of Tennessee Health Science Center
Le Bonheur Children's Research Foundation

LSU Superfund Research Center

THE UNIVERSITY of TENNESSEE HEALTH SCIENCE CENTER

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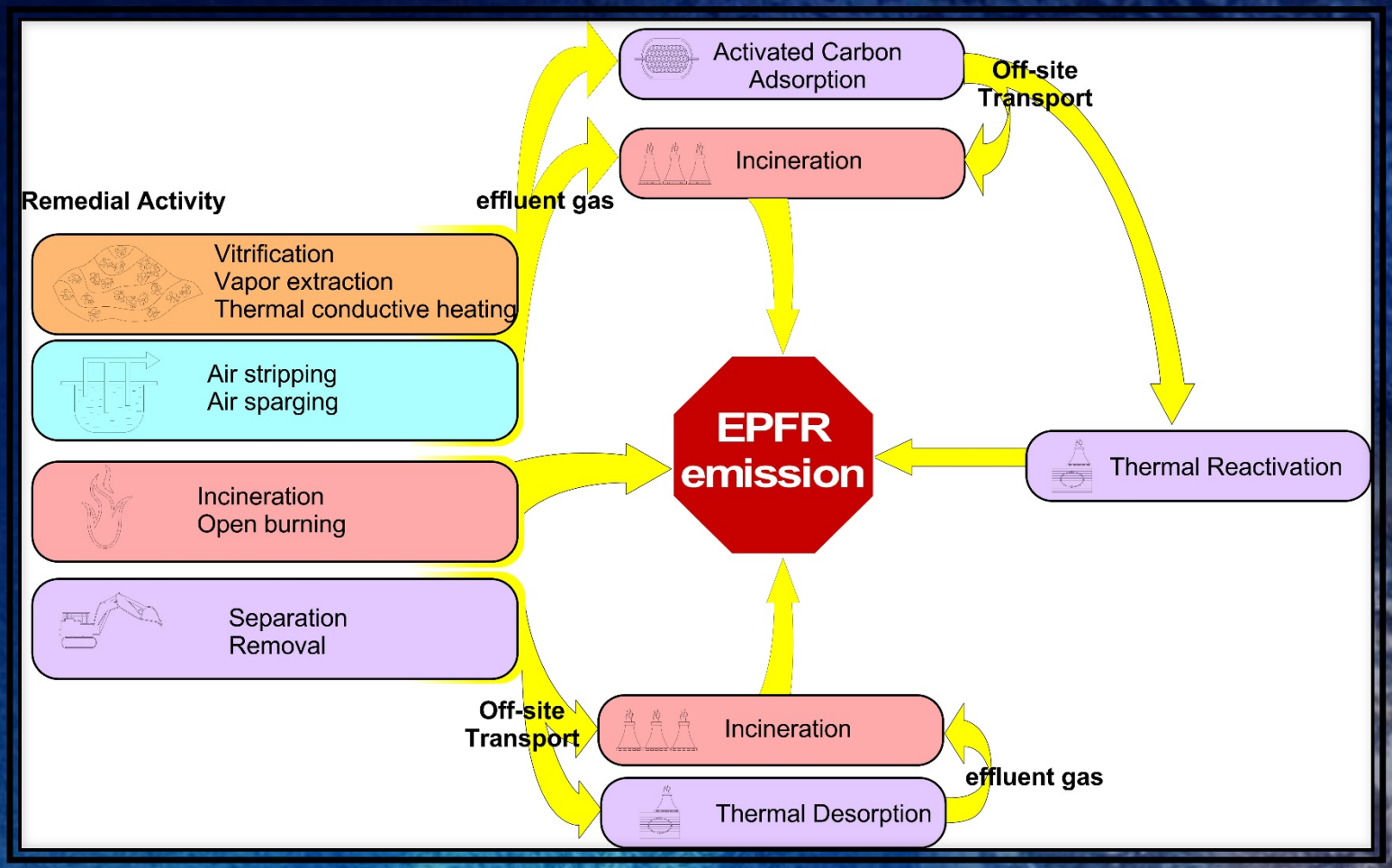
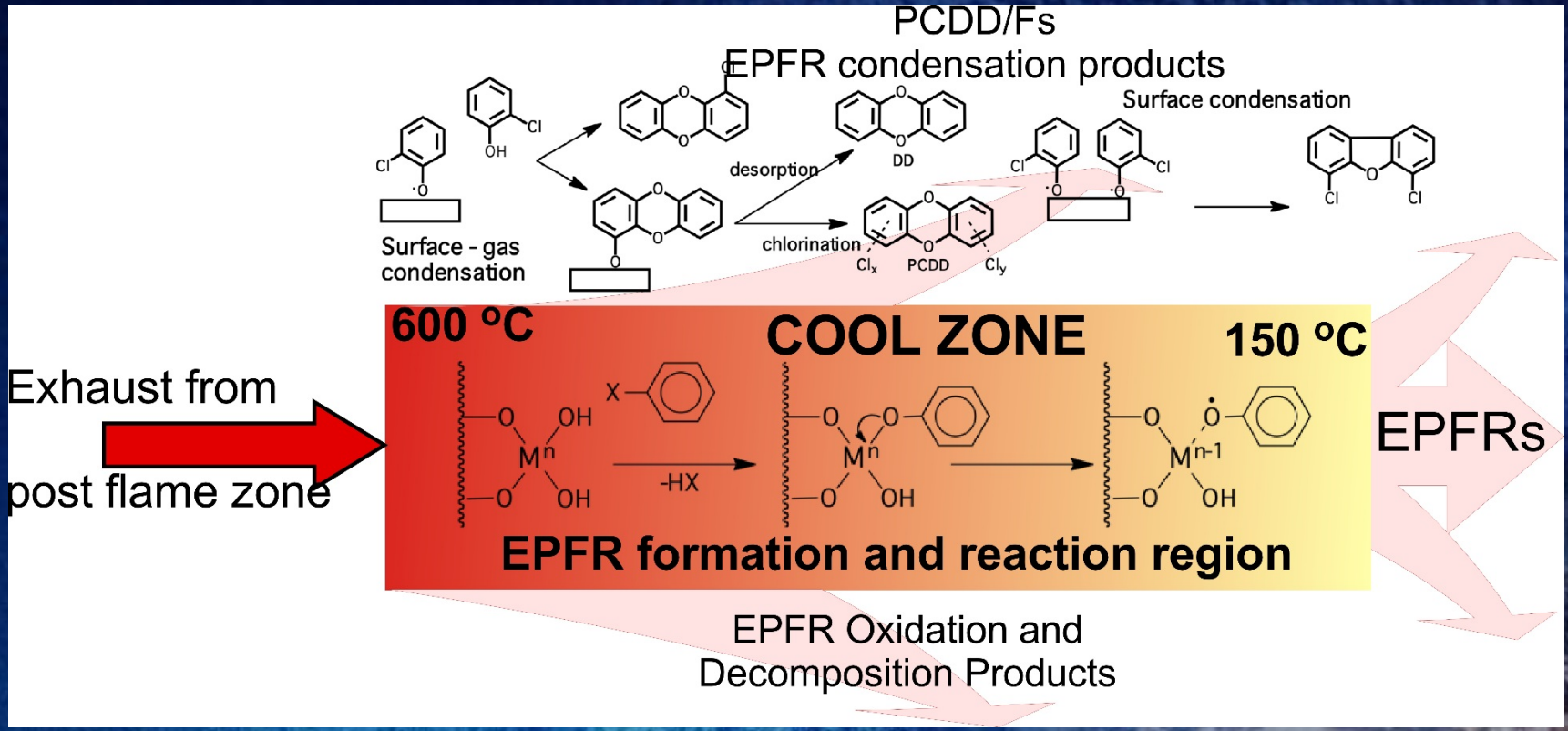
Environmental Cleanup Methods

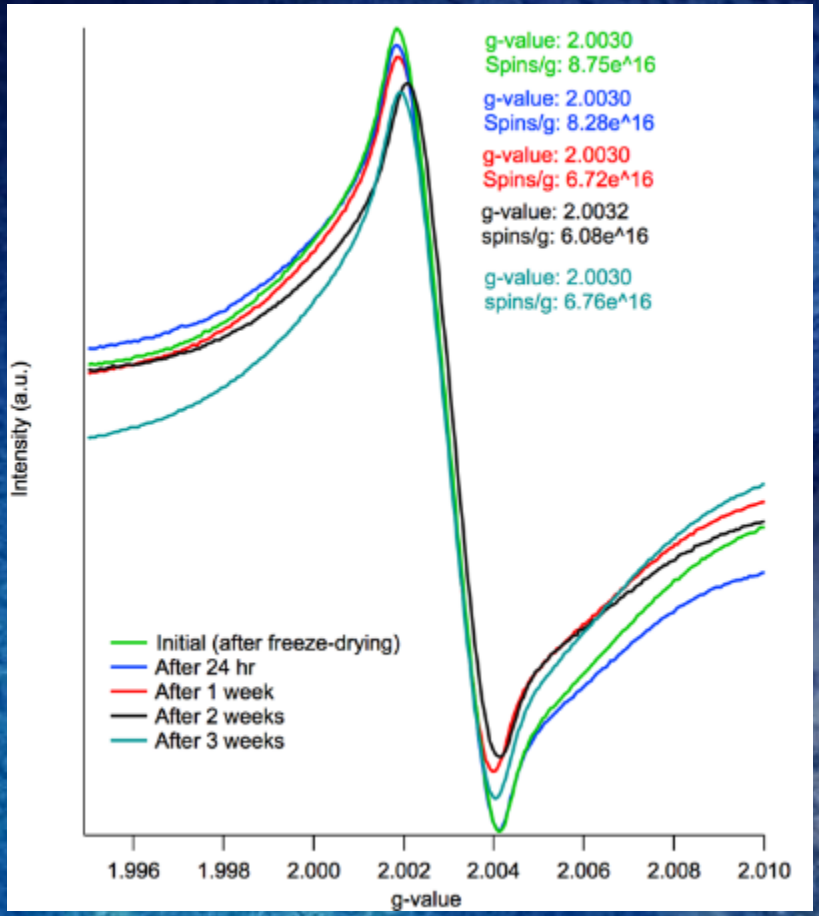


Formation of EPFRs

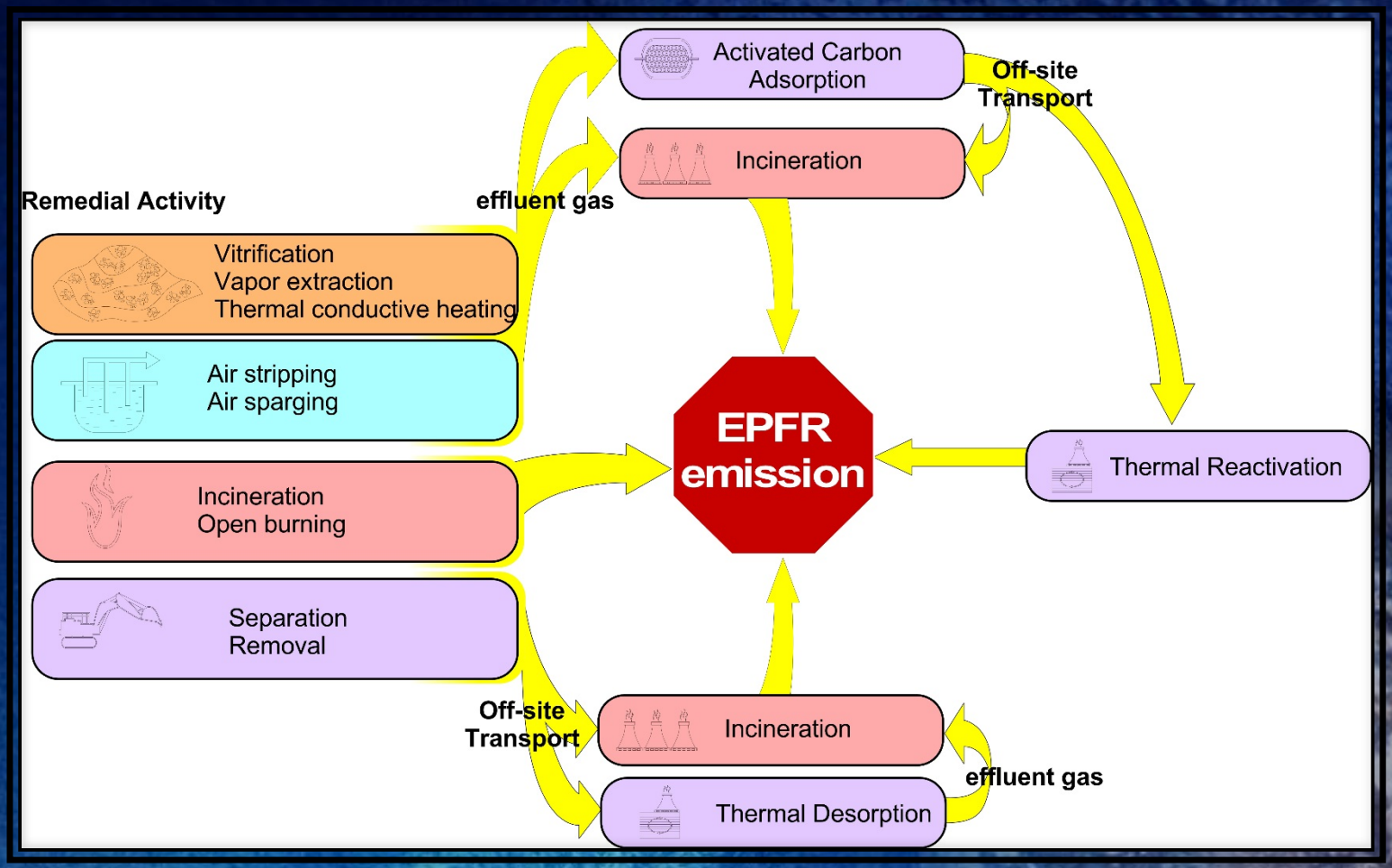


Barry Dellinger/Slawo Lomnicki

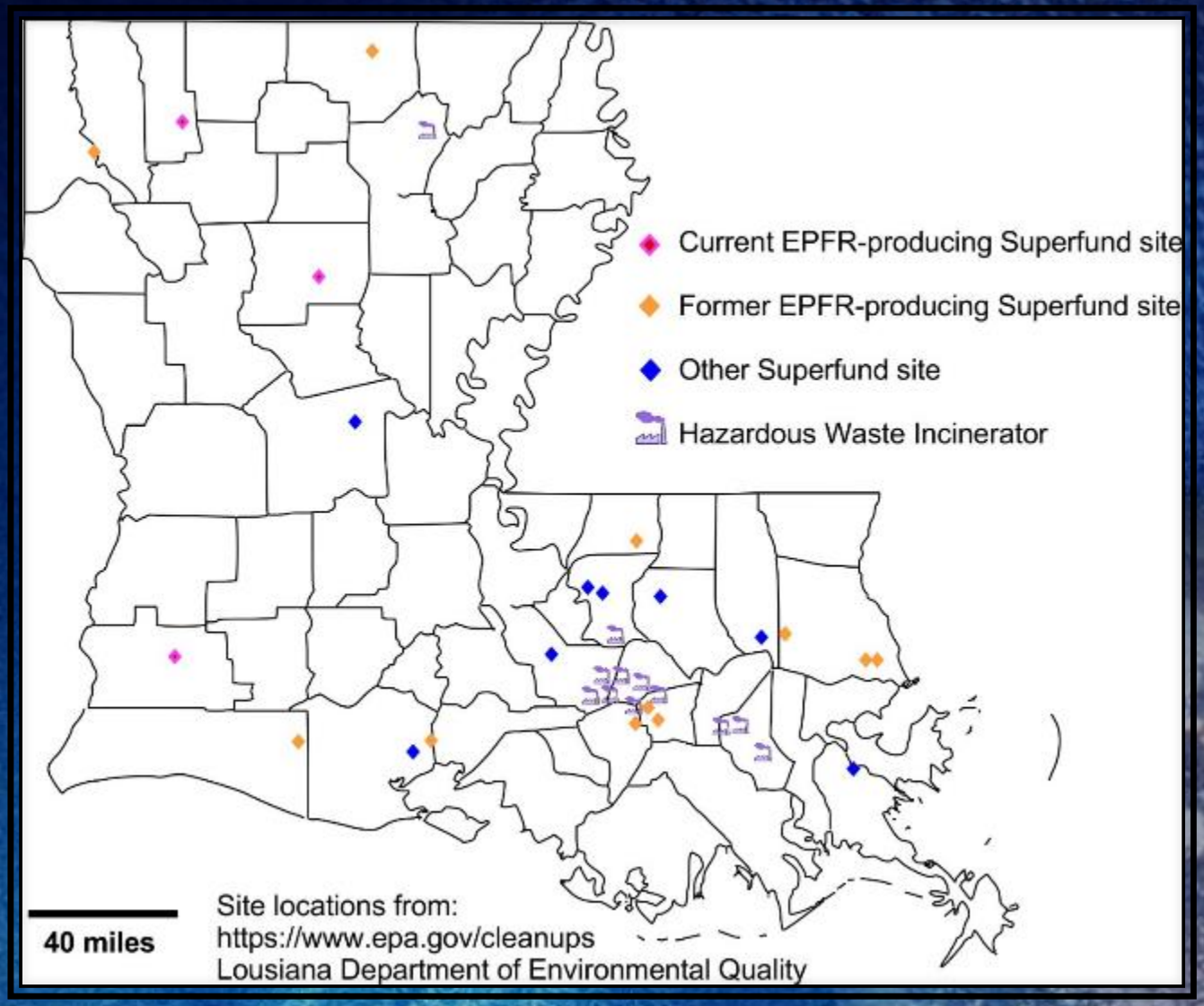
Fly Ash: A Source of Environmentally Persistent Free Radicals (EPFRs)



Environmental Cleanup Methods

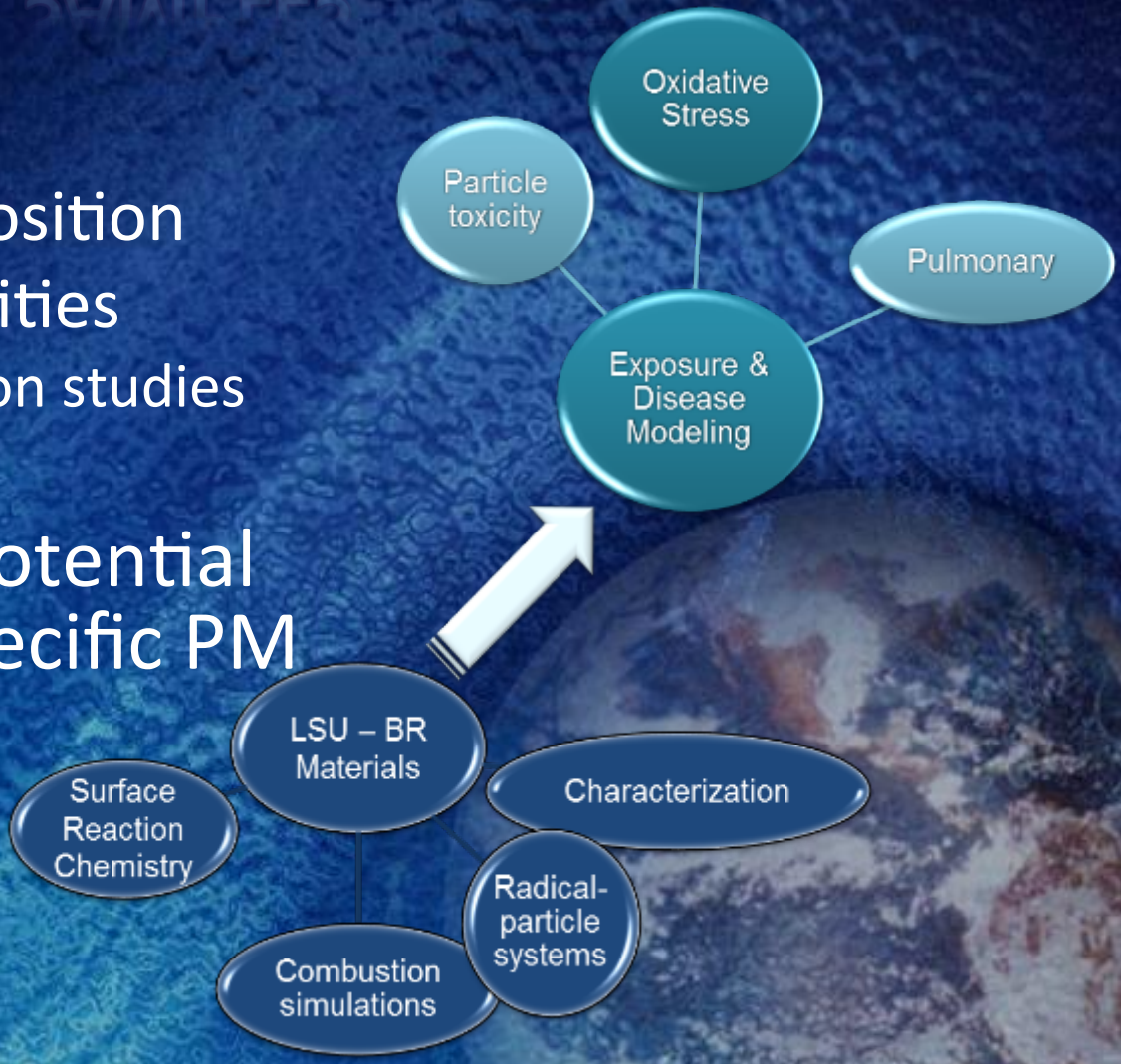


LA Hazardous Waste Sites

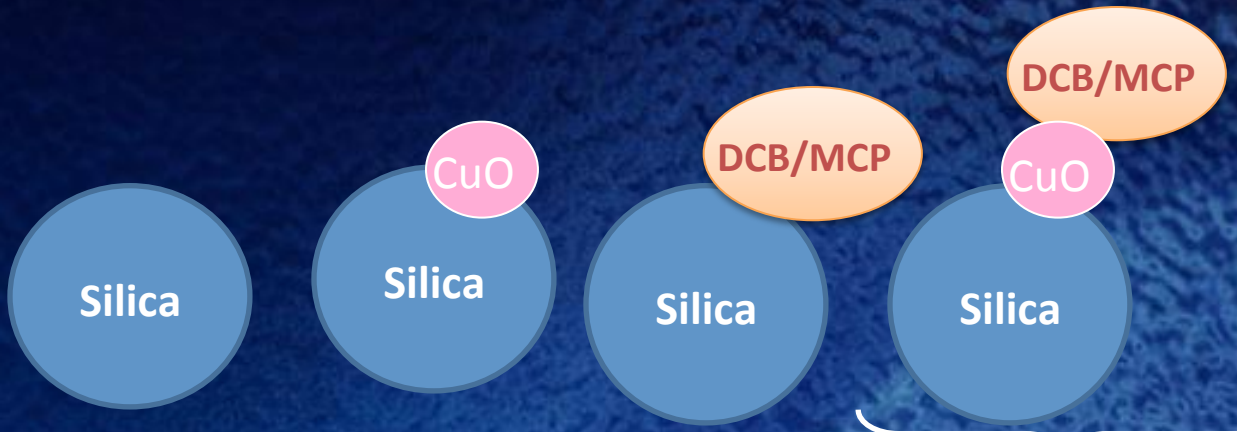


LABORATORY GENERATED COMBUSTION SAMPLES

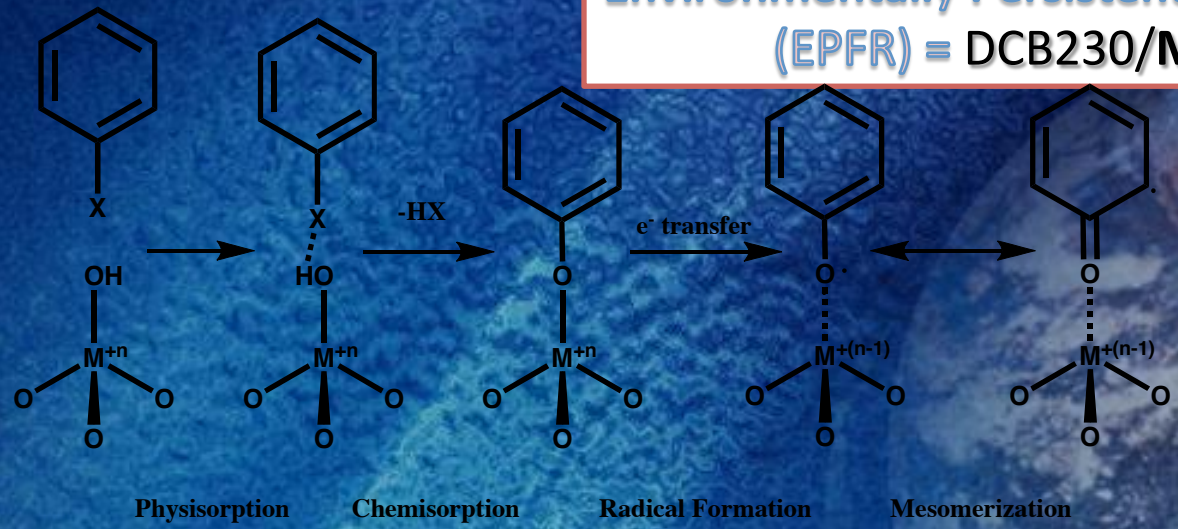
- Control
  - Size
  - Chemical composition
  - Sufficient quantities
    - In vivo inhalation studies
- More accurate assessment of potential risk posed by specific PM components
  - CHC/BHC
  - Radicals



Particle Systems



Environmentally Persistent Free Radical (EPFR) = DCB230/MCP230



CS tar: 1e16 radicals/g

EPFRs:1e14 - 1e16 radicals/g

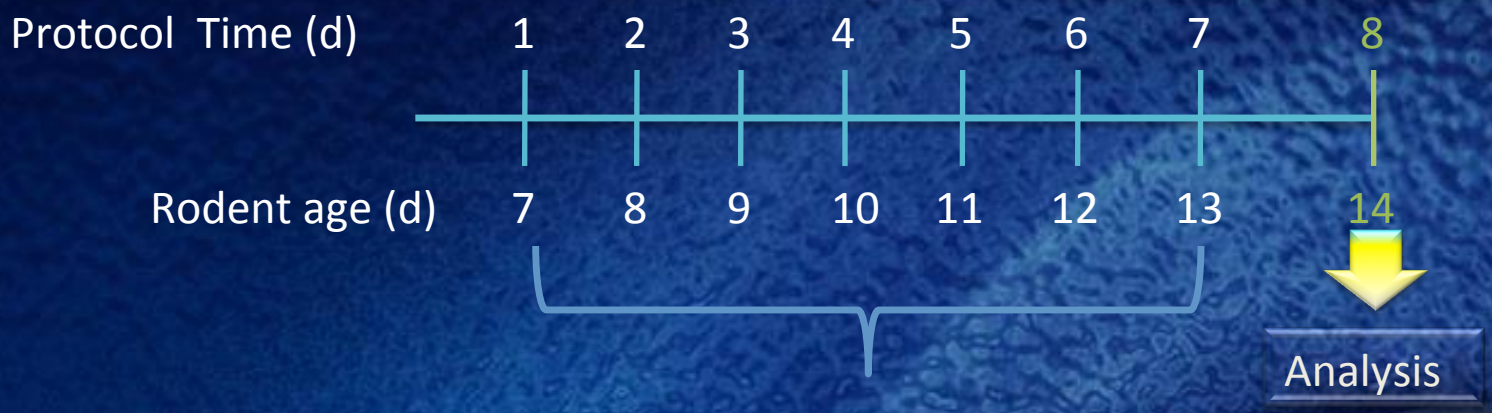
$PM_{2.5}$:1e16 - 1e17 radicals/g

Dellinger et al., 2007

Infants highly vulnerable to airborne exposures

- **Lungs & immune systems are still developing**
- **High respiratory rate**

In Vivo Acute Exposure Protocol



Study Endpoints

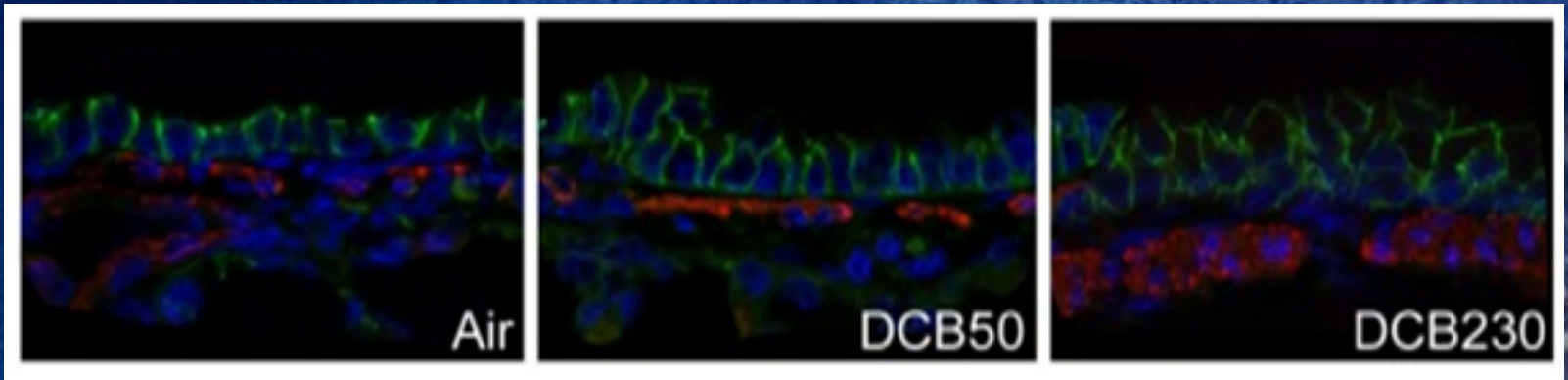
Lung Function
- AHR
- Resistance, elastance,

Lung Histology
- Cellular inflammation
- Mucus production

Inflammation
- BAL: cell type & numb

Window of Vunerability

- Structural Changes
  - Lung injury and destruction of epithelial barrier
  - Airway remodeling: EMT



Thevenot P, et al. AJRCMB. 2013. 48:188-97.

Window of Vunerability

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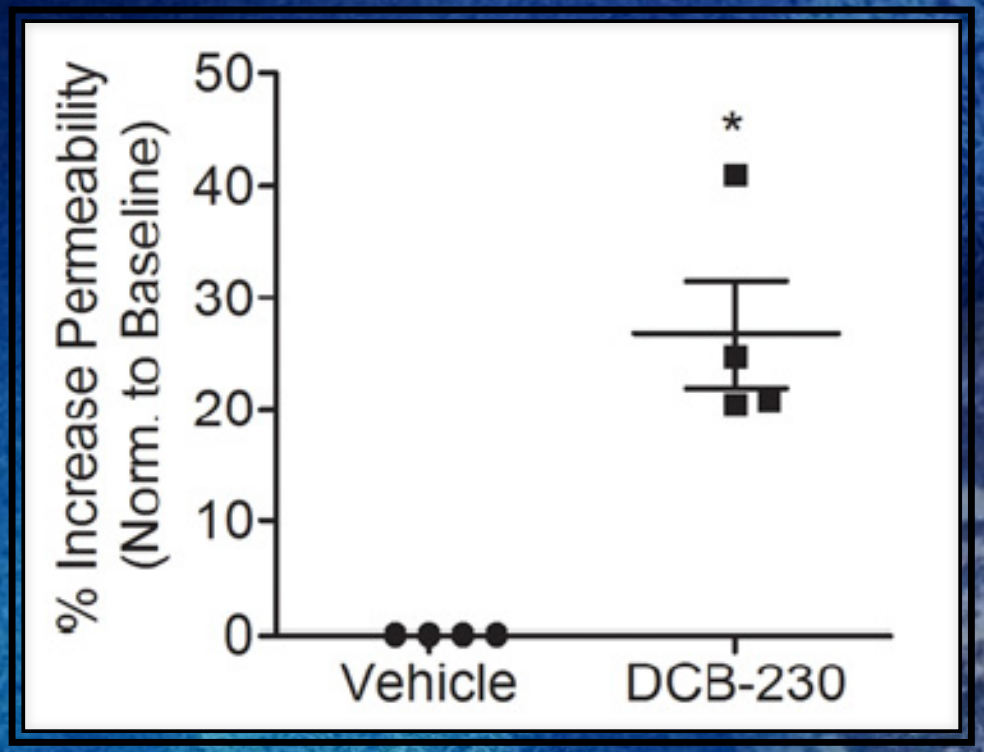


Thevenot P, et al. AJRCMB. 2013. 48:188-97.

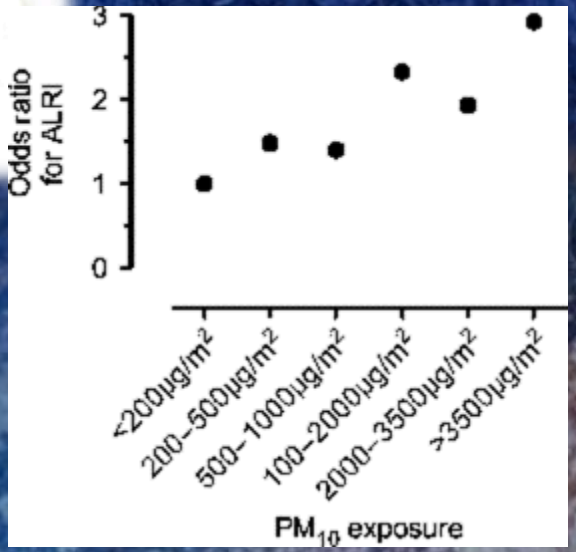
Summary of Results

- Infant exposures to EPFR-containing PM lead to long-term pulmonary consequences
  - Distinct pathologies
    - Inflammation
    - Epithelial disorganization (3dpe) – lung leak
    - Remodeling (w/i 4d exposure) – EMT
      - In vivo
        - » E cad + aSMA
        - » Bgal + aSMA
      - In vitro neonatal ALI
        - » E cad + aSMA
        - » Expression of genes associated with EMT: ↑Snai1 + aSMA and ↓E cad
  - Respiratory dysfunction
  - Uptake & Oxidative stress
    - ↑ 8-isoprostanes
    - ↓ GSH:GSSG ratio
- Relevance:
  - Mechanistically link PM exposure to airway remodeling
  - Loss of epithelial integrity (3-4dpe) suggests window of vulnerability to RTI

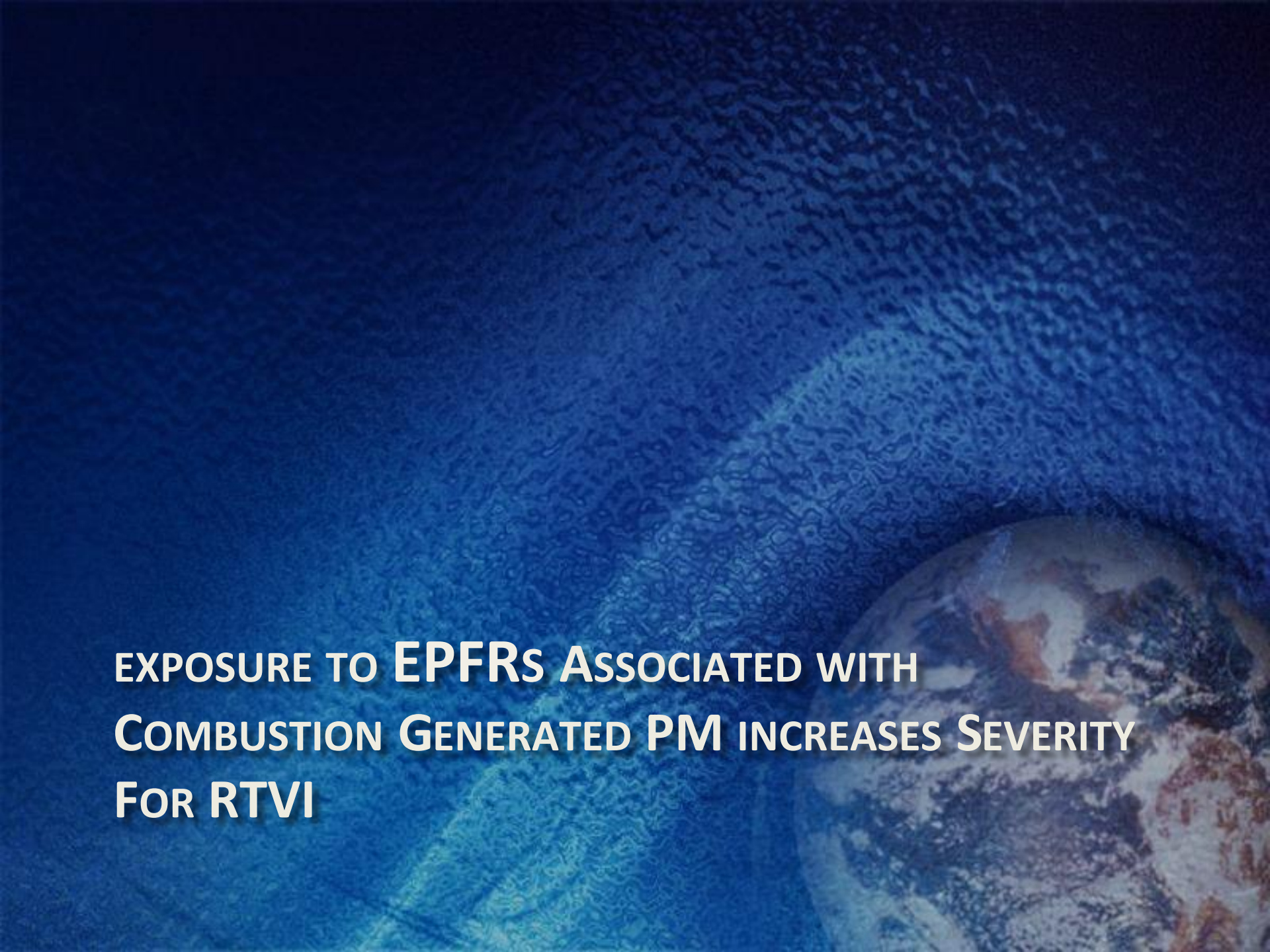
Thevenot P, et al. AJRCMB. 2013. 48:188-97.
Balakrishna S, et al. PFT. 2011;8:11.
Wang P, et al. AJRCMB. 2011. 45: 977-983

Every year, 1.96 million people die from ARIs as a result of indoor air pollution.

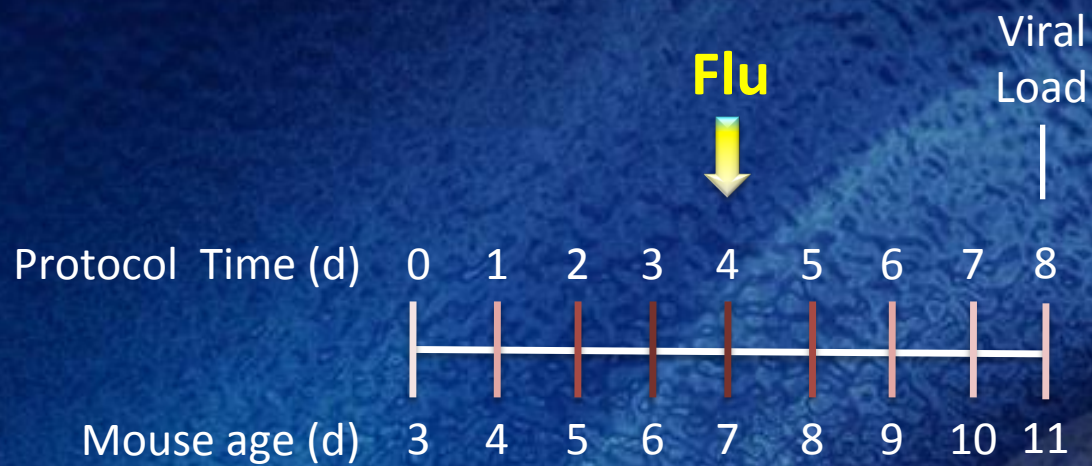
Source: ARIAtlas.org, World Lung Foundation 2010



Grigg. 2011. Clinical & Experimental Allergy. 41: 1072-1075

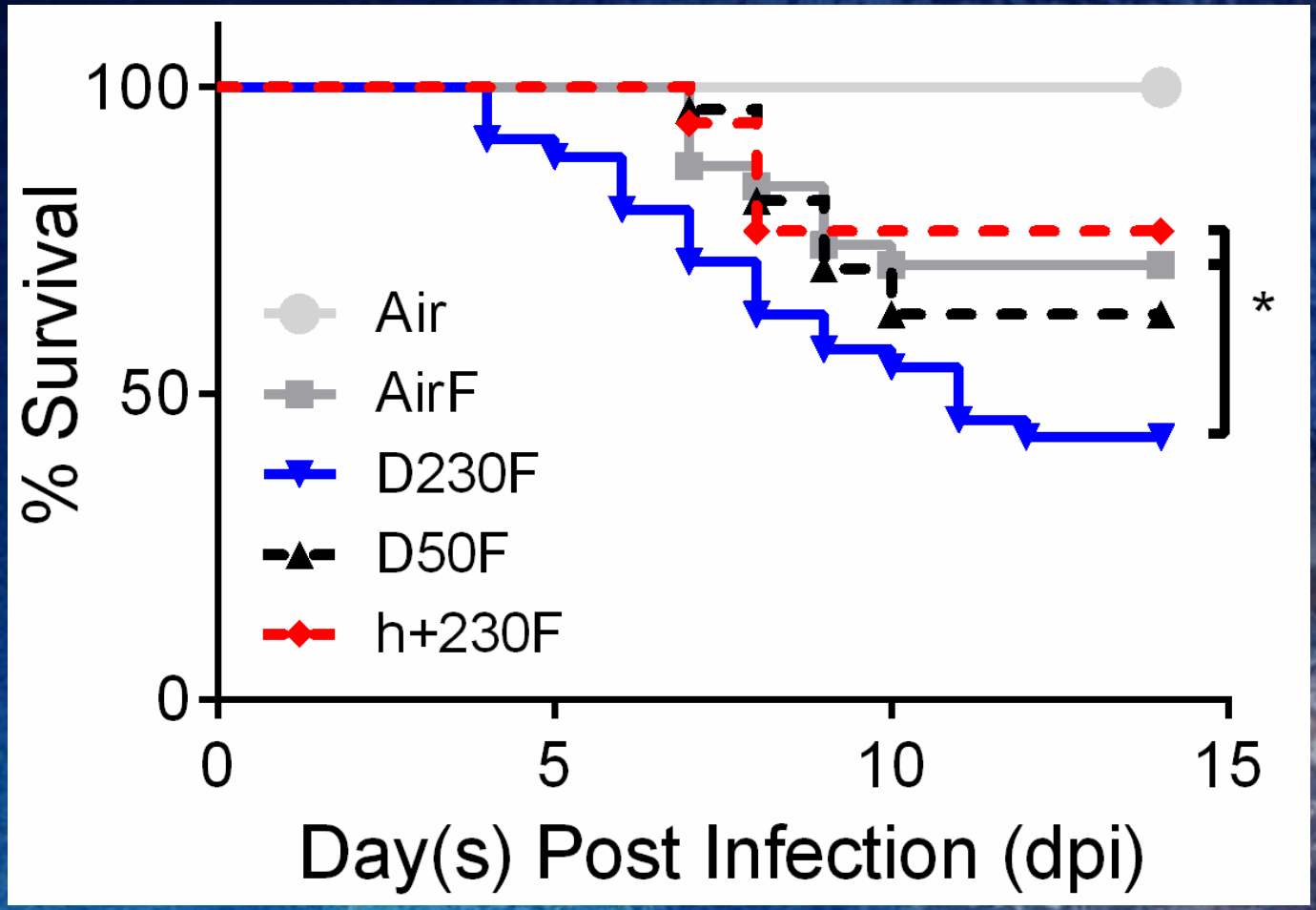

EXPOSURE TO EPFRS ASSOCIATED WITH
COMBUSTION GENERATED PM INCREASES SEVERITY
FOR RTVI

Exposure and Infection Protocol



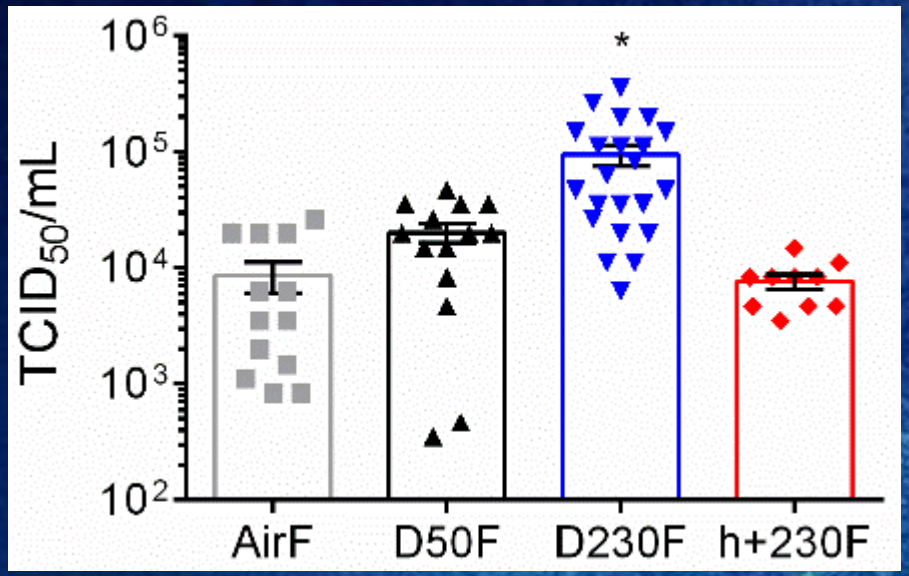
Lee, et al. PFT. 2014

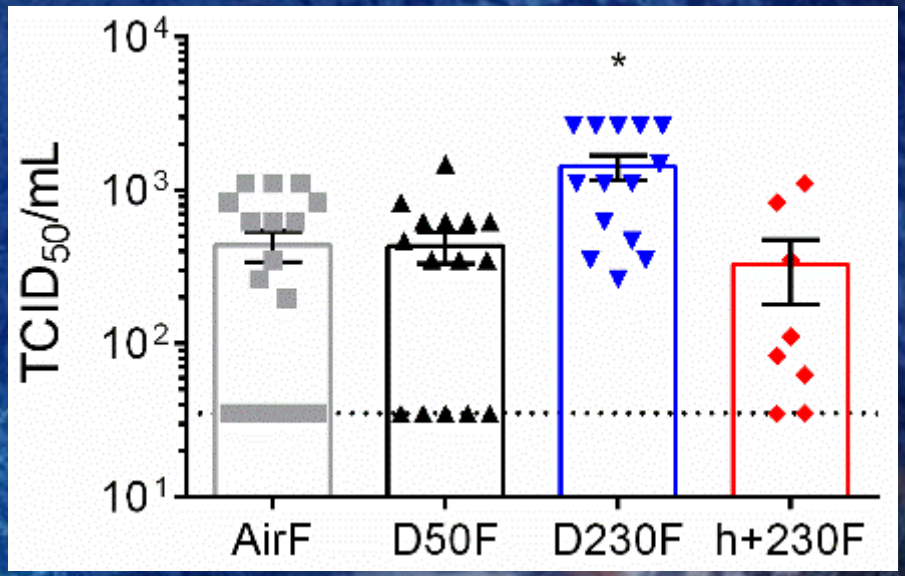
Influenza Mortality is Enhanced with EPFR Exposure



n = 16-35

EPFRs Increase Flu Viral Load & Delay Clearance





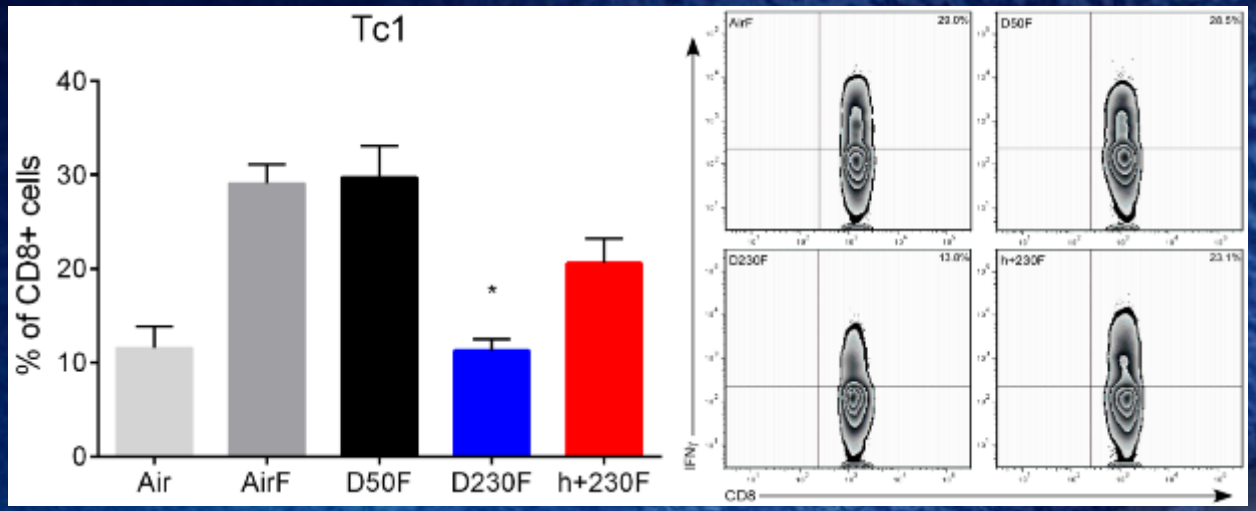
AirF	air	Flu
D50F	non-EPFR PM	Flu
D230F	EPFR PM	Flu
H+230F	hSOD2 + EPFR PM	Flu

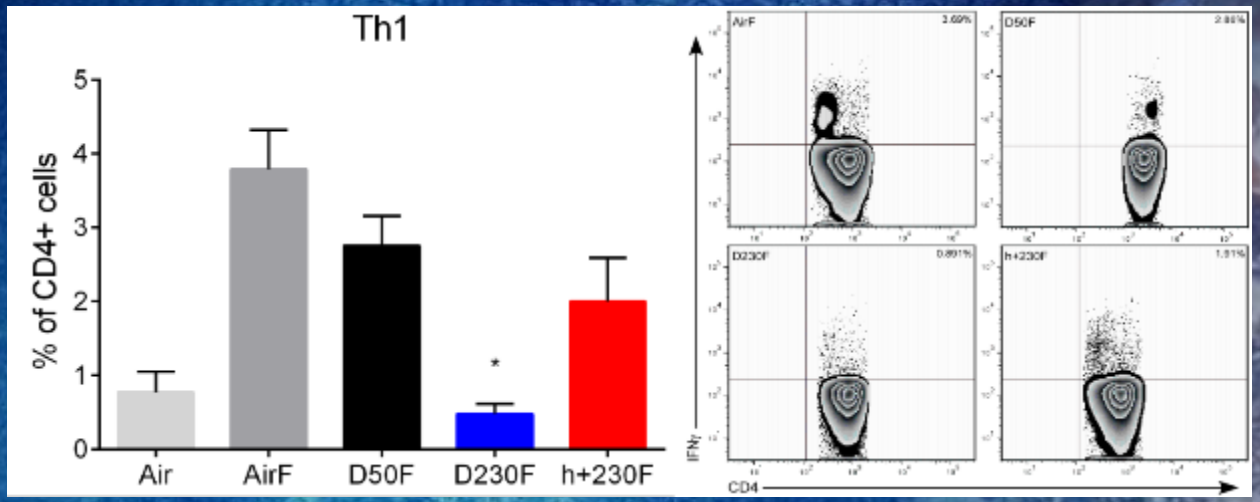
N=10 22

N=8 18

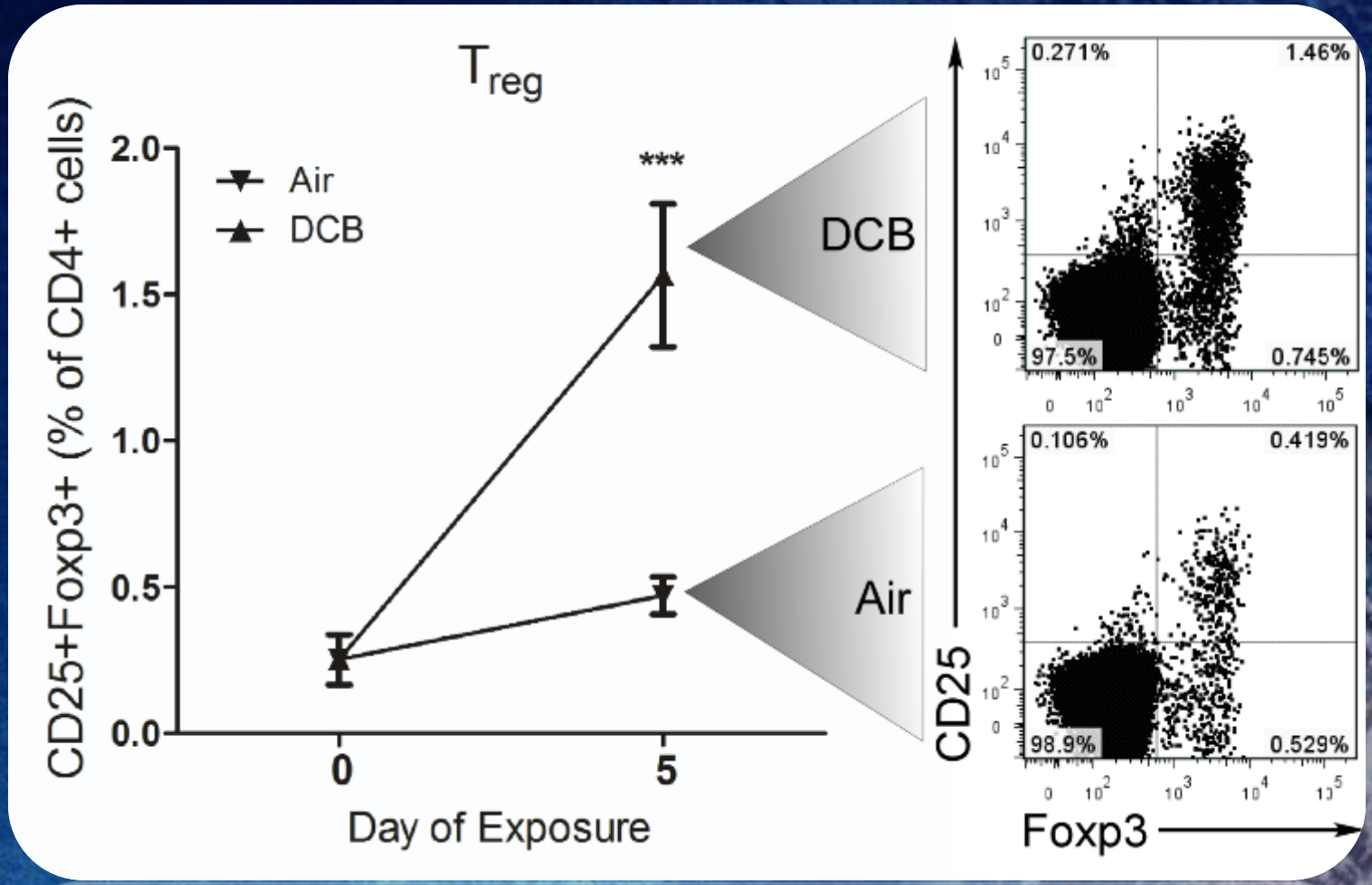
Lee, et al. PFT. 2014

Exposure to EPFRs Suppresses Protective Immune Responses





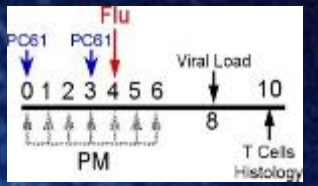
EPFRs Increase Tregs in the lung

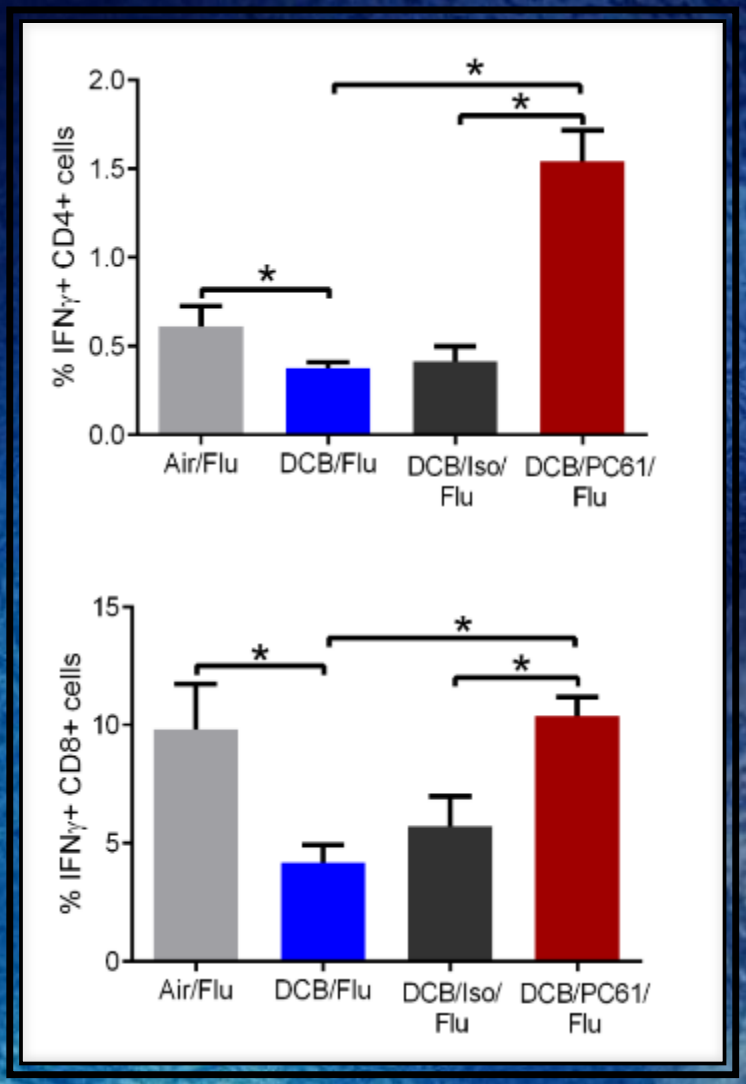


*p<0.05

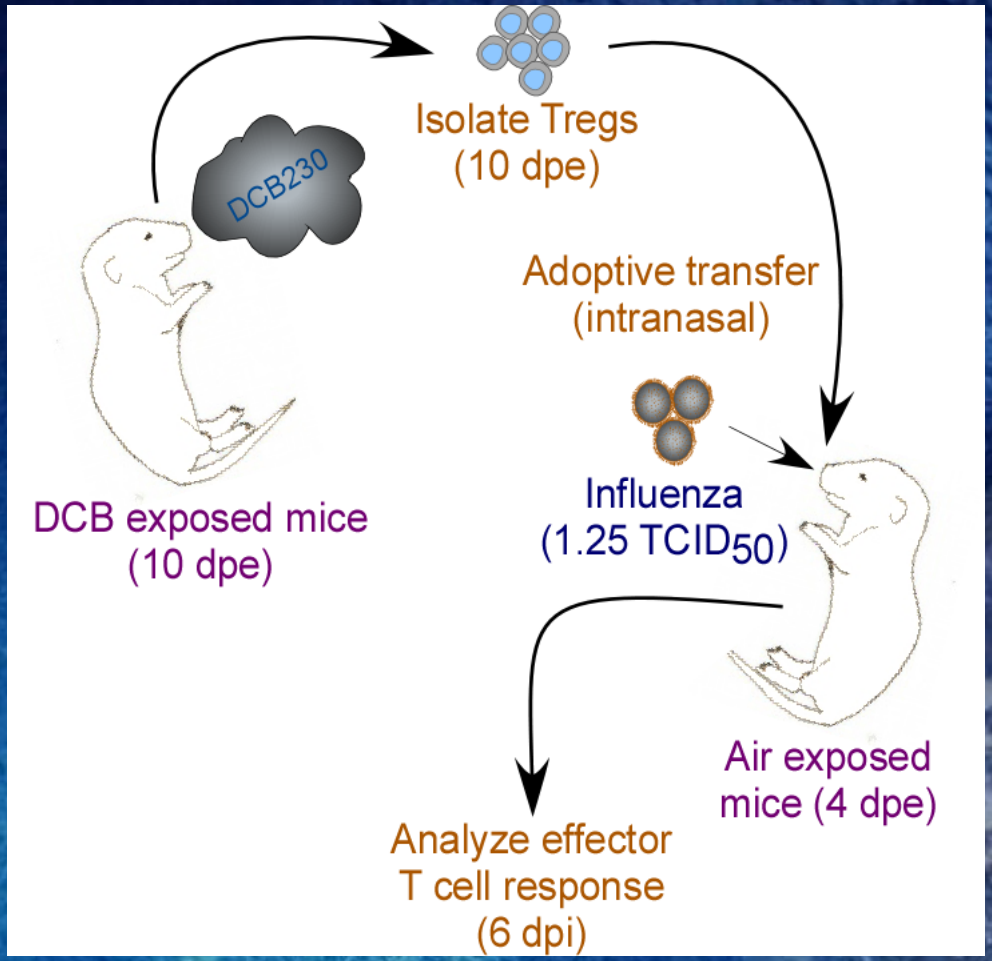
Saravia, et al. Mucosal Immunol. 2014

Absence of Tregs Restores Effector T cell Responses

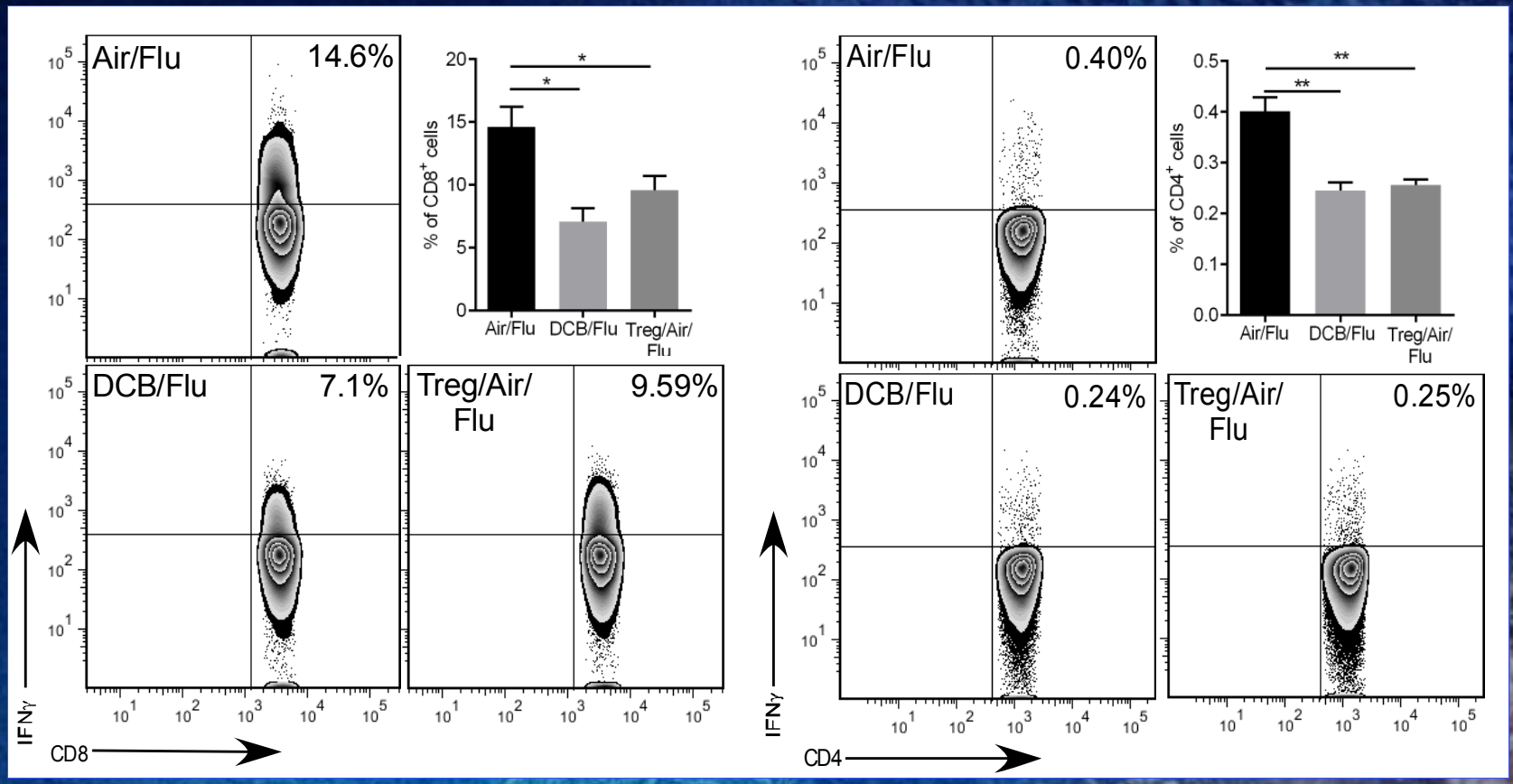




Adoptive Transfer of $Treg_{EPFR}$



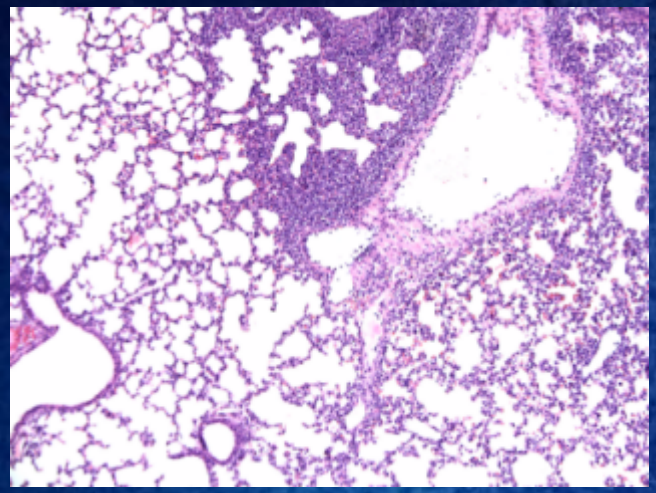
$Treg_{EPFR}$ Suppress Effector T cell Responses

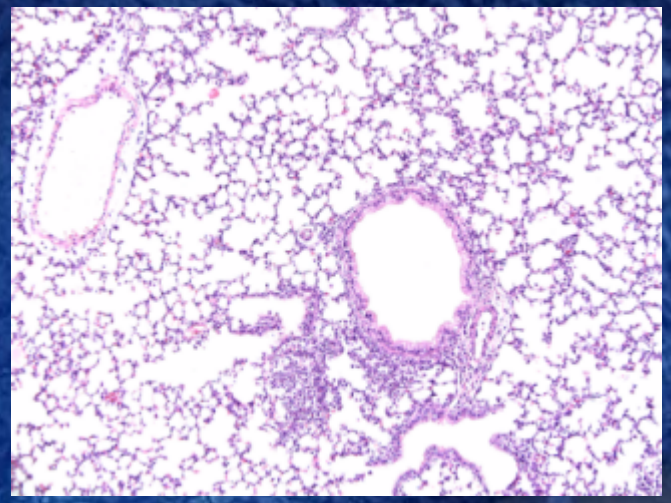


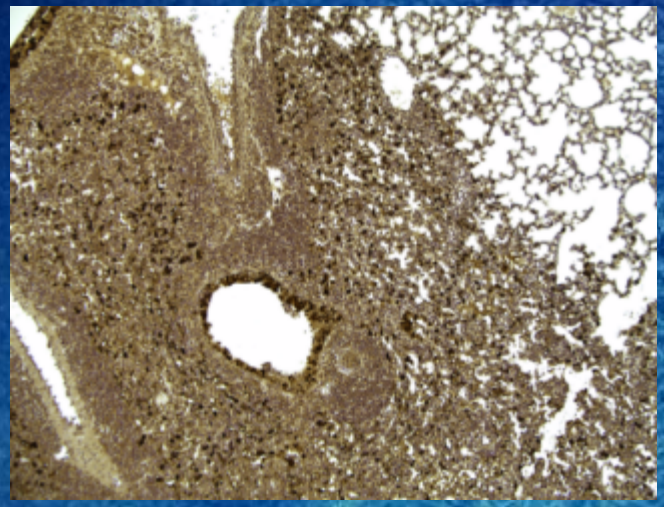
Absence of IL10 Reduces Influenza-Induced Pathology Following Exposure to EPFRs

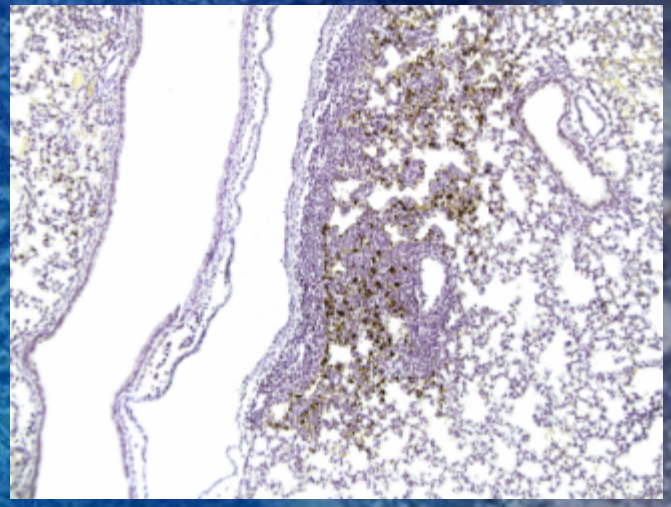
WT/DCB/Flu

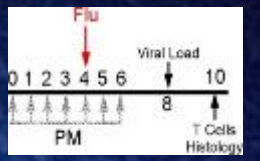
IL10KO/DCB/Flu





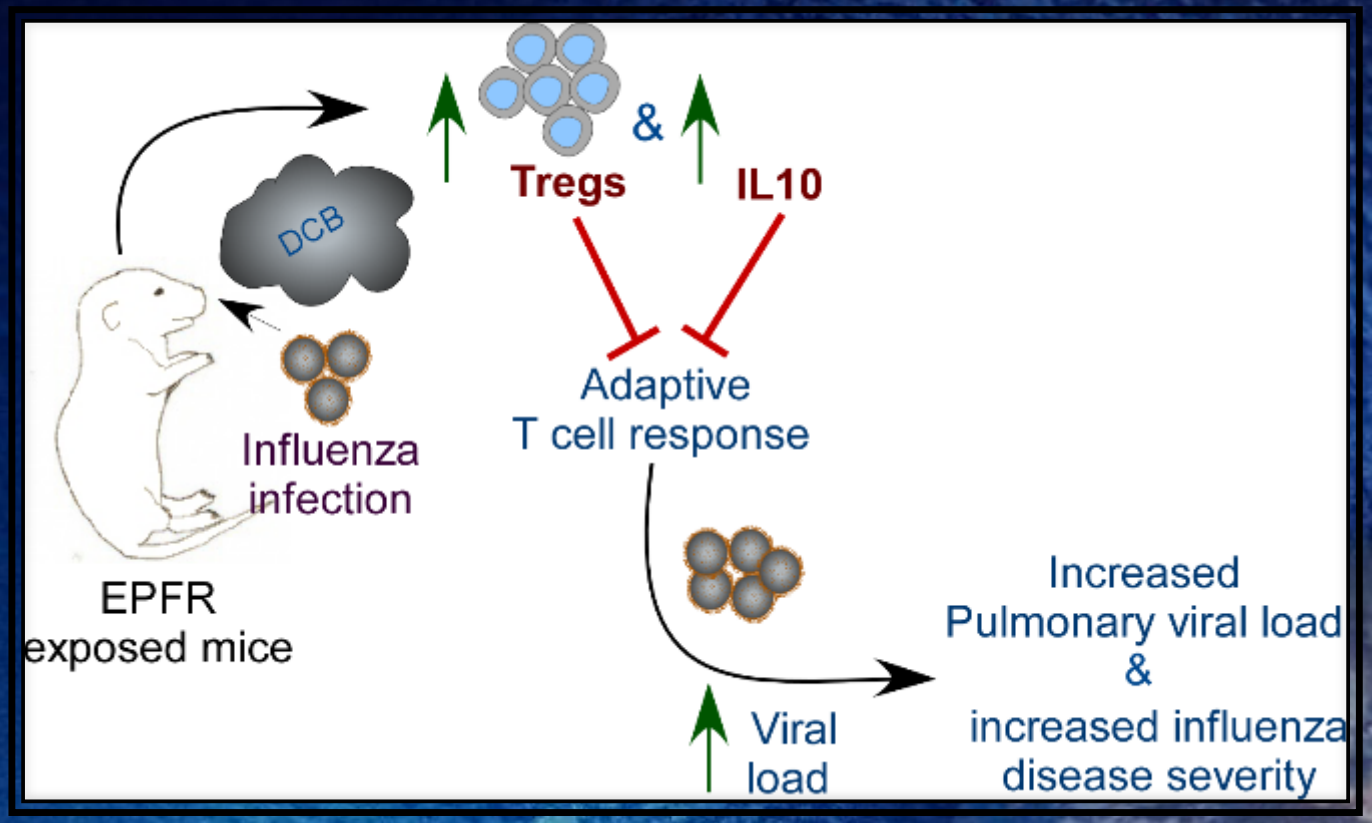






IL10$^{-/-}$

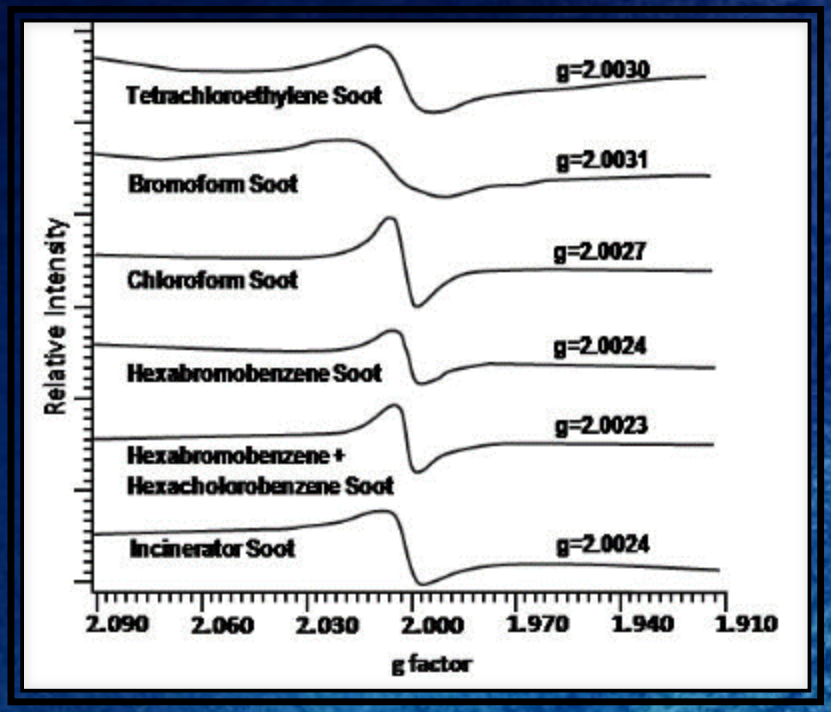
Summary

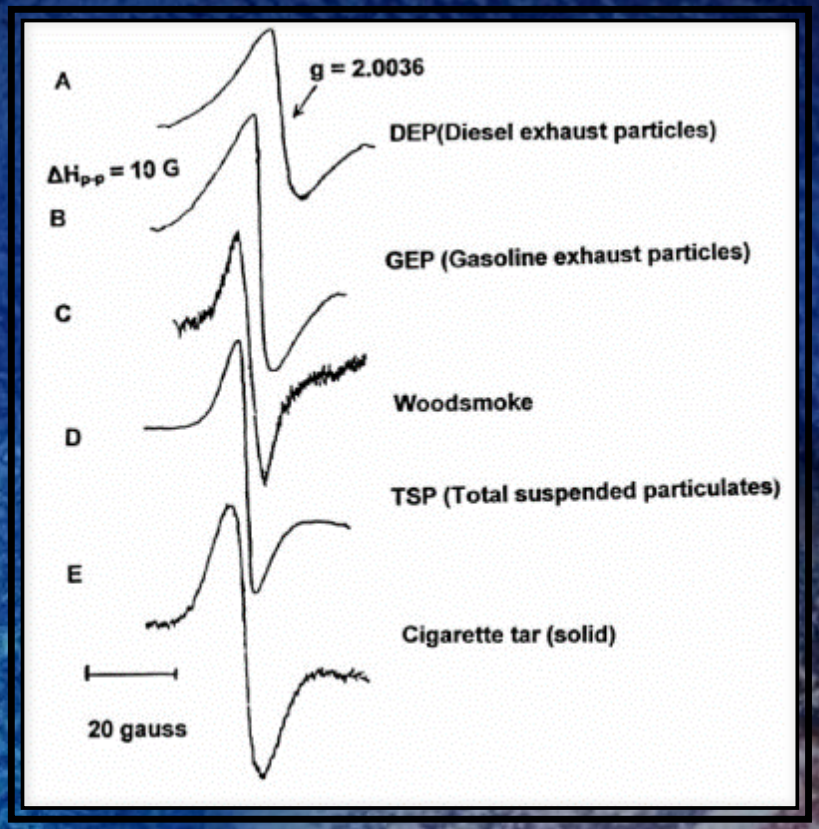


- Depletion of Tregs/IL10 in PM exposed mice increases protective T cell responses and reduces influenza morbidity & mortality
- IL10 alone recapitulates PM enhanced influenza morbidity


EPFRS –JUST A SUPERFUND PROBLEM?

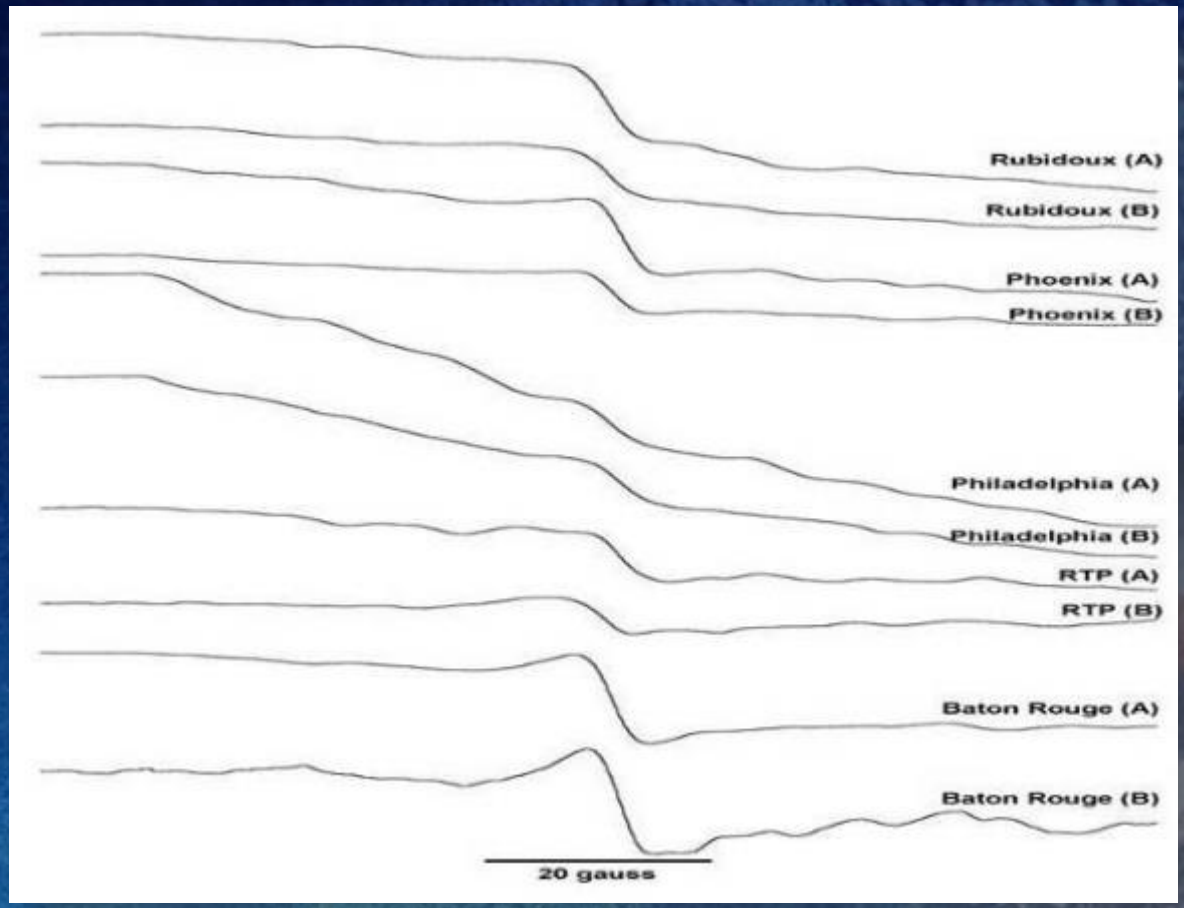
Combustion-Generated Particles Also Contain Detectable Radicals





A. Valavanidis 2004

Atmospheric Fine Particles Contain Persistent Semiquinone-type Radicals

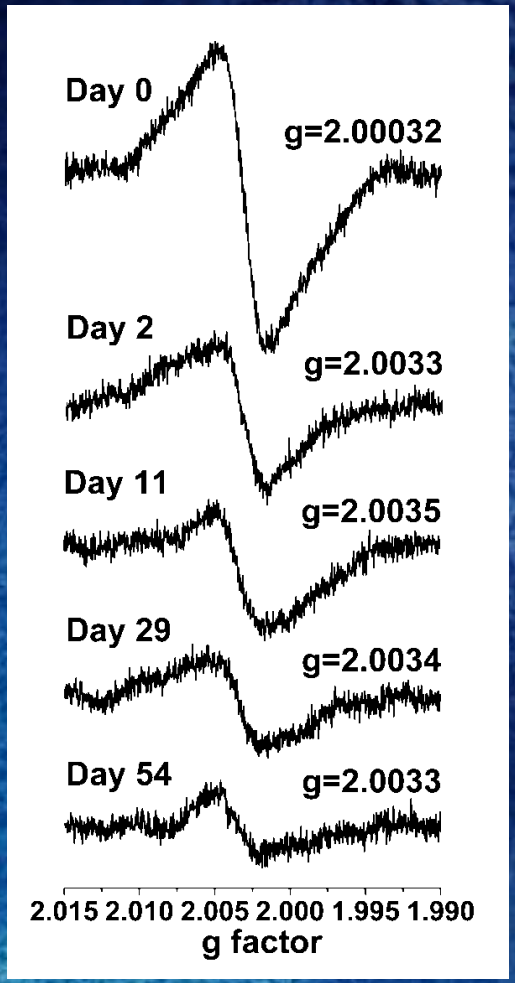


CS tar: 1e16 radicals/g

Barry Dellinger, LSU

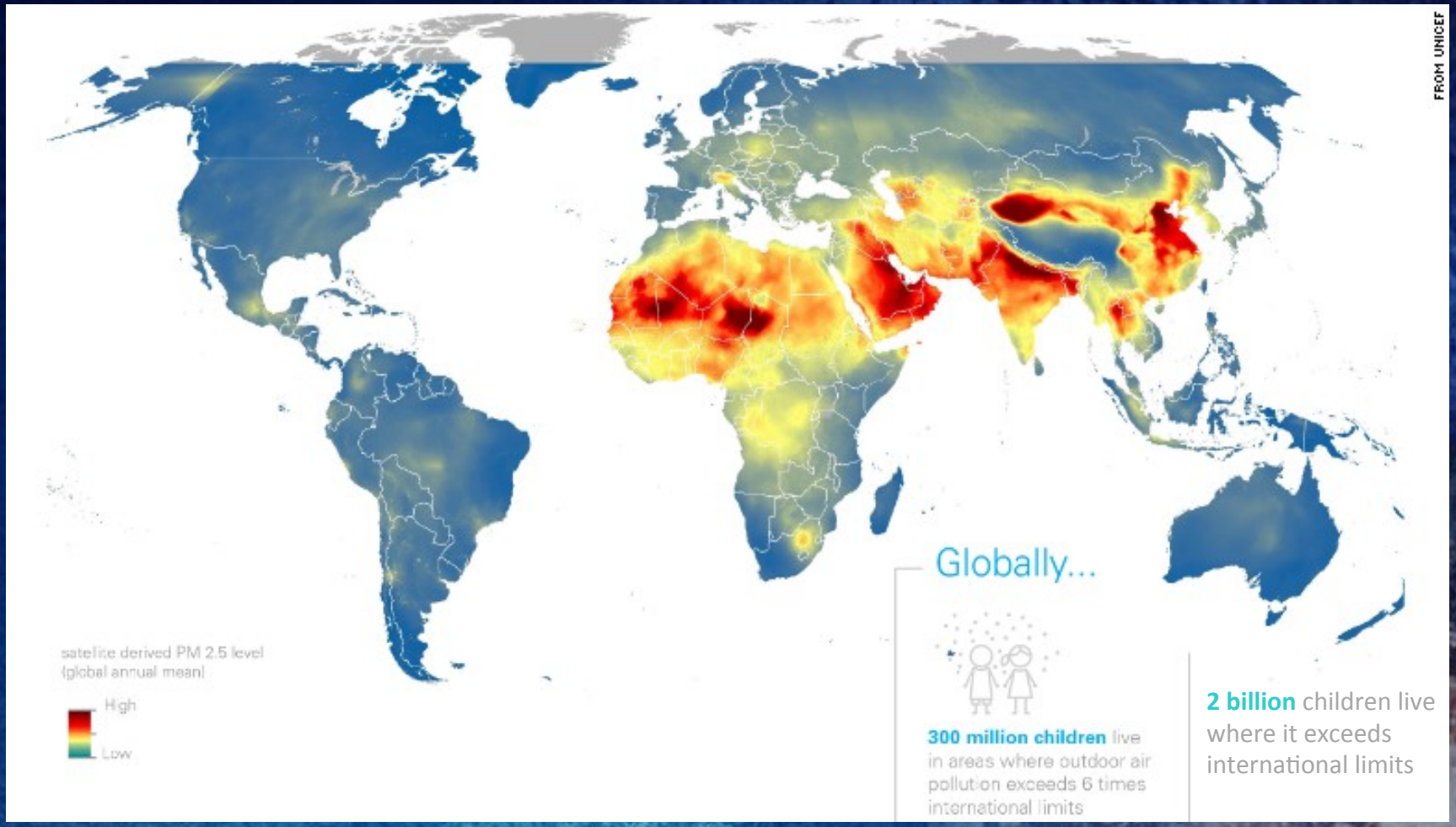
$PM_{2.5}$:1e16 - 1e17 radicals/g

EPFRs in Baton Rouge $PM_{2.5}$



$T_{1/2}$ = 21d

Satellite derived PM 2.5 level (global annual average), 2012-2014



A. van Donkelaar et al. 2016. Environ. Sci. Technol.

Population

2358 total cases of radiographic pneumonia (all three sites)

977 (41.4%) Pneumonia cases from Memphis

167 (17%) not properly geocoded or not included in Memphis Metropolitan Area (MMA)

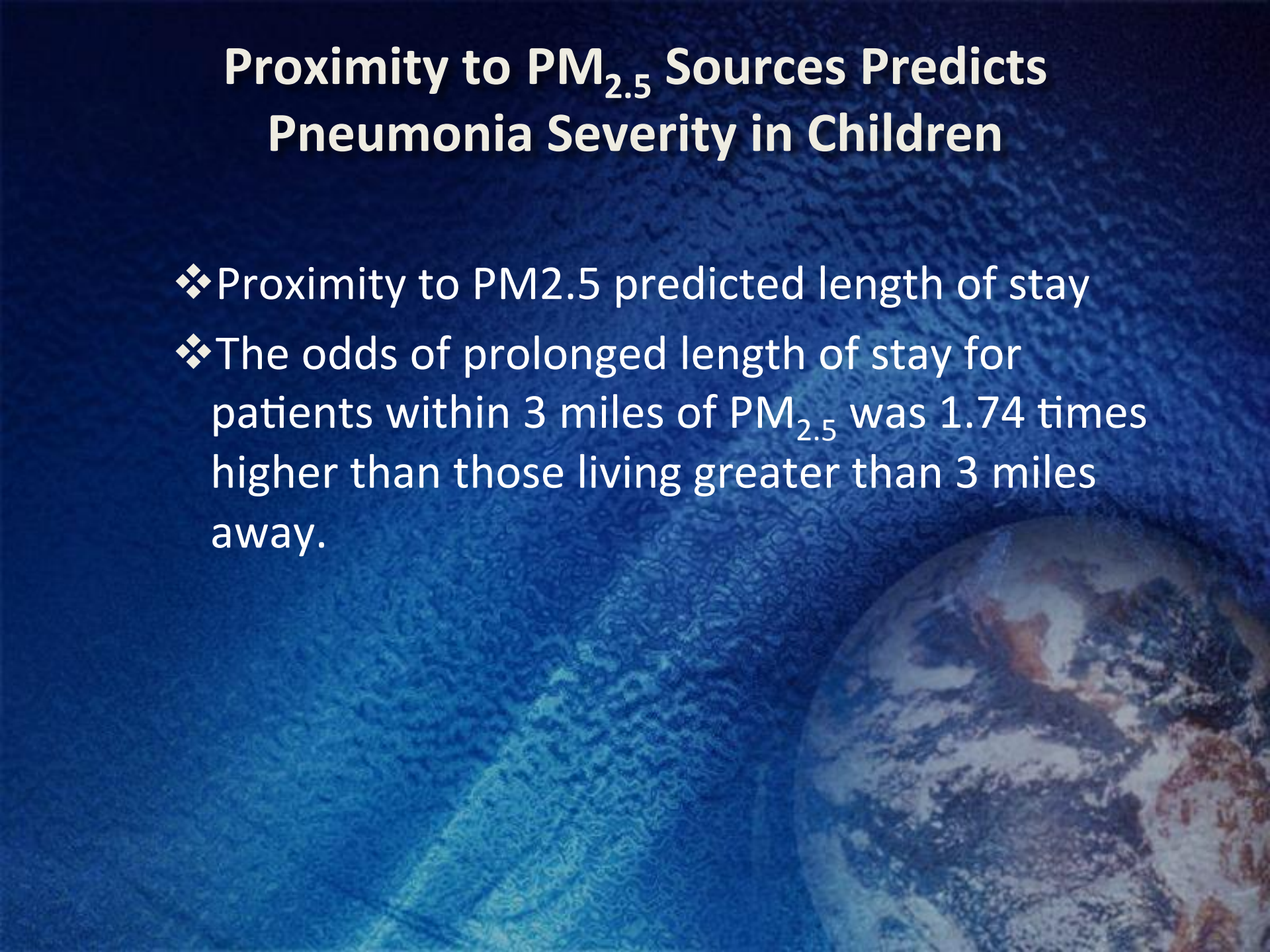
810 (83%)

387* (47.8%) with PLOS

114 (14.1%) admitted to ICU

Proximity to $PM_{2.5}$ Sources Predicts Pneumonia Severity in Children

- Proximity to PM2.5 predicted length of stay
- The odds of prolonged length of stay for patients within 3 miles of $PM_{2.5}$ was 1.74 times higher than those living greater than 3 miles away.



Conclusions

- EPFR exposure in neonates
  - Induces oxidative stress (Balakrishna et al. PFT. 2011;8:11).
  - Disrupts airway epithelium
    - Inducing EMT (Thevenot et al. AJRCMB. 2013)
    - Tolerogenic DCs (Saravia et al. Mucosal Immunol. 2014)
    - Reduces effector T cell responses (Lee et al. PFT 2014)
  - Active suppression of effector T cell responses to RTVI (e.g. Flu) (Jaligama et al. *In revision*).

- The existence of EPFRs in airborne PM2.5 represents a new paradigm for evaluating the toxicity of airborne PM.

Acknowledgements

- **Cormier Lab**
  - Asst Professor
    - Dahui You, PhD
  - Postdoctoral Fellows
    - **Sridhar Jaligama, PhD**
    - **Jagila Minso Wesley, MD**
  - Former Students/Postdocs
    - Jordy Saravia, PhD
    - **Greg Lee, PhD**
    - Paul Thevenot, PhD
    - Pingli Wang MD, PhD
    - Shrilatha Balakrishna, PhD
    - Baher Fahmy, PhD
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- **Tonny Oyana (UTHSC)**
- **Tammy Dugas (LSU-SVM)**

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  - Le Bonheur Foundation Grant to JMW

Le Bonheur Children's Hospital
Methodist Healthcare Family

LSU Superfund Research Center

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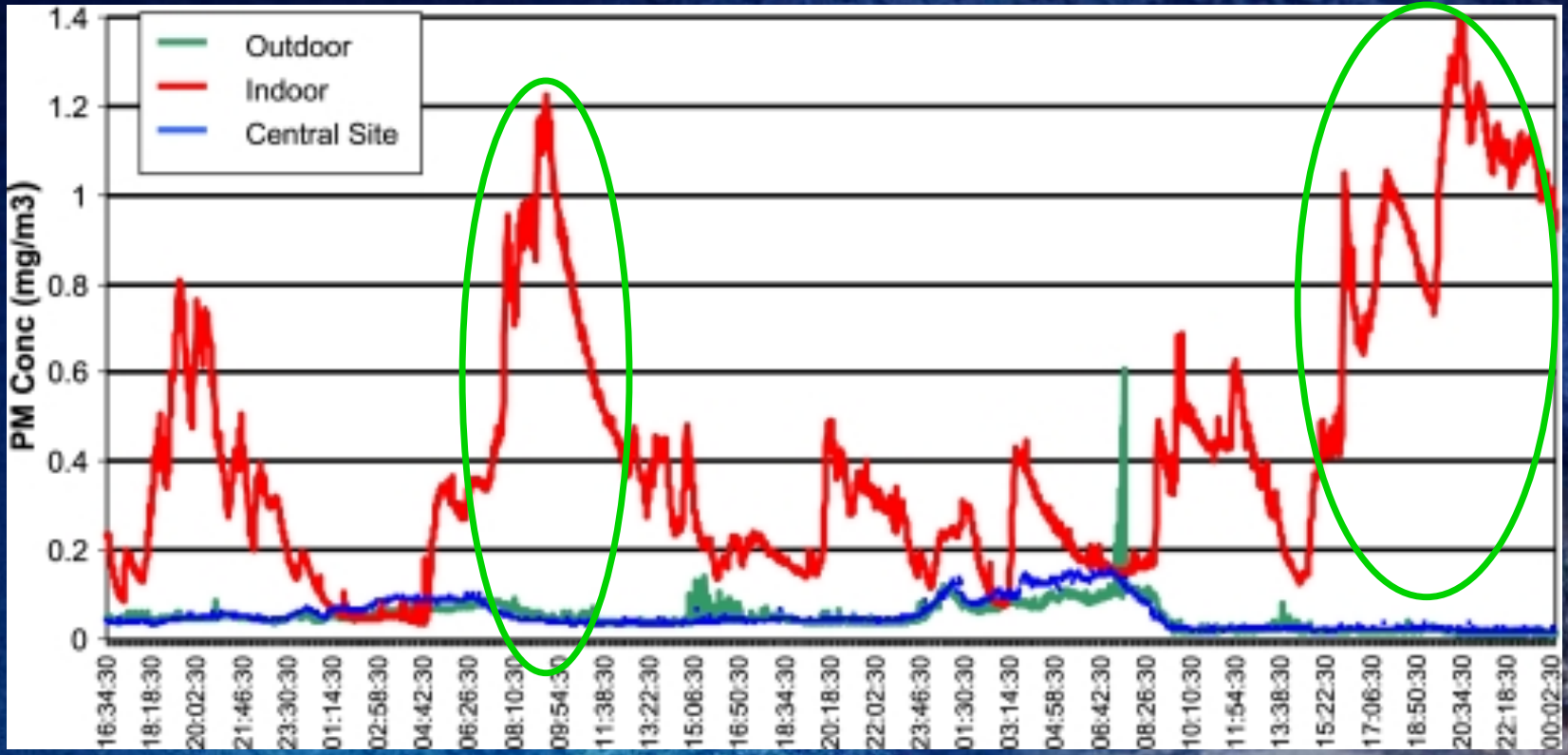
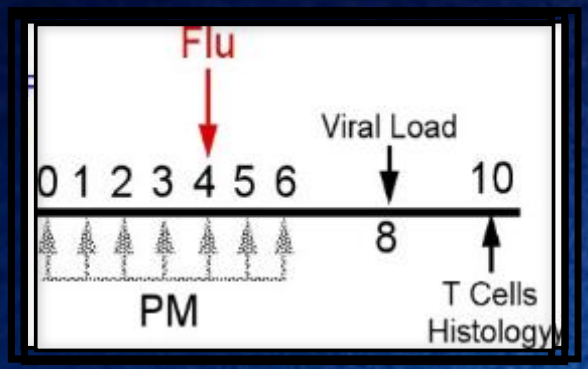
Not Just an Outdoor Concern



Breysse et al. 2010 Proc Am Thorac Soc.

Are Regulatory T Cells Responsible For Increase In Influenza Severity?



Time line:

- Exposure to PM: **3** days age
- Flu Infection: **4** days post-exposure (dpe)
- Peak viral load: **5** dpi
- Peak T effector cell response: **7** dpi
- Viral clearance: 8 dpi

Dose: 200 $\mu g/m^3$
Exposure: Inhalation route

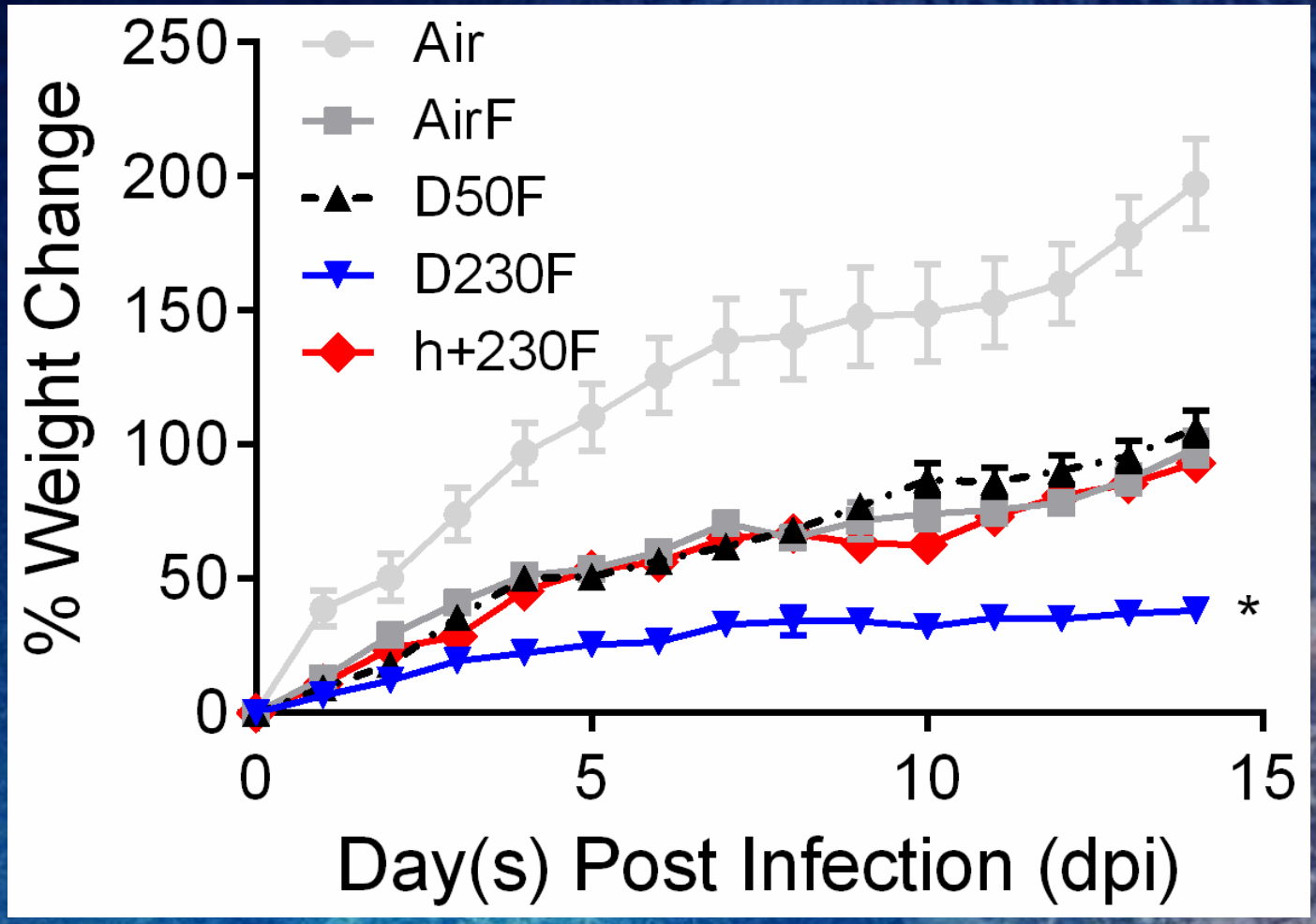
Influenza:
Mouse adapted human influenza strain A/PR/8/34

- Determine the kinetics of Treg induction upon exposure to PM

Treg-kinetics: Profile Tregs at

- 4 dpe (just prior to infection)
- 5 dpi (Peak viral load)
- 7 dpi (Peak effector T cell response)

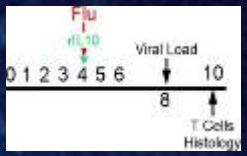
EPFRs Induce Greater Weight Loss in Influenza Infected Mice



n = 16-35

* indicates $p < 0.05$ compared to all other groups

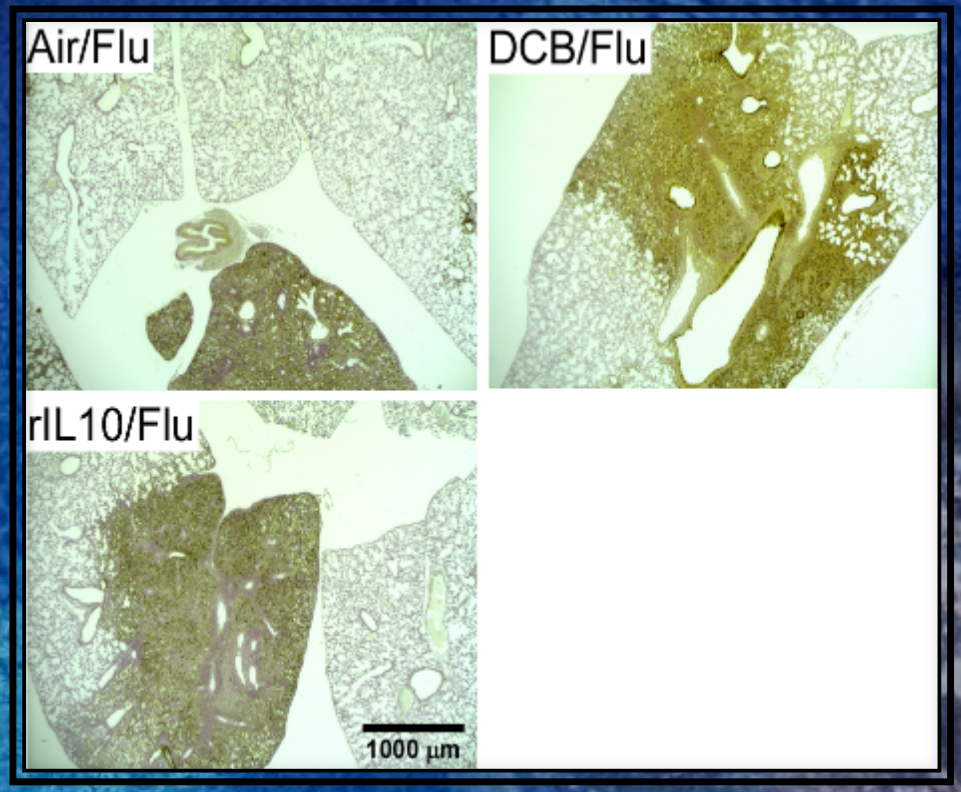
IL10 Alone Enhances Influenza Severity and Viral Load



rIL10

Body weight gain

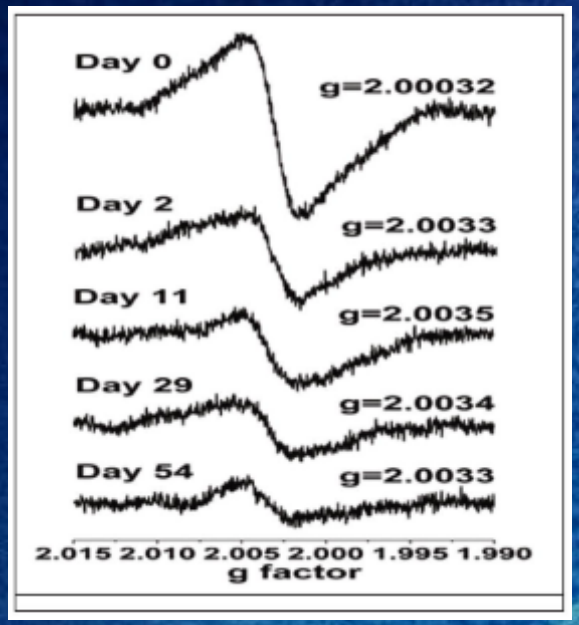
Viral load



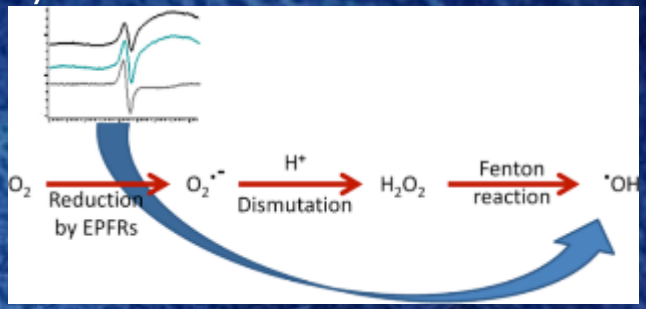
Particulate pollution and Health

Combustion generated ultrafine particulate matter containing Environmentally Persistent Free Radicals (EPFRs)

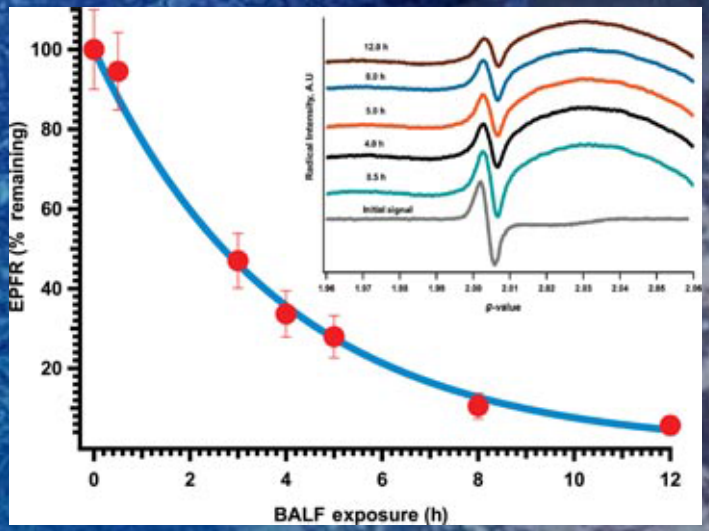
- Aromatic compounds chemisorb to surface of PM through transition metal oxides and form Environmentally persistent free radicals (EPFRs)



Persistence of EPFRs



Kelley et al., Chem Res Toxicol, 2013



Saravia *et la.,* 2012