

TOXICOLOGY STUDIES IN A MULTI-SITE AMBIENT PM RESEARCH EFFORT

A Basis for Discussion

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PROS OF TOXICOLOGY

- Precise control of exposure conditions (concentration, duration)
- Direct evaluation of host factors that may influence response (gender, disease, etc)
- Ability to directly assess contribution of components of pollutant mixtures



CONS OF TOXICOLOGY

- Use of “model” or surrogate PM atmospheres
- Use of relatively healthy human subjects
- Common need to extrapolate from animal or in vitro models to human ambient exposure



Types of Toxicological Studies

- Controlled inhalation exposures of human subjects
- Controlled inhalation exposures of animal models
- Controlled exposures of cells/tissues (in vitro)



IN VIVO EXPOSURES

Some Interpretative Issues

Susceptibility – dosimetry vs sensitivity

Exposure metric – mass or number concentration

Some Design Issues

Surrogate atmosphere – CAPs or what?

Inclusion or exclusion of ambient gases

Type of animal model



IN VITRO EXPOSURES

- Some Interpretative Issues
 - Relationship of dose to “reality”
 - Relevance of isolated cell/tissue response
- Some Design Issues
 - Method of exposure
 - Exposure “atmosphere”

SUMMARY OF ANIMAL CAPS INHALATION EXPOSURE PARAMETERS

■ Exposure Models

- Normal rats
- Bronchitic (SO₂ induced) rats
- Pulmonary hypertensive (MCT) rats
- Cardiomyopathic hamsters
- Dogs (normal; coronary artery occlusion)



SUMMARY OF ANIMAL CAPS INHALATION EXPOSURE PARAMETERS

- Exposure Duration
 - Ranges from single 1 hr to 6 hr/d for 3 days

SUMMARY OF ANIMAL CAPS INHALATION EXPOSURE PARAMETERS

■ Biological Endpoints

□ Pulmonary

- Lavage parameters – differentials, viability, cytokines
- Pulmonary function testing
- RNA analysis for mRNA
- Reactive Oxygen Intermediates

□ Pulmonary immunological

- Bacterial burden of instilled bacteria
- Bacterial killing

□ Cardiovascular

- Pulmonary artery lumen size (constriction assay)
- Peripheral blood cell differentials
- EKG analysis



SUMMARY OF HUMAN CAPS INHALATION EXPOSURE PARAMETERS

- Exposure Subjects
 - Normal adults (18-40 yr)
 - Elderly adults (> 60yr)
 - Asthmatic adults



SUMMARY OF HUMAN CAPS INHALATION EXPOSURE PARAMETERS

- Exposure Durations
 - 2 – 3 hr, some with exercise

SUMMARY OF HUMAN CAPS INHALATION EXPOSURE PARAMETERS

■ Biological Endpoints

□ Pulmonary

- Lavage parameters – differentials, viability, cytokines
- Pulmonary function testing
- Cell adhesion molecule levels
- Inflammatory markers – IL-6, IL-8, etc.

□ Cardiovascular

- Peripheral blood cell differentials
- EKG analysis (Holter)
- Peripheral blood cytokines (IL-6, 8)
- Peripheral blood fibrinogen levels
- Brachial artery diameter



SUMMARY OF IN VITRO AMBIENT AIR DERIVED PM EXPOSURES

- PM Type

- Aqueous extracts of ambient PM
- Total or various residual organic solvent extracts of ambient PM

SUMMARY OF IN VITRO AMBIENT AIR DERIVED PM EXPOSURES

■ Exposure Models

- Human epithelial cell lines (BEAS-2, A549)
- Human neutrophils (lavage derived)
- Human alveolar macrophages (lavage derived)
- Murine alveolar macrophages (lavage derived)
- Murine macrophage cell line (RAW264.7)
- Myofibroblasts

SUMMARY OF IN VITRO AMBIENT AIR DERIVED PM EXPOSURES

■ Biological Endpoints

- Cytokine release
- Activation of NF- κ B
- Alveolar macrophage phagocytic activity
- Cell signaling activity
- Reactive Oxygen Intermediates release
- Heme-oxygenase expression (oxidative stress)
- Intracellular glutathione levels (oxidative stress)



Multi-Site Study

- Consistency across all sites with respect to
 - Exposure mode
 - Experimental design (duration, etc)
 - Biological assays
 - Biological models (animal)
 - Human subjects



OUTLINE OF MULTI-SITE TOXICOLOGY STUDY

- Ambient PM Collection/Exposure System
 - Fine PM concentrator (0.1-2.5 μm)
- PM Exposure Metric
 - Mass and number concentration on size-fractionated basis

OUTLINE OF MULTI-SITE TOXICOLOGY STUDY

■ Analysis of Exposure Atmosphere

□ PM

- Elemental Carbon
- Organic Carbon
- Sulfate
- Nitrate
- Acidity
- Trace metals (transition metals)

□ Gases

- CO
- NO₂
- SO₂
- O₃

OUTLINE OF MULTI-SITE TOXICOLOGY STUDY

■ Experimental Design

- Three exposure groups for each exposure model/subject type

- Control (clean air filtered for PM and scrubbed for ambient gases)
- Ambient gases only (ambient air filtered for PM)
- Total ambient air (exposure to PM with ambient gases)

OUTLINE OF MULTI-SITE TOXICOLOGY STUDY

■ Experimental Design

□ Animal

- Exposure for 3 hr/d up to 30 days in winter and summer at each site
- Subgroups (n=10 each) sacrificed immediately after 1, 15 and 30 d of exposure and at 15 d after last exposure

□ Human

- Exposure for 3 hr twice each season
- Exposure groups consist of 10 subjects/atmosphere

OUTLINE OF MULTI-SITE TOXICOLOGY STUDY

■ Exposure Models

□ Animal (A)

- Normal rats (young adult)
- Compromised animals
 - Old rats
 - SH (spontaneously hypertensive) rats

□ Human (H)

- Normal adults
- Compromised subjects
 - Asthmatics
 - History of mild cardiac disease

OUTLINE OF MULTI-SITE TOXICOLOGY STUDY

■ Biological Endpoints

□ Pulmonary

- Pulmonary function (A,H)

□ Biochemical/Cellular (markers of inflammation/injury)

■ Lavage Analysis (A)

- Cell differentials
- Cell viability
- Total protein
- LDH
- ROI from macrophages
- Cytokines

□ Histological (A) (general pathological evaluation)

OUTLINE OF MULTI-SITE TOXICOLOGY STUDY

- Biological Endpoints - Cardiovascular
 - Physiological (A via telemetry, H-via Holter)
 - EKG analysis
 - Heart Rate Variability
 - Interval analysis
 - Beat rate
 - Waveform analysis
 - Histological (A)
 - Lesions related to inflammation, necrosis, etc.
 - Systemic Blood (A, H)
 - Plasma
 - C-reactive protein
 - Fibrinogen or clotting factors
 - Formed Elements
 - Cell differentials
 - Platelet adherence/activation
 - ROI for leukocytes

OUTLINE OF MULTI-SITE TOXICOLOGY STUDY

- Genomics/proteomics?
 - What does it mean in the context of issues related to PM?
 - Are we at this stage of investigation at this point?



OUTLINE OF MULTI-SITE TOXICOLOGY STUDY

- Dosimetry
 - Exhaled vs inhaled mass/number concentrations



ISSUES FOR FURTHER DISCUSSION

- Dose – Response Paradigm (which endpoint?)
- Threshold for response (which endpoint?)



A THOUGHT OF THE DAY

- A discovery is generally an unforeseen relation not included in theory, for otherwise it would be foreseen.
 - Claude Bernard (1865)