Presentation 12 – Johnnye Lewis

Inhalation of Uranium Oxides to Mimic Gulf War Exposures:

Deposition and toxicity in brain, lung, and kidney

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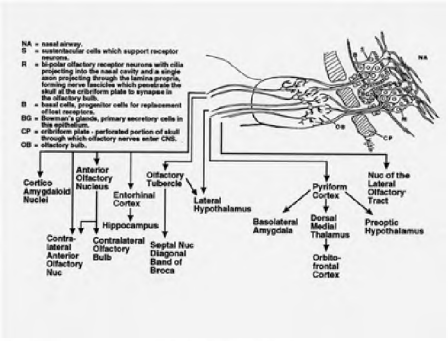
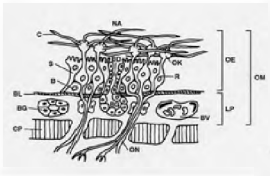
Background of Team

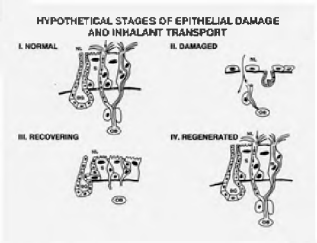
Nose-Brain Barrier



Epithelial structure, damage, & uptake



Normal Epithelium



Perspective on Gulf War Exposures and Disease

What were unique characteristics of Gulf War exposures?

- **Multiple toxicant exposures (+neurotoxicants)**
- **Inhalation – major route of exposure**
- **Potential for sensitization**
  - Many irritants – dusts, smoke, petroleum combustion products
- **Acute and chronic exposures both likely**

DU as a contributor

- **Potential for exposures to DU aerosols**
  - Tank-Impact – High concentration, acute (15 min) exposure
  - March-Through – Low concentration – single day
  - Clean-up – Low concentration – up to 30 day
  - Maintenance – Very low concentration – longer duration
- **Aerosols resulted from impact, combustion, resuspension**
  - Estimates of exposure inconsistent
    - Varied from 300 micrograms to >25 grams
    - Estimates of solubility and respirability varied
  - Respirable fraction could move suspended for hours
- **Other heavy metals neurotoxic and neuroimmunotoxic**

Assembly of multidisciplinary team

...collaborative history

- Inhalation toxicology of metals and neurotox – *J. Lewis, DABT & J. Karlsson (UNM)*
  - **Olfactory uptake of inhaled metals to CNS – *Nose-Brain Barrier***
  - **Neurochem/anatomy of neurodegenerative disease**
- Generation of complex respirable aerosols – *E. Barr (LRRI)*
  - **>20 years of aerosol generation history – metals, solvents, rads**
  - **Studies of factors influencing respiratory tract deposition**
- Quantitative localization of metals – *G. Bench (LLNL)*
  - **Micro-PIXE analysis – high resolution, low detection limit, single-scan – multiple metal analysis; History with biologic tissues**
- Pathology of kidney and lung – *F. Hahn, DAVP (LRRI)*
  - **Historical work in DU shrapnel implantation**
  - **Pathology of inhaled metals and radionuclides**

Hypotheses

1. *Inhalation of uranium aerosols during the Gulf War from combustion of DU-containing weapons resulted in CNS deposition and subsequent neurodegeneration in a subset of those exposed*
2. *Transient conditions which compromise the olfactory epithelium will enhance the entry of uranium and the subsequent development of neurodegeneration*
3. *Markers of neurodegeneration will be correlated with the concentration and pattern of deposition of uranium within the CNS following inhalation exposure*

Scope:

- Expose rats by inhalation to aerosols of uranium varying in solubility, to tantalum oxide, or to air
  - *Tank-Impact Scenario:*
    - **Acute (15 min) – high-level concentrations (500 mg/m^3)**
  - *March-Through Scenario:*
    - **Short duration – moderate concentration (1 mg/m^3 - 6 hrs)**
  - *Clean-Up Scenario:*
    - **Longer duration – moderate concentration (1 mg/m^3/ 6 hrs/ 30 days)**
  - *Maintenance Scenario:*
    - **Long-term – low concentration (0.01 mg/m^3/ 6 hrs/ 30 days)**

Scope (cont'd):

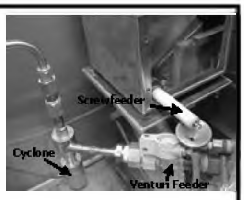
- Expose with and without concommitant respiratory tract inflammation
- Localize U & assess pathology in CNS, lung, kidney
- Assess time course of response through serial sacrifices at 0, 30, 180, and 360 days post-exposure

Importance of team experience

- Relevant exposure methods
  - Magnitude of effect, target organ, and overall toxicity dependant on route and pattern of exposure
- Physiologic exposures
  - Physiological defense mechanisms can be swamped by excessive exposure concentrations
- Intra regional localization *in situ*
  - Non-homogeneous deposition can be diluted by inclusion of non-affected tissues
- History with sensitization protocols

Glove Box Enclosure System

Aerosol Generation System



Exposure Chamber Pass Box

96-Port Nose-Only Exposure Chamber

EXPOSURE

Ed Barr, MSEE
Lovelace Respiratory Research Institute

Uranium atmosphere

Tank-impact scenario

Compound	Conc [mg/m³]	Size Distribution MMAD, μm	GSD
Ta_2O_5	548	2.1	1.9
UO_2	329	1.6	1.7
DUOx	609	2.0	1.4
UO_2	572	2.4	1.4
$UO_2 + UO_3$	305	2.0	1.5
Air			

To test sensitization, endotoxin used to induce nasal inflammation

- $UO_2 + UO_3$ + endotoxin
- DUOx + endotoxin
- Air + endotoxin

U analysis via Nuclear Microscopy

Graham Bench

**Center for Accelerator Mass Spectrometry,
Lawrence Livermore National Laboratory,
Livermore, CA 94550**

Brain Tissue Prep

Dissection:

Three sagittal planes identified to encompass brain regions of interest

PIXE at early time points – frozen tissue only

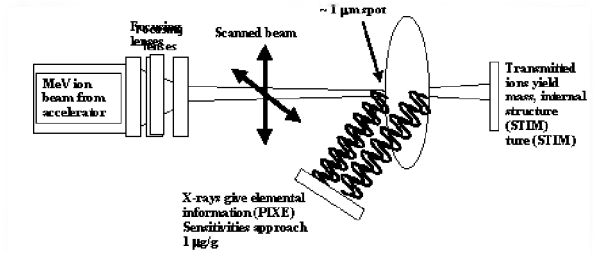
Longer survival, fixation

Preparation of tissue sections for PIXE analysis

- Bilateral sagittal 10 micron cryosections mounted on nylon foils and freeze-dried
- Adjacent sections mounted onto glass slides for immunohistochemical analysis or cytological staining.
- Adjacent slide-mounted sections stained cytologically, photographed and regions of interest identified

- Marked images sent as portable document files (pdf) along with the freeze dried sections to LLNL for analysis.

Nuclear microscopy: spatially resolved elemental and mass quantitation



PIXE - Proton Induced X-ray Emission

STIM - Scanning Transmission Ion Microscopy

Element profiles within identified regions measured with PIXE

- Structures on adjacent stained sections identified on serial freeze dried section with a x 40 optical microscope
- PIXE utilized 3 MeV protons to produce x-ray spectra.
- STIM measured the residual energy of the proton beam after it had passed through the sample.
- Beam spot sizes varying between 0.2 x 0.2 and 0.5 x 0.5 mm were used to irradiate brain regions for 15 microcoulombs of charge.
- X-ray spectra were analyzed and the incoming and outgoing energies of the proton beam as it traversed the sample were used to convert x-ray yields to concentration in units of mg/kg using the the PIXEF analysis package (PIXEF: *The Livermore PIXE Spectrum Analysis Package. A.J. Antolak and G. Bench. Nucl. Instr. and Meth.* ***B90****, (1994), 596-601).*

Deposition at 4 hr post-exposure

Tank-Impact Scenario

Kidney metal uptake

At 4 hr post "tank-impact" exposures, only UO_3 animals showed detectable U in kidneys

Max concentration expected at 7 days post exposure

Pooled tissue to increase sensitivity - UO3 early deaths

Day 6 death (n=5)	34.2 ± 2.1 mg/kg U dry weight
Day 7 death (n=3)	34.6 ± 1.7 mg/kg U dry weight
Day 8 death (n=3)	24.6 ± 1.7 mg/kg U dry weight
Day 10 and 13 death (n=2)	23.4 ± 1.3 mg/kg U dry weight.

Uranium uptake in brains

4 hr post-15 min high dose exposure

Characterization of MDLs across Brain Regions Analyzed

Structure	MDL	SE	95% CI Lower	95% CI Upper
CPu	2.53	0.05	2.43	2.63
Glomeruli	2.60	0.03	2.53	2.66
Mitral	2.62	0.03	2.56	2.69
SN	2.55	0.05	2.45	2.65
Sp	2.67	0.05	2.58	2.76
Tu	2.43	0.05	2.33	2.53
Overall	2.58	0.02	2.54	2.62

No detectable uptake – regardless of form

Pathology at 4 hr post-exposure

Tank-Impact Scenario

(Moribund sacs & deaths at <14 d included)

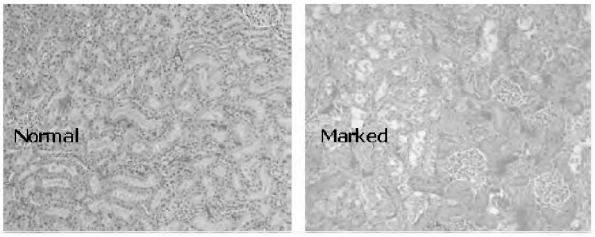
Early Deaths and Moribund Sacrifice of Rats After Inhalation Exposure to UO_3

Days Post Exposure	Number[a] M/F	Histologic Score[b] Kidney Tubular Necrosis M	F	Lung Uremic Pneumonia M	F
2	1/0	0	-	0	-
4	0/1	-	4	-	2
6	0/5	-	3.8	-	3.4
7	0/3	-	3.3	-	4
8	1/2	4	4	3	4
10	0/1	-	3	-	3
13	1/0	3	-	3	-
	3/12				

[a]Initial number at risk: 34 M; 34 F
[b]2 = Mild; 3 = Moderate; 4 = Marked

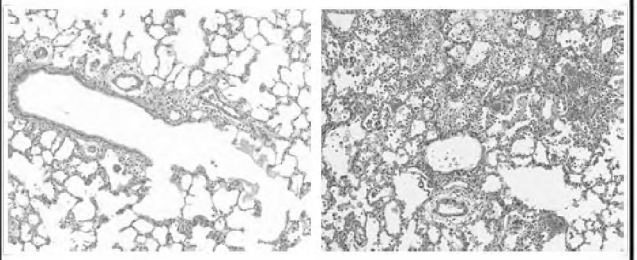
Renal Tubular Necrosis

More soluble UO_3 resulted in renal tubular necrosis and uremia



Uremic Pneumonia

Mild vs. Marked, Fatal



- **Uremic pneumonia was the immediate cause of death**
- **Females had a higher death rate**

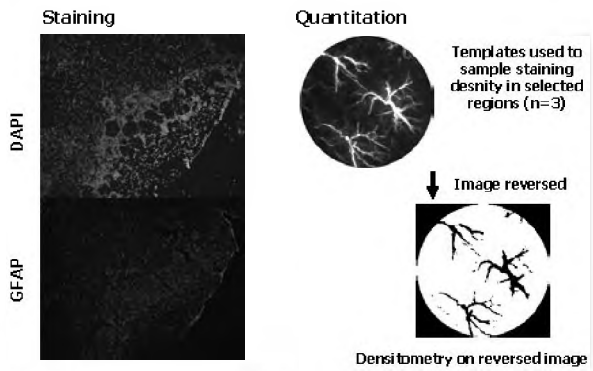
Lung Pathology – 4 hr post *Tank-Impact*

Exposure Atmosphere	Number M/F	Lung Histologic Score (Ave)[a] Alveolar Macrophage Particles M/F	Alveolar Macrophage Hyperplasia M/F
Air	4/4	0 / 0	0 / .25
UO_2	3/3	1.3 / .66	1 / .66
UO_3	3/3	0 / 1	0 / .66
UO_2 +UO_3	3/3	0 / .66	1 / 1.3
TaO_2	3/3	.33 / 1	0 / .66
DUO	3/3	.33 / .66	.33 / .66
Endotoxin	3/3	0 / 0	1 / .66
UO_2 +UO_3 + Endotoxin	3/3	.66 / .33	.33 / 1.3
DUO + Endotoxin	3/3	0 / 1	1 / 1

[a] 1=Minimal; 2 = Mild

- Few particles found in sections, no concentration at broncho-alveolar junction
- Nearly all particles in alveolar macrophages
- Number of alveolar macrophages only minimally increased

GFAP methods



Brain inflammation – GFAP data

Tank-impact scenario

- Solubility related increase in GFAP
- Endotoxin increases GFAP response in all exposures

GFAP 4 hours post 15 min high dose exposure

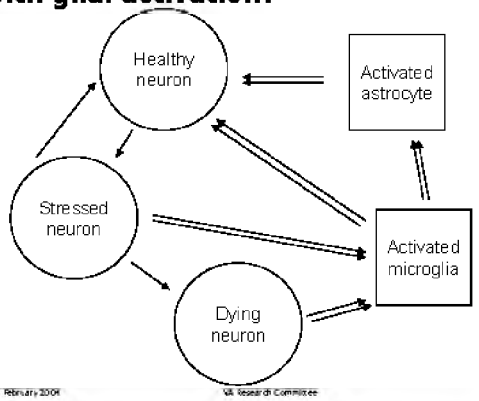
Tank-impact Scenario

Control DUOx UO3

What is the significance of glial activation?

- **Are glia synthesizing & releasing cytokines?**
  - Immunohistochemistry cytokine patterns
  - Protective or degenerative response?
  - Proinflammatory
    - IL-1, IL-6, TNFα or
  - Antiinflammatory
    - IL-10, TGFβ1
- **What are microglia doing?**
  - OX-42, daintain (AIF-1)

What is happening to neurons in regions with glial activation?



Low dose, longer duration U exposures

March-Through Scenario
Clean-up Scenario

Exposure Characterization

- **Nose-only inhalation, rats, male and female**
- **1 or 30 day exposure (6 h/day. 5 days/week)**
- **UO_2:UO_3, 1:1 mixture**
- **Target conc 1 mg/m3**
  - Actual conc 1.02 +/- 0.12 mg/m3
  - Size 1.66 +/- 0.01 micron
  - sigma-g 1.55 +/- 0.11
- **Sacrifices 0, 30, 180, 360 days post-exposure**

Results -- 4 hr post-exposure

- ***Clean-Up Scenario*** (30 d x 6 hr – 1 mg/m³ UO_3+UO_2)
  - no endotoxin – no uptake observed
- ***March-Through Scenario*** (1 d x 6 hr – 1 mg/m³ UO_3+UO_2)
  - no endotoxin – no uptake observed
- ***March-Through Scenario with nasal inflammation***
  - *2 of 6 animals show uptake in glomerulus, 1 also in deeper mitral cell layer*

Conclusions

- **Very Short/High Dose – *Tank-Impact* scenario**
  - no detectable CNS uptake regardless of solubility
  - Solubility-related neuroinflammation
  - Most soluble forms result in extensive renal deposition and renal toxicity
  - Females more sensitive to renal toxicity
- **Short-term/ Moderate Dose – *March-Through* Scenario**
  - Nasal inflammation increases the probability of CNS deposition and transport with low dose inhalation for 6 hr durations
- **Longer-duration/ Moderate Dose – *Clean-Up* Scenario**
  - No uptake observable in animals without inflammation

In progress

- **Characterization of nasal inflammation**
- **30 day exposure with inflammation**
- **Characterizing longer survival times**
- **Continued analysis of neurotoxicity at longer survival times**
- **Additional exposures at lower doses (*Maintenance* Scenario)**

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