

## Microdistribution and Long-Term Retention of $^{239}\text{Pu}(\text{NO}_3)_4$ in the Respiratory Tracts of a Hanford Worker and Experimental Beagles

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### Specific Aims

- ▶ **Specific Aim 1** - Long-term retention of plutonium nitrate ( $^{239}\text{Pu}(\text{NO}_3)_4$ ) in the lungs of an exposed Hanford worker and beagles is due to the accumulation of  $^{239}\text{Pu}(\text{NO}_3)_4$  by alveolar macrophages and sequestration of  $^{239}\text{Pu}(\text{NO}_3)_4$  in fibrotic scar tissue (**physically bound**)
- ▶ **Specific Aim 2** - Inhaled  $^{239}\text{Pu}(\text{NO}_3)_4$  in both humans and beagles may change chemical form to one that is less soluble resulting in **chemically bound** plutonium



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## Absorption Characteristics of Plutonium-Nitrate

- ▶ ICRP states that inhalation of soluble forms of Pu results in rapid translocation
- ▶ Pu-nitrate - Type M (Moderately Rapid) absorption characteristics
- ▶ Lifetime follow-up in exposed Pu worker indicate higher doses than predicted for Type M
- ▶ Changes in functional group (i.e. shape, solubility, SA, chemical compound)

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## Pu-Nitrate Study at PNNL (1975-1992)

- ▶ Beagles as an experimental model
- ▶ Single Inhalation of Pu-Nitrate aerosol
- ▶ Held for life-time care and observation
- ▶ Designed to study effects of inhaling aerosols  $^{239}\text{Pu}(\text{NO}_3)_4$  (176 dogs total)
- ▶ Six dose groups, ranging from 0.062kBq to 202kBq (20 dogs each)

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## Accidental Inhalation of Soluble Plutonium at Hanford (Case 0269)

- ▶ Operator at Hanford (REDOX)
- ▶ Inhaled 58kBq of a  $^{239}\text{Pu}(\text{NO}_3)_4$  aerosol mist
  - Died at age 79 from prostate cancer, 38-y after the inhalation
- ▶ Urinalysis results, fecal data, and terminal lung and lymph node concentrations were fitted (James et al., 2007)
  - Intake ~ 58 kBq
  - Bound fraction,  $f_b \sim 8\%$
- ▶ ~27 Bq of  $^{239/240}\text{Pu}$  remained in the lungs at the time of death
  - ICRP 66 predicts zero for Type M material

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Estimated Dose to Thoracic Lymph Nodes and Lungs in Dogs and a Human Exposed to  $^{239}\text{Pu}(\text{NO}_3)_4$

Dog/Human Case	Initial Lung Deposition (kBq)	Lung Weight (g)	Lymph Node Weight (g)	Time after exposure (days)	Dose to Lung (Gy)	Dose to Lymph Nodes (Gy)
Beagle #1	3.7	82.49	0.735	5289	0.599	1.728
Beagle #2	7.1	125.4	1.117	4041	0.765	1.655
Beagle #3	8.6	93.5	0.833	3861	1.243	2.563
Beagle #4	9.2	116.6	1.039	4431	1.064	2.544
Beagle #5	9.5	123.2	1.098	3197	1.041	1.749
Beagle #6	9.9	156.2	1.392	4720	0.855	2.185
Beagle #7	10.3	166.1	1.48	4385	0.836	1.976
Beagle #8	10.9	110	0.98	4452	1.334	3.205
Beagle #9	12.8	110	0.98	3730	1.565	3.109
Beagle #10	13.1	116.6	1.039	3767	1.516	3.044
Beagle #11	17	126.5	1.127	3118	1.825	2.985
Beagle #12	89.2	169.4	1.509	1278	6.571	4.038
Beagle #13	92.8	116.6	1.039	1041	9.548	4.661
Human Case #0269	58	1120	1.7	14054		

- 5 control beagles and 1 human control

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## Accidentally Exposed Hanford Worker

Organ or Tissue	Activity, Bq Sample <sup>-1</sup>		Concentration, Bq g <sup>-1</sup>	
	<sup>239+240</sup> Pu		<sup>239+240</sup> Pu	
	Meas.	s	Meas.	s
Lung (R)	1.53E+01	2.04E-01	2.39E-02	3.18E-04
Tracheal/Bronchial Node	2.37E-02	8.65E-04	3.65E-02	1.33E-03
Trachea	8.86E-02	2.65E-03	1.05E-02	3.15E-04

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## Histology Staining:

- ▶ Apoptosis – TUNEL
- ▶ Cellular structure – H&E
- ▶ Connective tissues & Interstitial fibrosis – Masson's Trichrome Stain
- ▶ Elastic Fibers – Elastic Van Giesen
- ▶ Glycogen detection – PAS
- ▶ Pro-fibrotic activity – IL-4, IL-13, TGF- $\beta$
- ▶ Pulmonary Interstitial Collagen Fibers
- ▶ Tissue destruction/ regenerative processes – MMP-9
- ▶ VEGF

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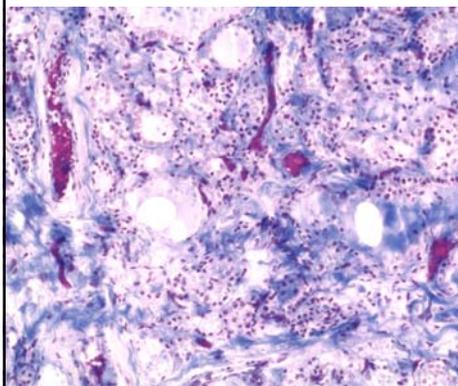
## Sectioning of Tissues

- ▶ 5-6 tissue blocks per exposed dog
  - 2 LN and 3-4 lung samples
  - 2 tissue blocks per control dog
- ▶ 15 tissue blocks prepared from formalin fixed lung tissues (Case 0269)
  - 6 tissue blocks prepared by pathologist
- ▶ 9 slides prepped per block for autoradiography
- ▶ 16 slides prepped per block for staining protocols

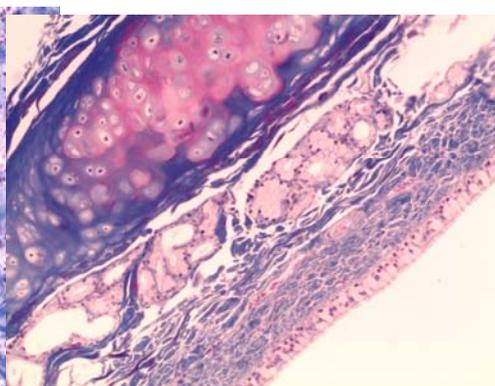


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Trichrome (Human Case 0269)



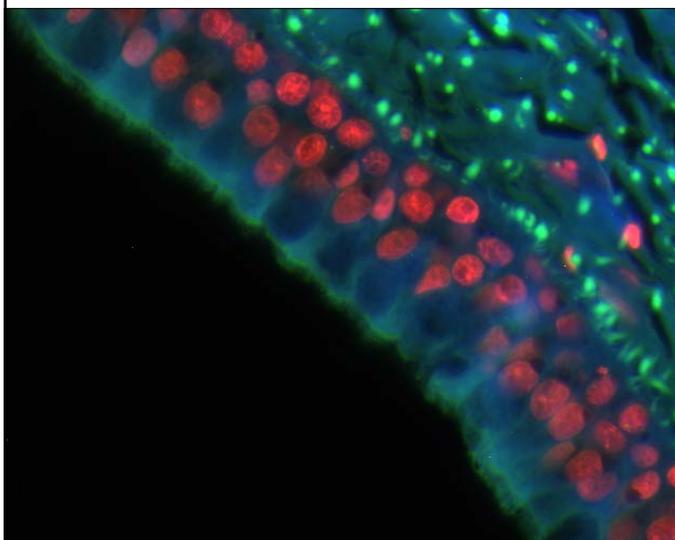
Trichrome (Dog 267 nCi)



- ▶ Collagen
  - Blue
- ▶ Nuclei
  - Black
- ▶ Muscle/cytoplasm/keratin
  - Red

Magnification X10

## Apoptosis (Dog 98 nCi)



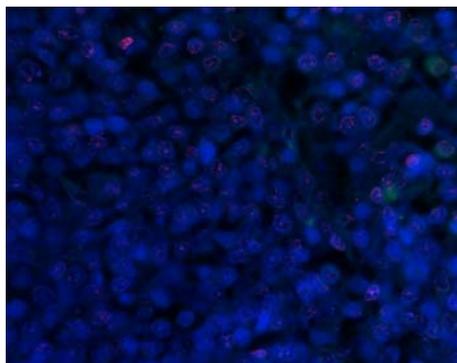
Bronchiole with mucus cells

A lot of apoptosis in the mucus cell layer

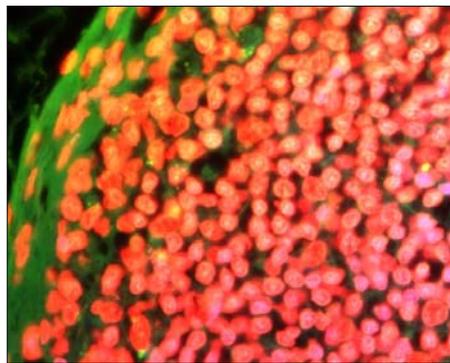
- ▶ Apoptosis
  - Red
- ▶ Normal Nuclear Material
  - Blue
- ▶ Cytoplasm
  - Green

Magnification X60

### TUNEL: Apoptosis (Dog Control)

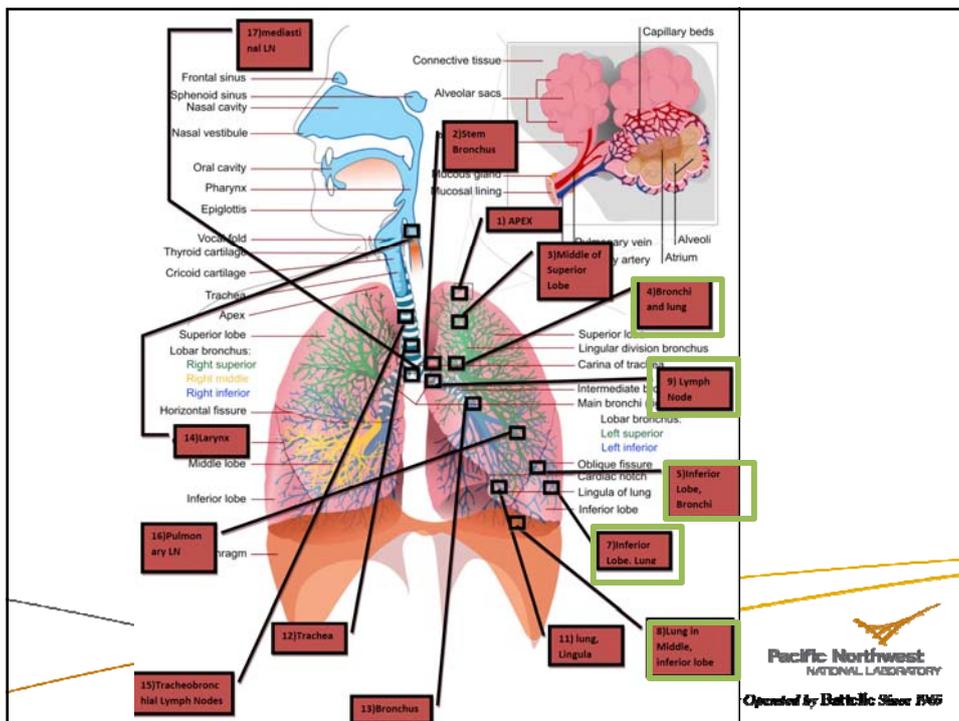


### TUNEL: Apoptosis (Dog 267 nCi)



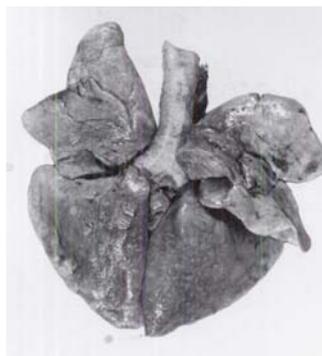
Lymph Node  
Magnification X60

- ▶ Apoptosis
  - Red
- ▶ Normal Nuclear Material
  - Blue
- ▶ Cytoplasm
  - Green

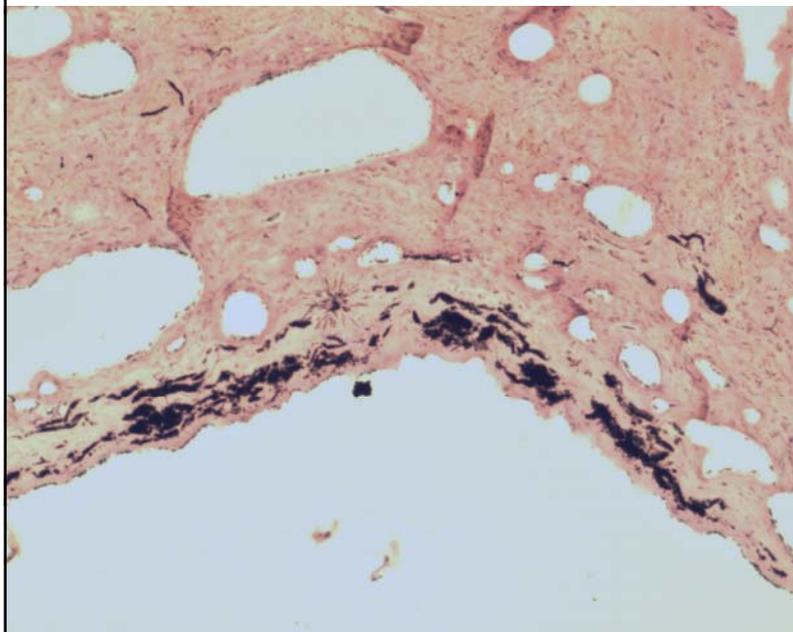


## The Autoradiography Process

- ▶ Slide Preparation
- ▶ Application of Emulsion
  - Ilford K5 (Most Sensitive)
  - 20 $\mu$  thick
- ▶ Development
  - Kodak D19
- ▶ Fixing
  - Ilford Rapid Fixer
- ▶ Staining
  - H&E
- ▶ Analysis

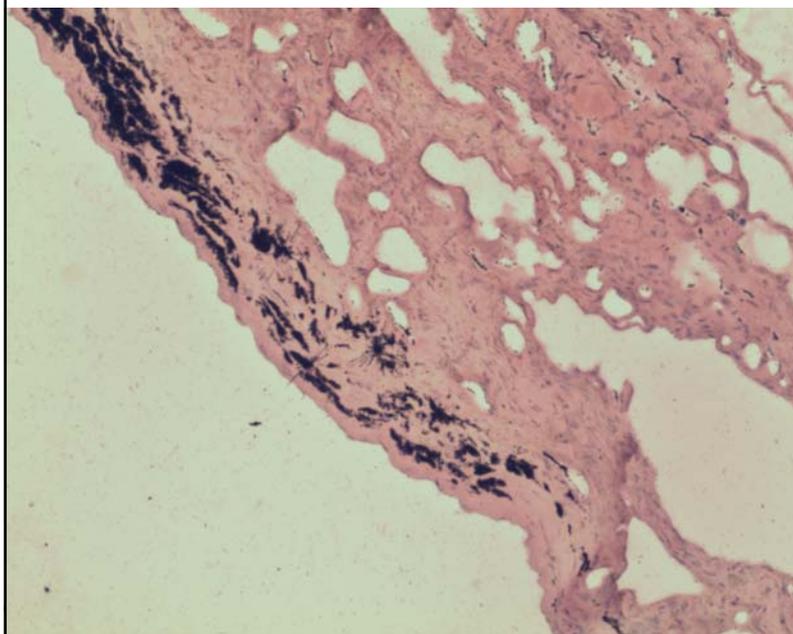


**Human Case 0269**



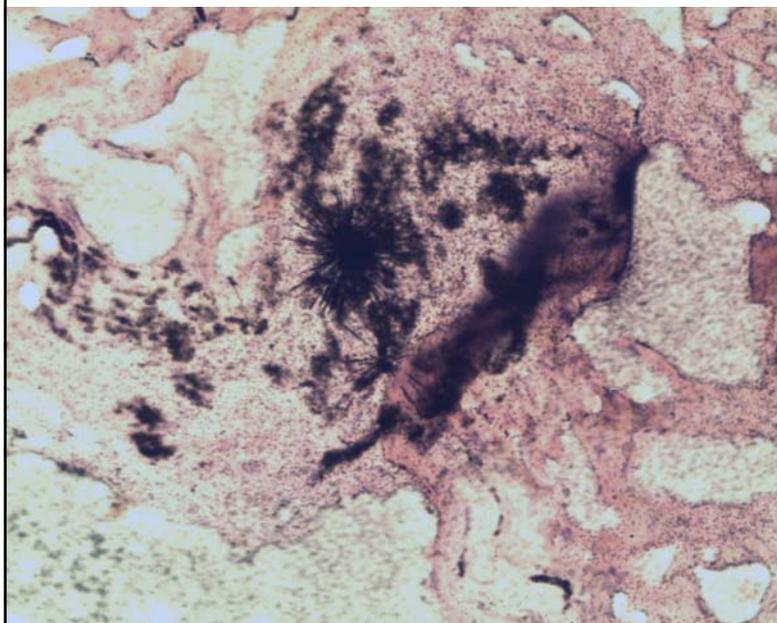
  
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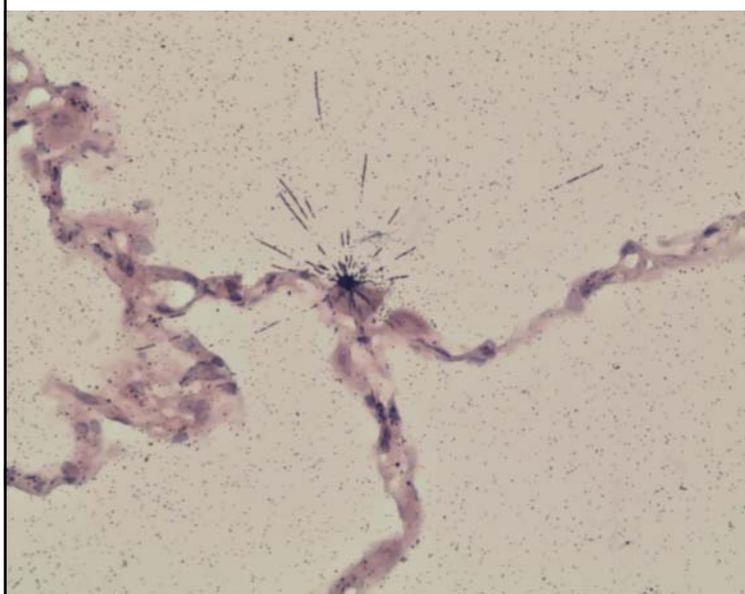
  
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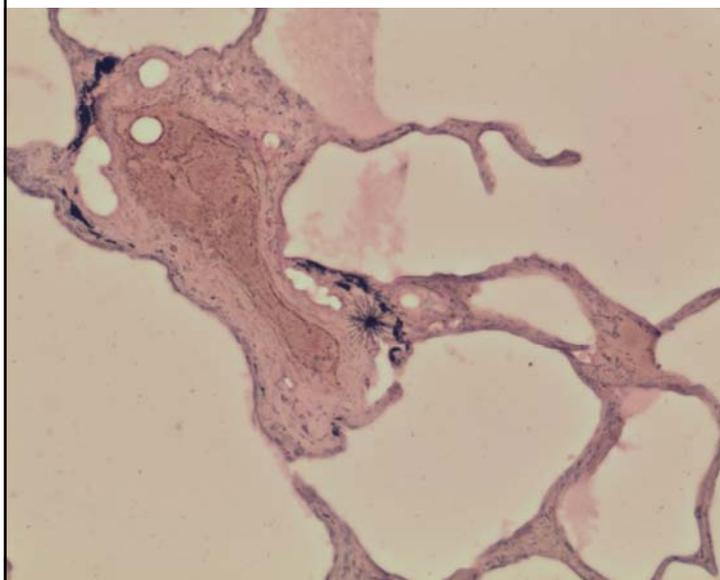
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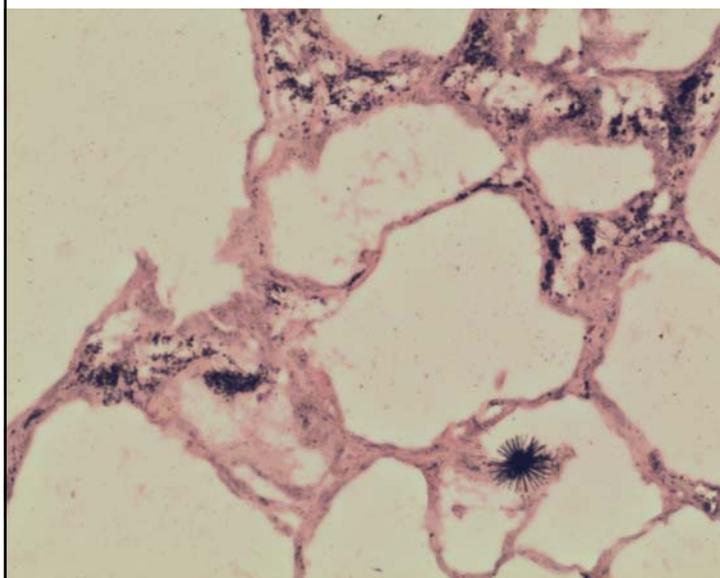
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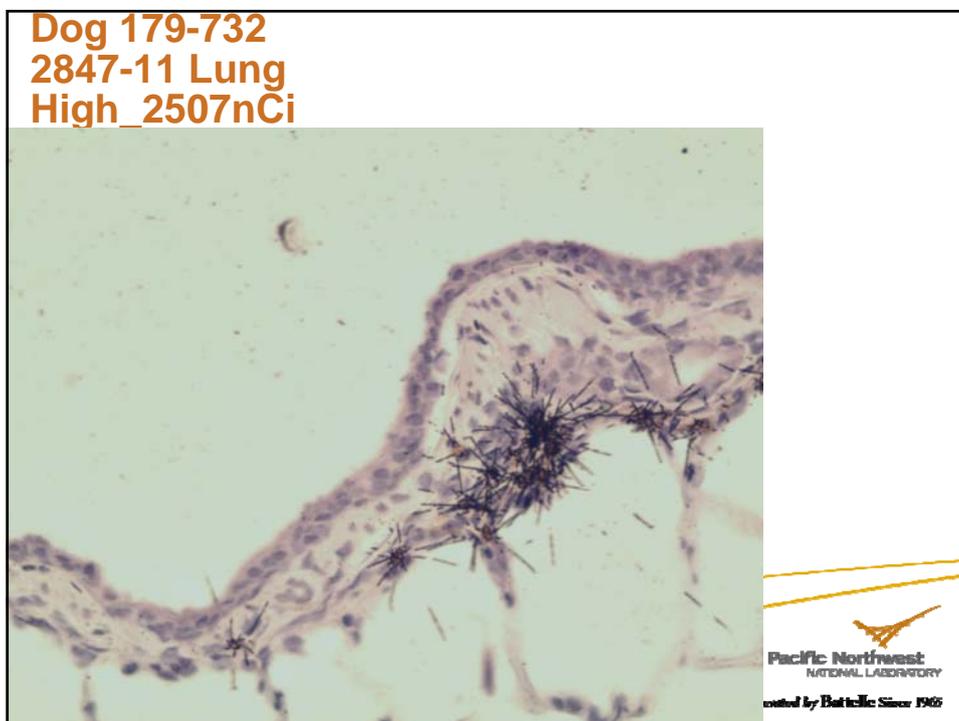
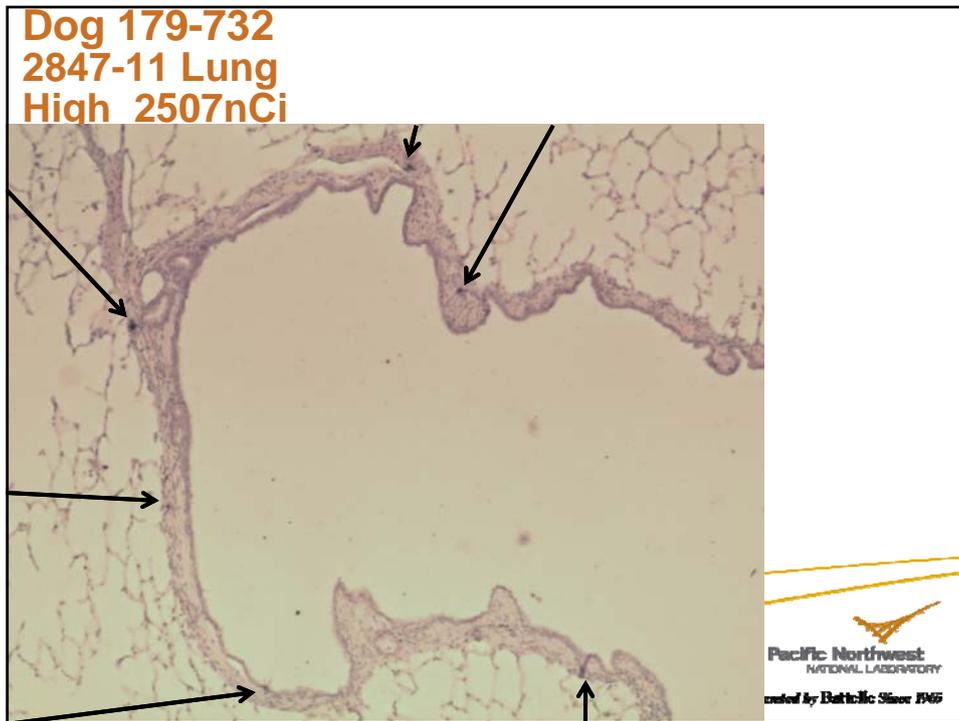


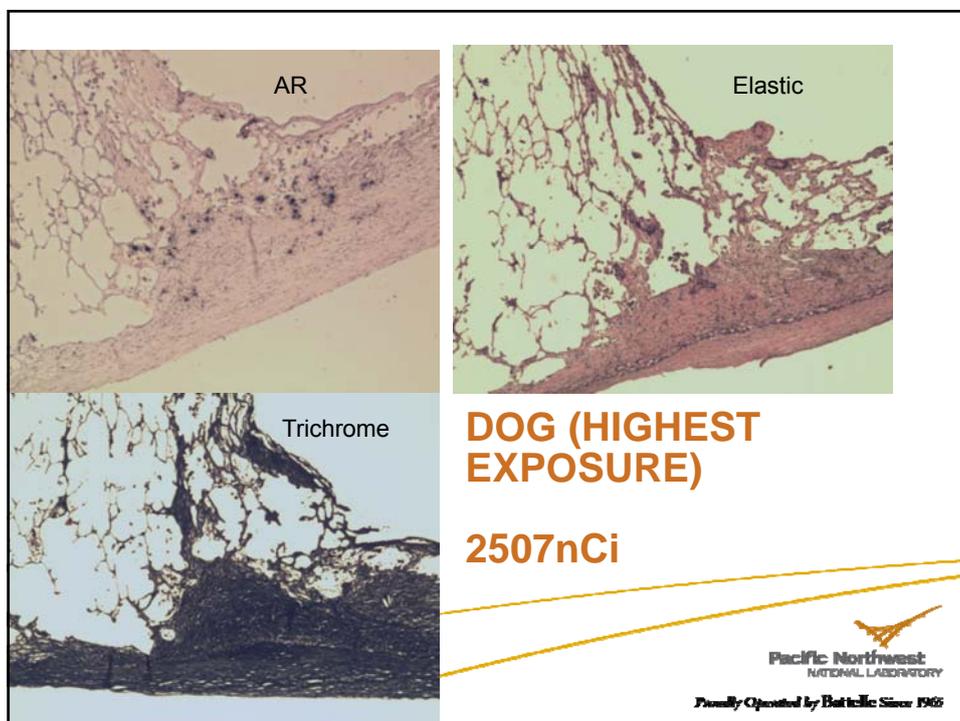
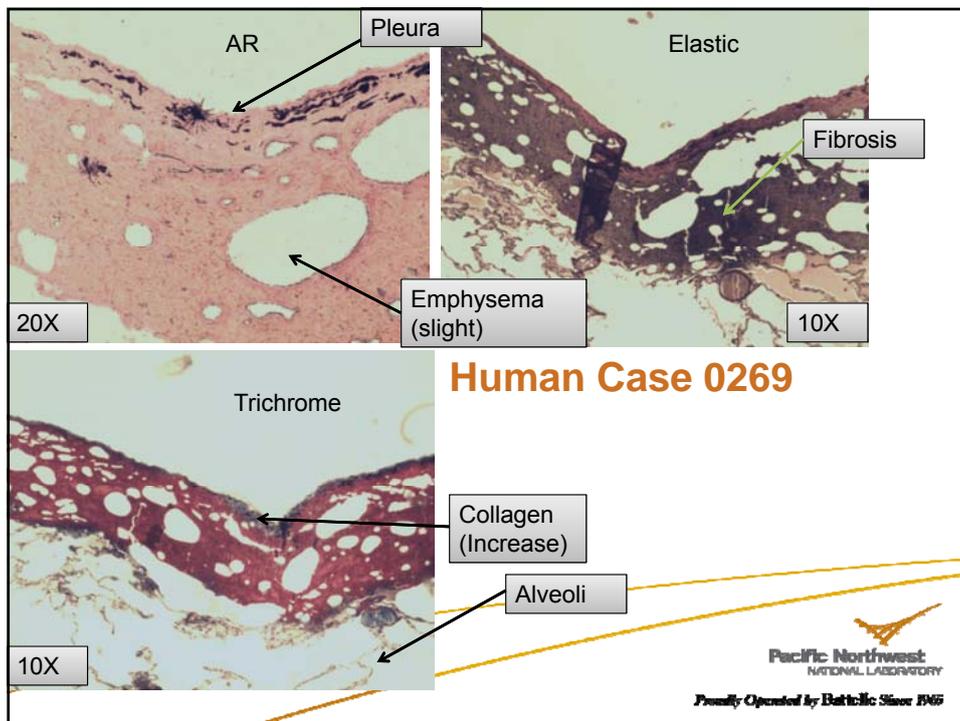
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## Results for Human Case 0269

### ► Samples prepared at autopsy by pathologist:

- Lymph Nodes:
  - No visible alpha activity
  - Carbon deposits throughout
  - This is expected due to Pu not being in particulate form
    - ◆ Otherwise greater translocation would have occurred
- Lung:
  - Alpha activity non-uniformly distributed throughout all specimens
  - Very low concentrations of Pu found associated with bronchi and in all cases none of the tracks penetrated into the more sensitive epithelial cells
  - Encapsulated Pu within scar tissue common in sub-pleural regions
  - Association of Pu with carbon deposits thought to be from cigarette residue

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## Results for Human Case 0269

### ► Formalin-fixed samples sectioned by Xihai and myself:

- Extreme tissue degradation
- Low alpha activity present
- Lung structure almost completely destroyed and often unidentifiable

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## Results for Exposed Beagles:

### ▶ In progress...

- Appears to be more efficient clearing of plutonium
  - Low concentrations in alveolar regions
  - Primarily associated with pleura
- Lower frequency of plutonium encapsulated by scar tissue
- Plutonium is frequently found bound to hemosiderin pigments
- Plutonium seems to be more commonly associated with interstitial tissue of the conducting bronchioles than in the human case

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## Current Status of Project...

- ▶ Autoradiography completed...
- ▶ Histology staining completed...
- ▶ Currently analyzing autoradiographs
  - Counting tracks? % bound to X vs. % bound to Y?
- ▶ Writing thesis
- ▶ Beagle samples currently at Argonne National Lab undergoing XFM/XRF
  - Scintillation counting to determine activity prior to getting beam time

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## Thanks to my committee:

- ▶ Dr. William Morgan
- ▶ Dr. Anthony James
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- ▶ Dr. Sergei Tolmachev
- ▶ Dr. Antone Brooks

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