



Armed Forces Institute of Pathology

DEPLETED URANIUM PROGRAM

Repository and Chemical Analysis of Biological Samples

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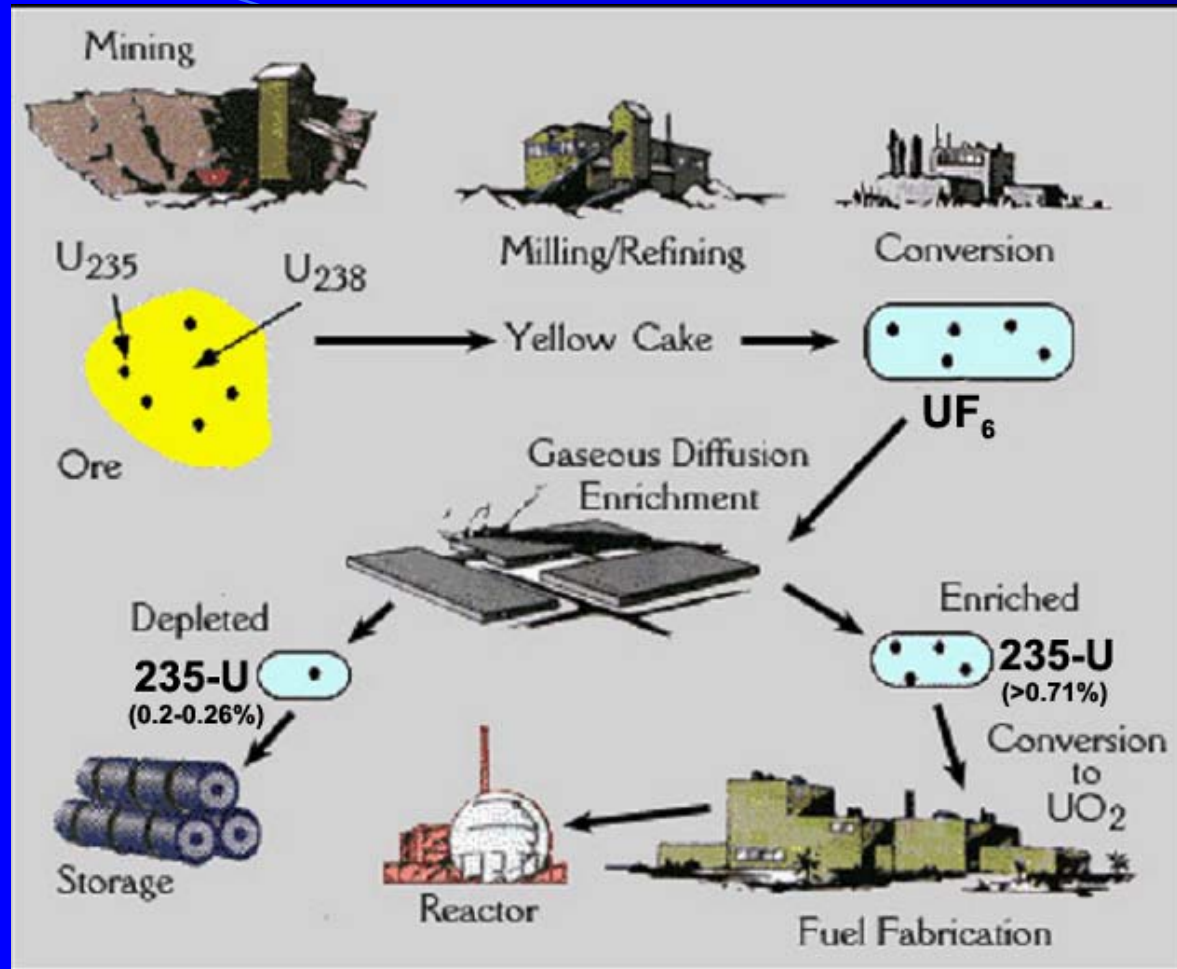
Depleted Uranium Registry

- Histopathological effects and analytical examination of embedded DU fragments in tissue removed from shrapnel wounds.
- Supports the VA-Baltimore Center medical surveillance program by measuring the levels of uranium in patient's urine and identifies the specific source of exposure by accurately measuring uranium isotopic ratios.
- Archive samples to provide a link between clinical research, pathology and toxicological analysis.
- Maintain an intra-lab quality assurance program on DU analysis (participating labs : CDC and AFIP)



What is depleted uranium?

Left-over (by-product) of the industrial ^{235}U enrichment process



• Natural U

- 0.0055% ^{234}U
- 0.72% ^{235}U
- $10^{-7}\%$ ^{236}U
- 99.274% ^{238}U

• Depleted Uranium (DU)

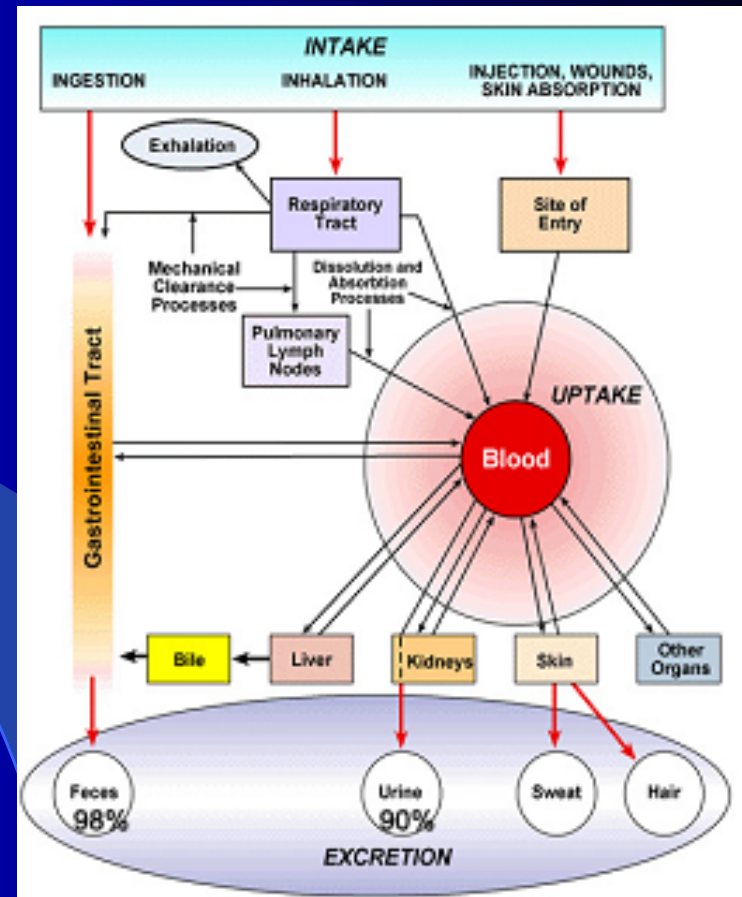
- 0.00061% ^{234}U
- 0.203% ^{235}U
- small amount ^{236}U
- 99.29% ^{238}U



DU Exposure Assessment – *Clinical, Pathological and Chemical Samples*

• Chemical Pathology and Analytical Assessment of U and DU in:

- Tissues
- Urine
- Whole blood
- Semen
- Embedded fragments
- “Lavage” or wound irrigation fluids



Kidneys, bone, liver: Soluble form of uranium dioxide

Lungs: Insoluble form of uranium dioxide

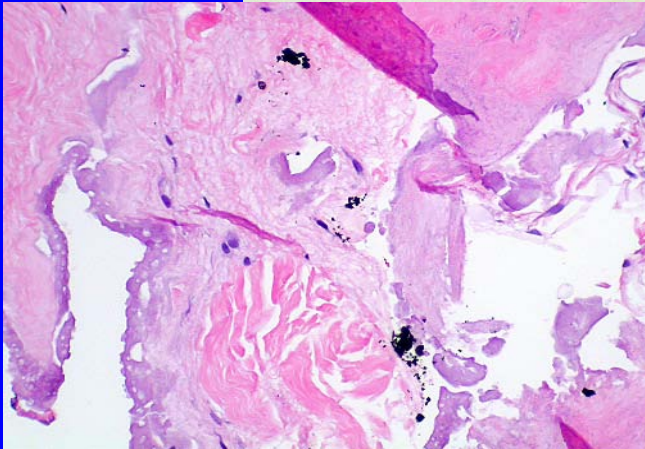


DU Exposure Assessment – *Tissues and Fragment Analysis*

Case 1

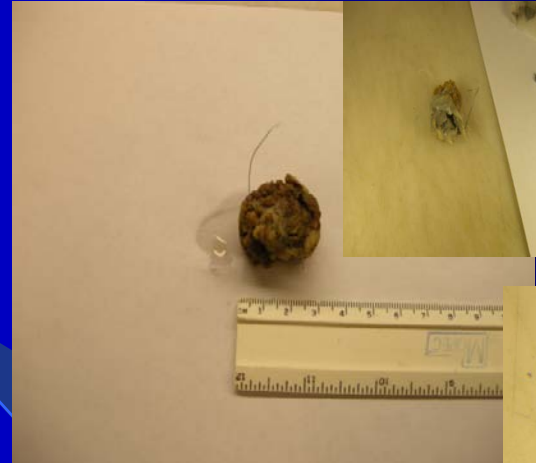


Gulf War veteran struck with 'friendly' fire while inside a vehicle reinforced with DU. Shrapnel removed from right leg after 12 years.

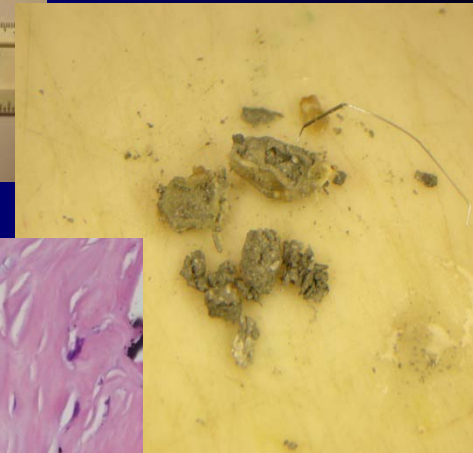


Dense fibrosis of surrounding tissue and scattered black metal fragments (x10)

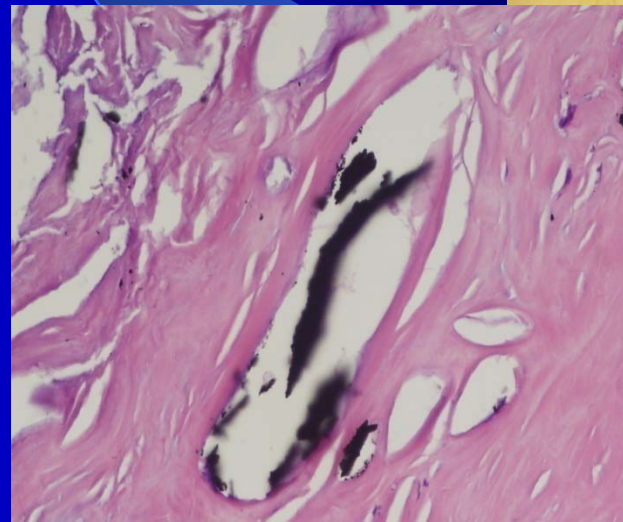
Case 2



Gulf War veteran;
Fragment recently removed
April 2010

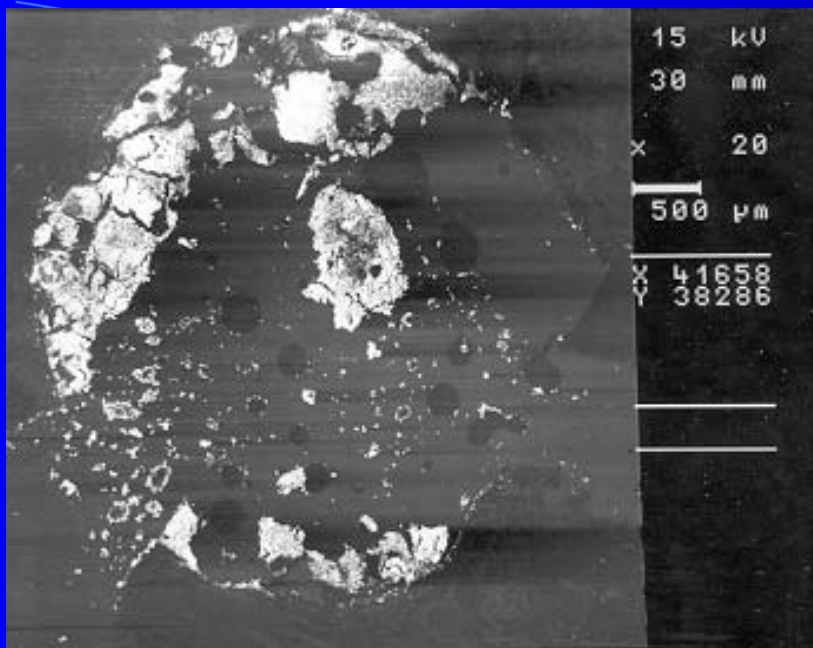


Fibro-adipose
soft tissue
showing a
typical foreign
body reaction

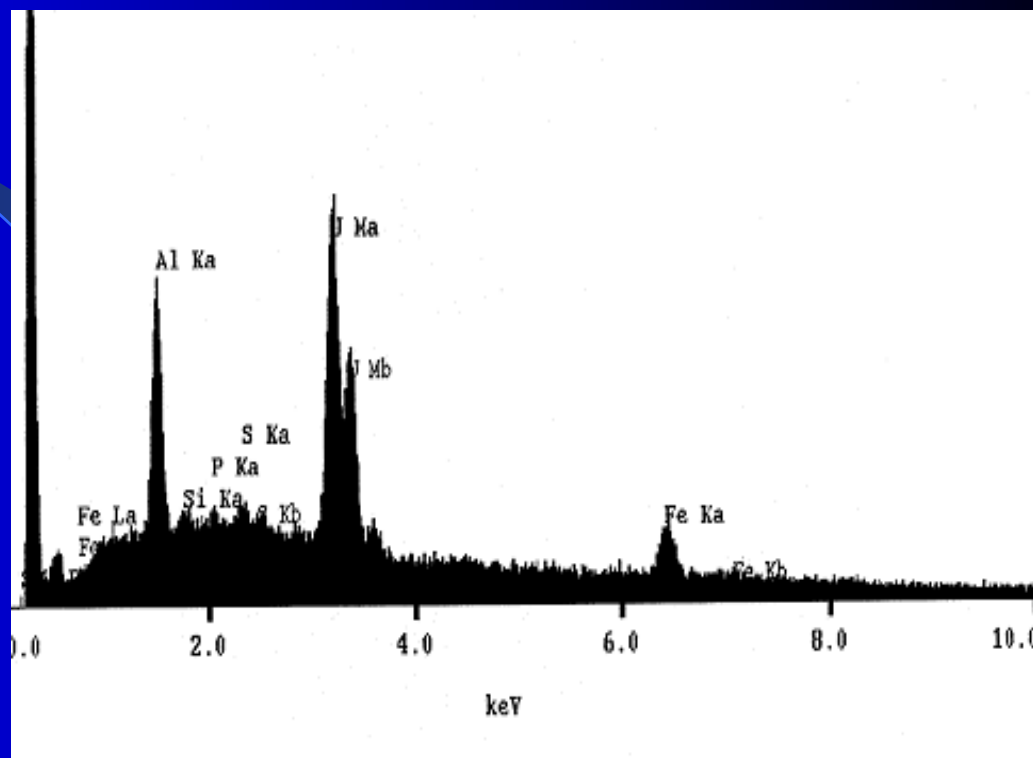




Tissue Analysis - Microscopy



Scanning Electron Microscopy photomicrograph of DU inclusion in tissue



Energy dispersive x-ray microanalysis of DU inclusion in tissue shows U, Al, Fe

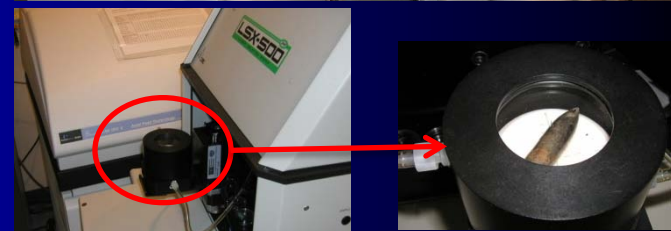


AFIP Depleted Uranium (DU) and Embedded Metal (EMF) Fragment Program

Analyzing fragment composition is an essential function of the AFIP DU and EMF Program

- Chemical analysis of removed fragments
 - Surface chemistry
 - Total fragment composition (over 35 metals)
 - Metals: Al, As, Cd, Cr, Co, Cu, Fe, Mn, Ni, Pb, Sn, Sb, Ti, U (DU), W, Zn
 - Plastics/polymer components: Isocyanate, Acrylics, Diethylhexylphthalates

Instrumentation and methodology available: ICP-OES, ICP-MS (quadrupole), HR-ICP-MS, Microscopy (IR and laser-based), SEM-EDXA, X-ray fluorescence



Laser ablation ICP-MS



X-Ray fluorescence spectrometry



Sample preparation for determination of total uranium and isotopic uranium ratios

• Urine

- Total uranium – 4x dilution, 500 ng/L ^{233}U internal standard; analyzed by ICP-MS
- $^{235}\text{U}/^{238}\text{U}$ ratio – 4x dilution for samples above 25 ng/L; APEX preconcentrated, analyzed by sector-field ICP-MS
- $^{235}\text{U}/^{238}\text{U}$ ratio – for $10 \text{ ng/L} < \text{total U} < 25 \text{ ng/L}$ we use 20mL urine, 2ml nitric acid ashed for 8 hours at 150°C followed by 3 hour at 475°C , analyzed by HR-ICP-MS
- $^{236}\text{U}/^{238}\text{U}$ precipitated by adding ammonium hydroxide, precipitate diluted with 2% nitric acid, APEX preconcentrated, and analyzed by HR-ICP-MS

Anal Bioanal Chem (2005) 382: 73–79
DOI 10.1007/s00216-005-3173-9

ORIGINAL PAPER

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Florabel G. Mullick · Katherine Squibb
Melissa A. McDiarmid · Jose A. Centeno

**Uranium analysis in urine by inductively coupled plasma
dynamic reaction cell mass spectrometry**



Sector-Field
HR-ICP-MS



Sample preparation for determination of total uranium and isotopic uranium ratios

● Blood

- Total Uranium – 0.5 – 1.0 mL WB microwave digested in concentrated nitric acid + hydrogen peroxide, 4 ug/L In as internal standard, analyzed by sector-field HR-ICP-MS
- $^{235}\text{U}/^{238}\text{U}$ ratio – 0.5 – 1.0 mL WB, digested in concentrated $\text{HNO}_3/\text{H}_2\text{O}_2$ – APEX preconcentrated, analyzed by sector-field HR-ICP-MS.





Sample preparation for determination of total uranium and isotopic uranium ratios

- Semen

- Total Uranium – dry ashed by concentrated nitric acid in muffle furnace, cooled to room temperature, reconstituted with 2% HNO_3 . 500 ng/L ^{233}U as internal standard, analyzed by sector-field HR-ICP-MS.





Limits of Detection

AFIP DU Program

- Urine

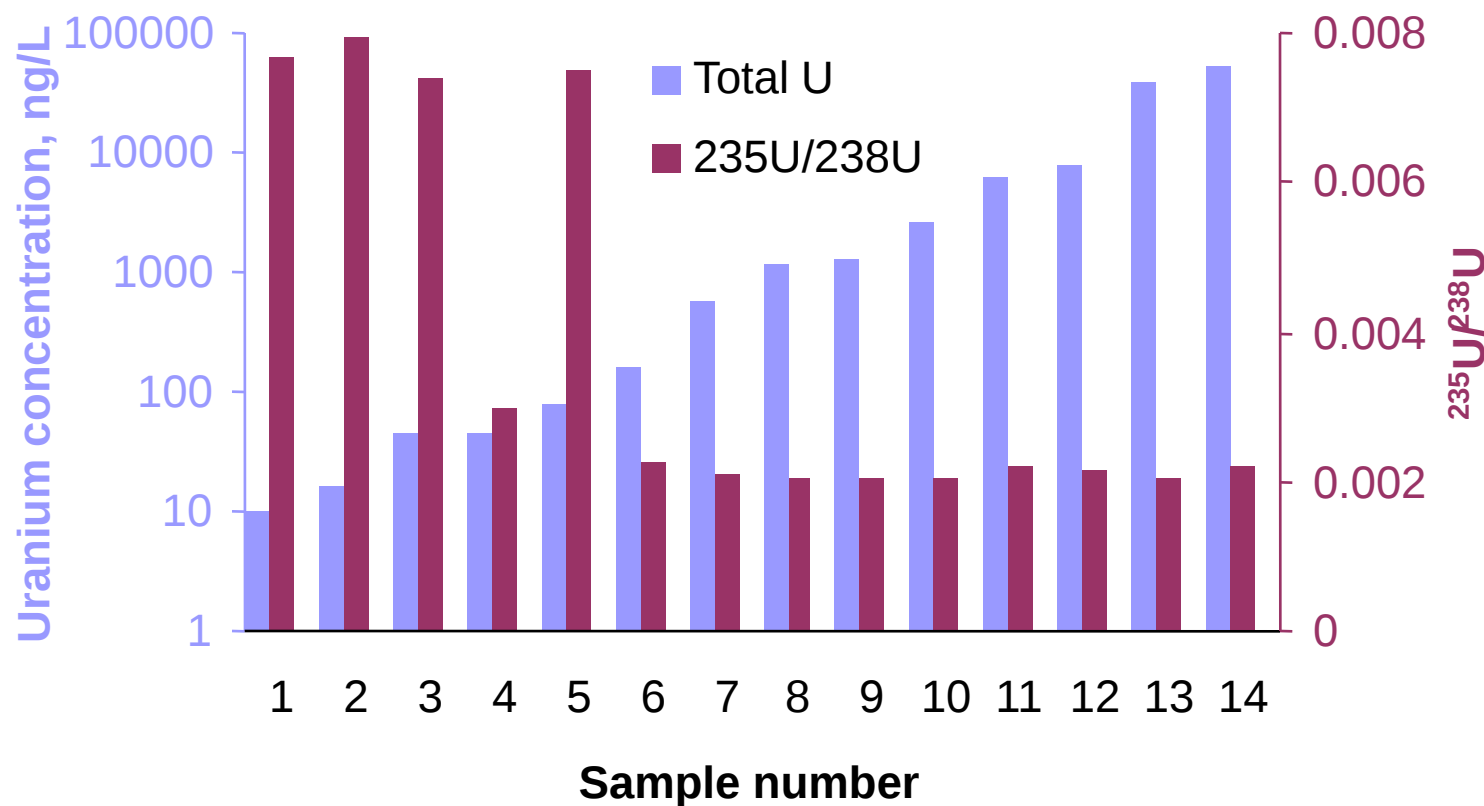
- Total U – 0.4 ng/L
- Isotopic U ratio – 10 ng/L total U

- Blood

- Total U – 3 ng/L based on 1.0 mL blood
- Isotopic U ratio – 60 ng/L total U for 1.0 mL blood

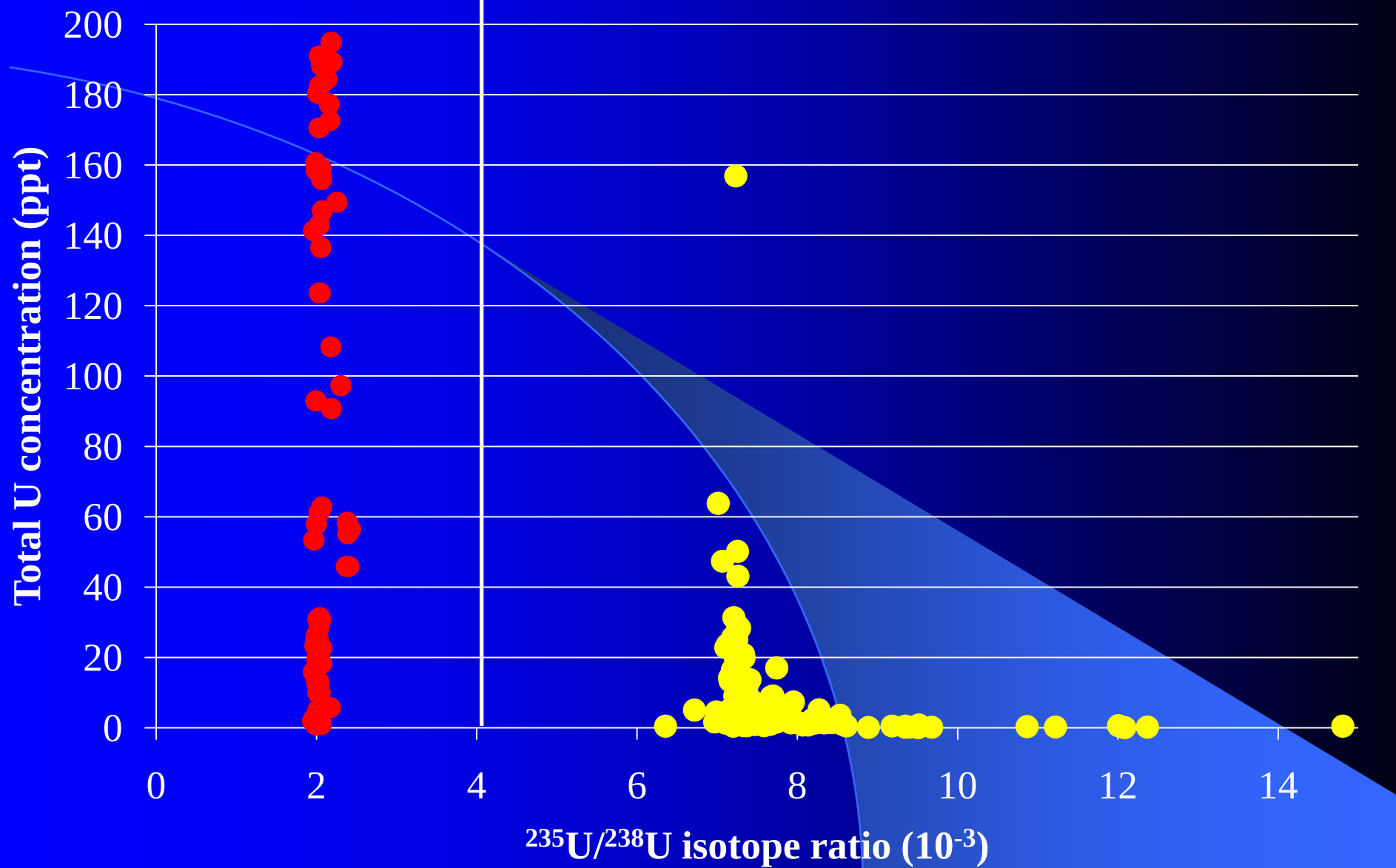


Total uranium and DU measurements in urine



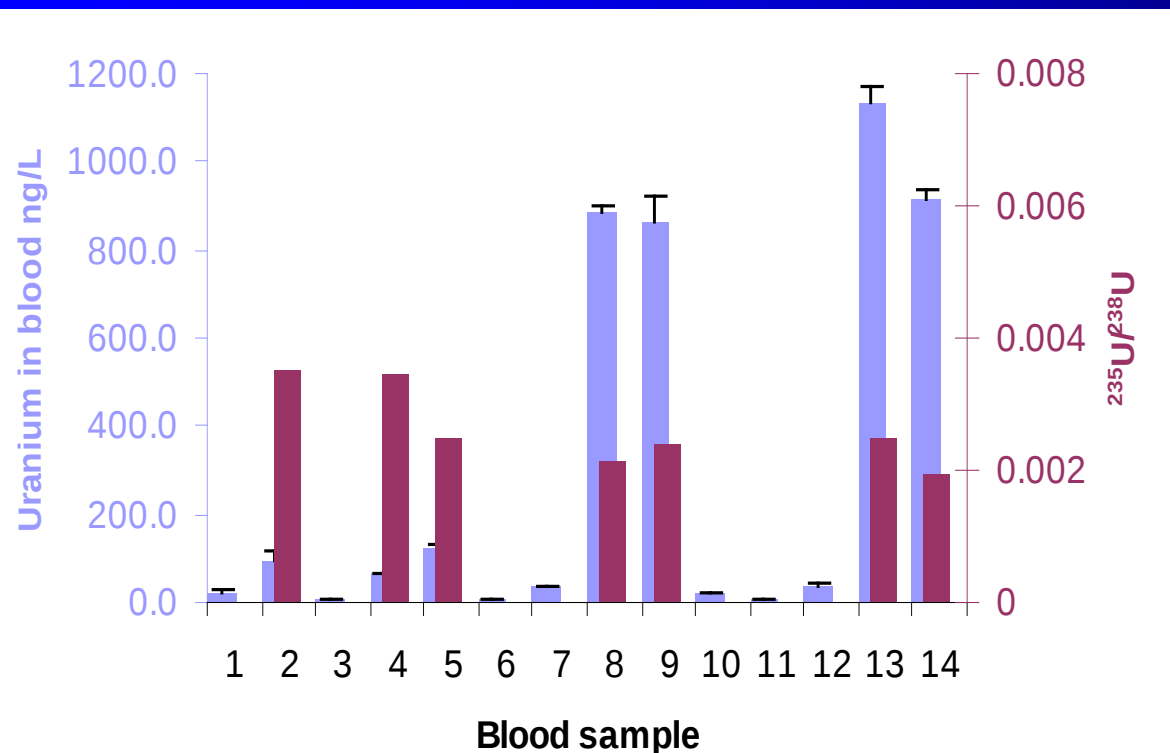
Depleted
Uranium

Natural Uranium





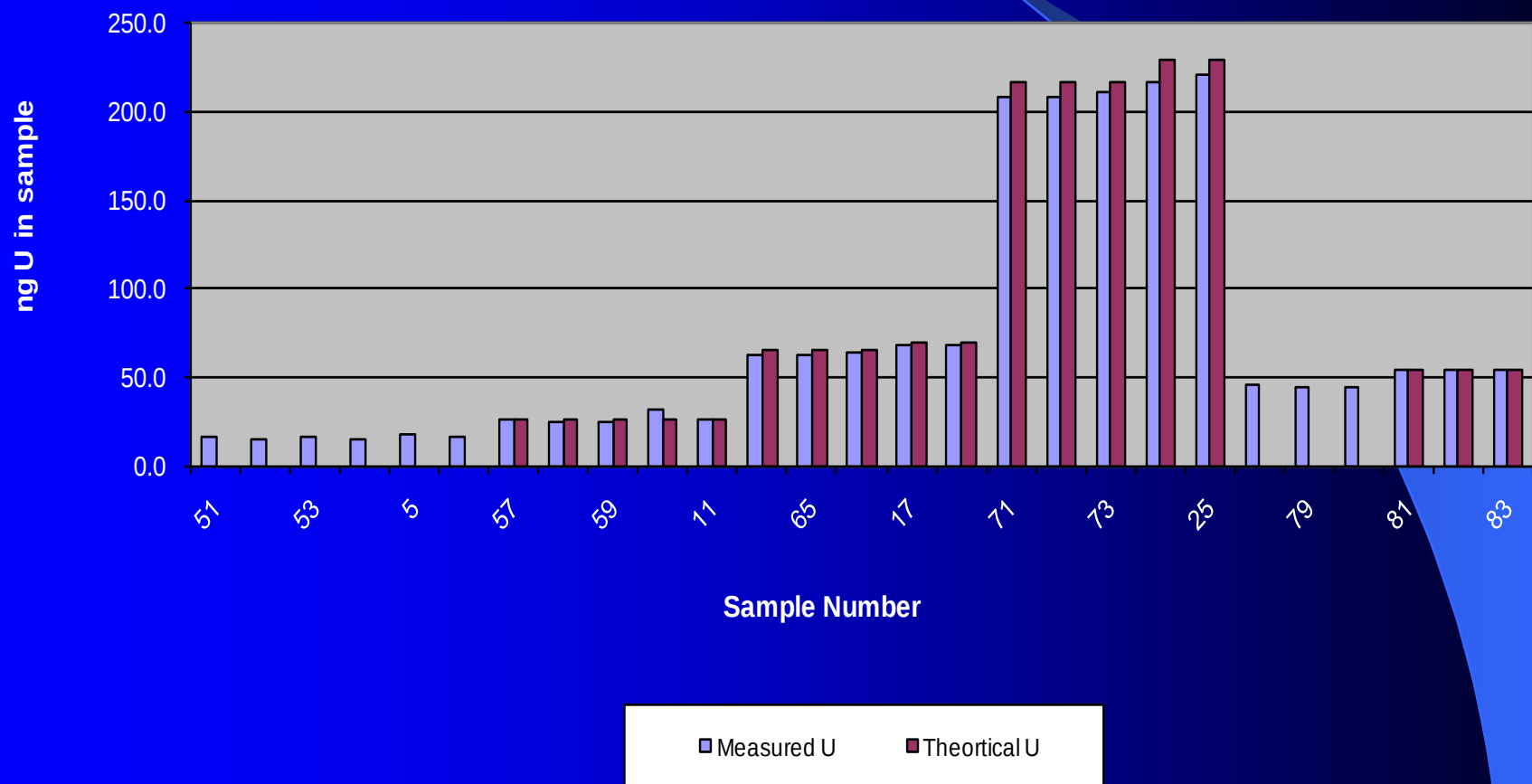
Total uranium and DU measurements in blood



ID #	Total U, ng/L	$^{235}\text{U}/^{238}\text{U}$
1	20	
2	97	0.0035
3	7	
4	66	0.0035
5	123	0.0025
6	9	
7	38	
8	882	0.0022
9	867	0.0024
10	20	
11	9	
12	38	
13	1134	0.0025
14	915	0.0020

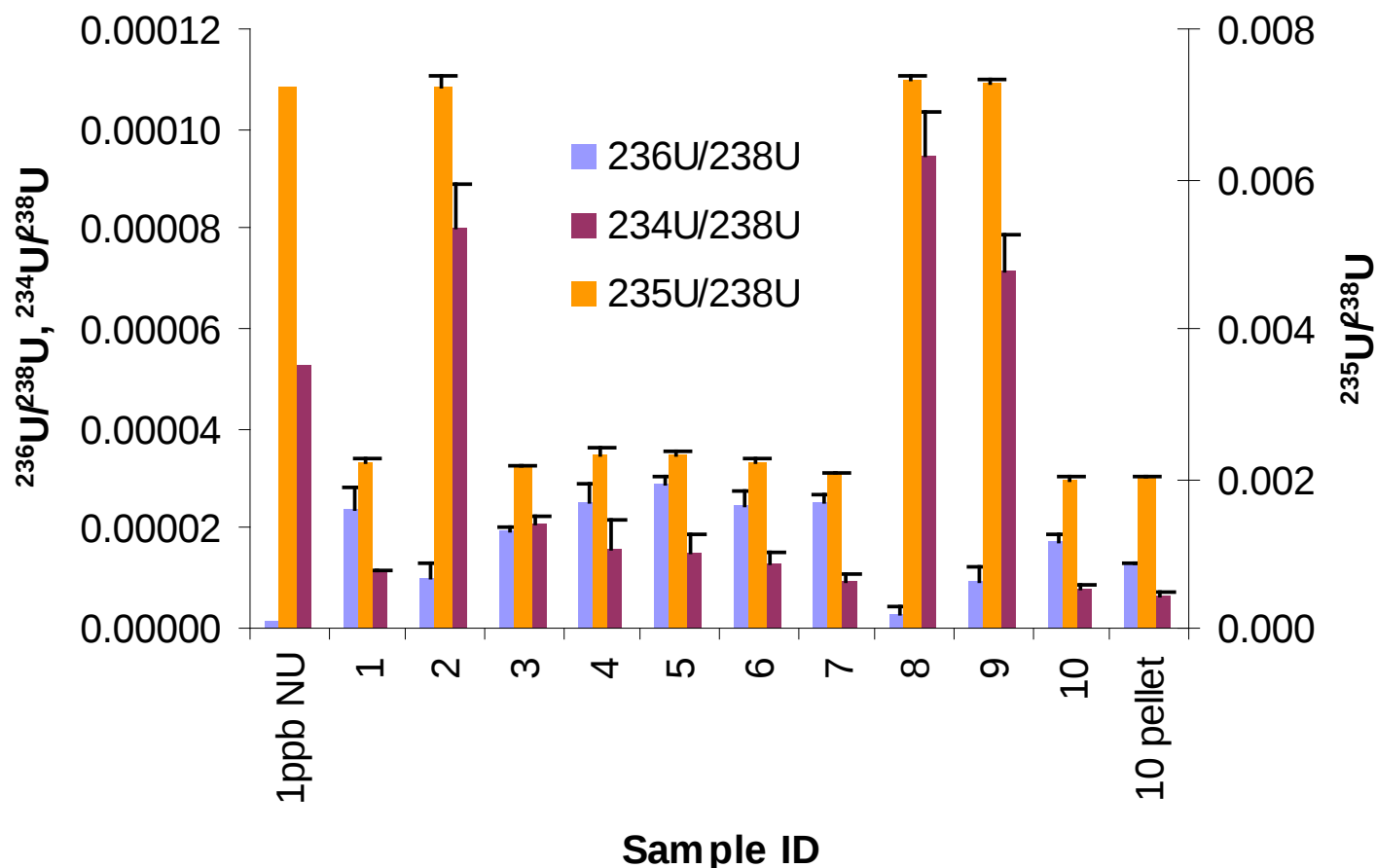


Semen





Uranium isotope analysis in urine





AFIP Depleted Uranium Program

Key Contributions

1. Maintain the ONLY DoD-VA chemical and medical surveillance program on DU exposure and health effects
2. The AFIP DU Registry has provided support to:
 - a. 442nd National Guard Military Police Co. Orangeburg, NY
 - i. Over 100 urine samples analyzed in one week by the AFIP Depleted Uranium Testing Program;
 - ii. Total and Isotopic Ratios were determined demonstrating that these soldiers were NOT exposed to DU;
 - iii. The AFIP results provided the information and support to address the health concerns from Soldiers, their families and Army physicians
 - b. Detected one case of exposure to retained fragments of DU based on a total urinary U of 48 ppt.



April 2004

AFIP-DBT Scholarly Activities on DU and Embedded Metal Fragments

Recent AFIP Publications on DU and Metal Fragments:

1. van der Voet, **Centeno JA**". Metals. In Side Effects of Drugs, Annuals 30 (Aronson JK, Editor), Elsevier BV, 2008 (ISBN: 0378-6080).
2. van der Voet GB, Todorov TI, **Centeno JA**,* et al. Metals and Health – A Clinical and Toxicological Perspective on Tungsten and Review of the Literature. *Military Medicine* 2007;172:1002-1005.
3. Ejnik JW, Todorov TI, Mullick FG, Squibb K, McDiarmid M, **Centeno JA***. Uranium analysis in urine by inductively coupled plasma dynamic reaction cell mass spectrometry. *Anal Biochem Chem* 2005;382:73-79.
4. Todorov TI, Ejnik JW, Mullick FG, **Centeno JA***. Chemical and Histological Assessment of Depleted Uranium in Tissues and Biological Fluids. In Depleted Uranium – Properties, Uses and Health Consequences. Miller A, editor, 2007, CRC Press. Chapter 6, pp. 85-103.
5. Mullick FG, Pestaner JP, Ejnik JW, **Centeno JA***. Health Effects from Depleted Uranium Exposure. *Histopathology* 2002;41 (Suppl. 2):320-340.
6. **Centeno JA** (Working Group member and Contributing Author). IARC Monograph, Surgical Implants and Other Foreign Bodies, Vol 74;1999: ISBN: 92-832-1274-6.
7. Todorov TI, Xu H, Ejnik JW, Mullick FG, Squibb K, McDiarmid MA, Centeno JA. Depleted Uranium Analysis in Blood by Inductively Coupled Plasma Mass Spectrometry. *J. Anal. At. Spectrom.* 2009;24:189-193. DOI: 10.10339/b816058a.



Acknowledgments

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MAJ Benjamin Potter, CWIP, WRAMC-USUHS

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Navy-Environmental Health Effects Lab – Ohio
Armed Forces Institute of Pathology
American Registry of Pathology

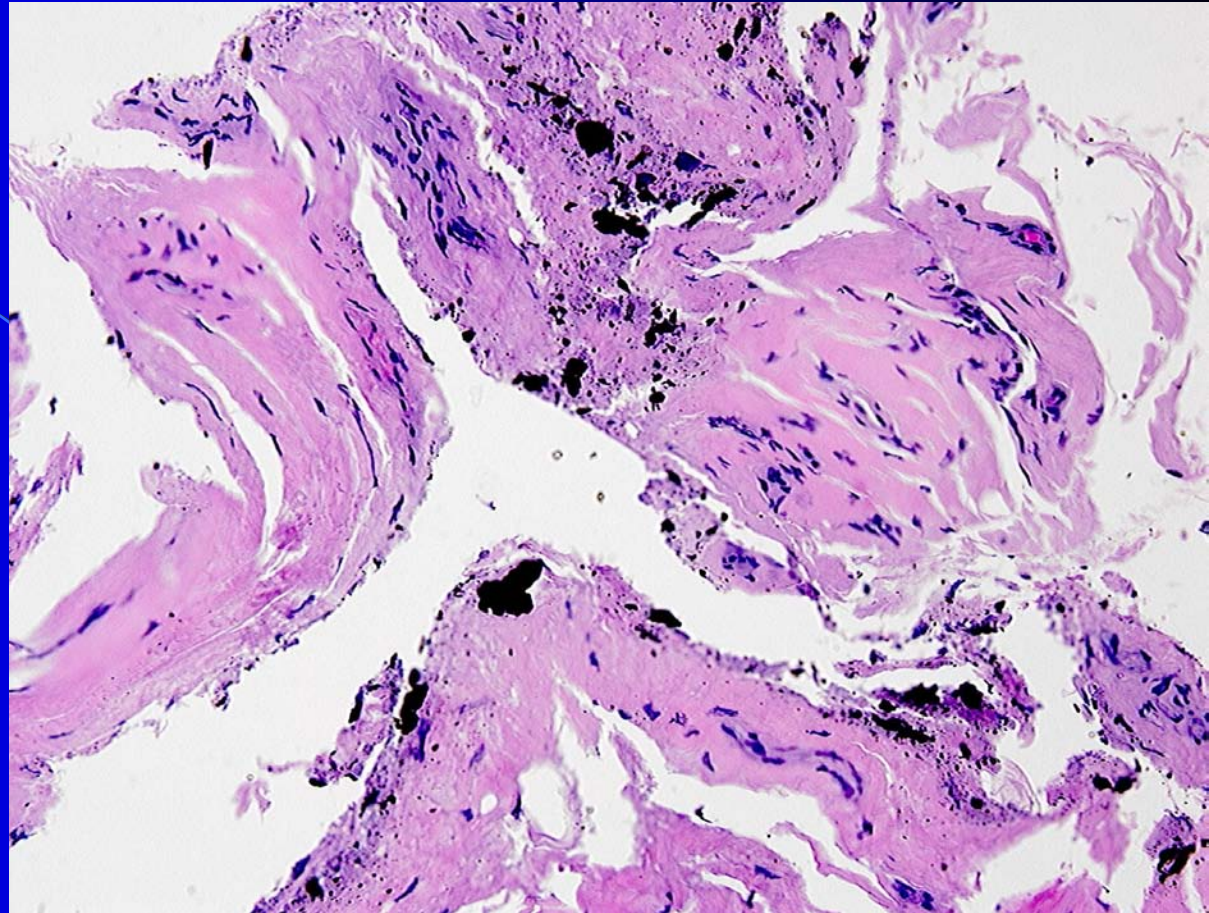
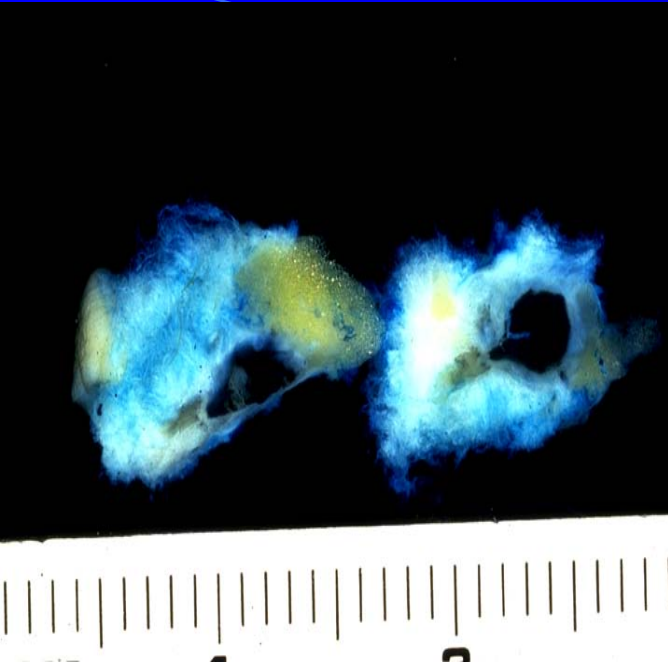
Thank you!

Questions and/or suggestions?





Tissue Analysis - Histology



Dense fibrous tissue (capsule) surrounding the shrapnel composed of paucicellular fibrous tissue, and scattered fragments of black material.



AFIP

SUMMARY

Histopathologic effects of embedded DU:

EARLY: Foreign Body reaction; acute → chronic

Scavenger cells → remove the 'invader'

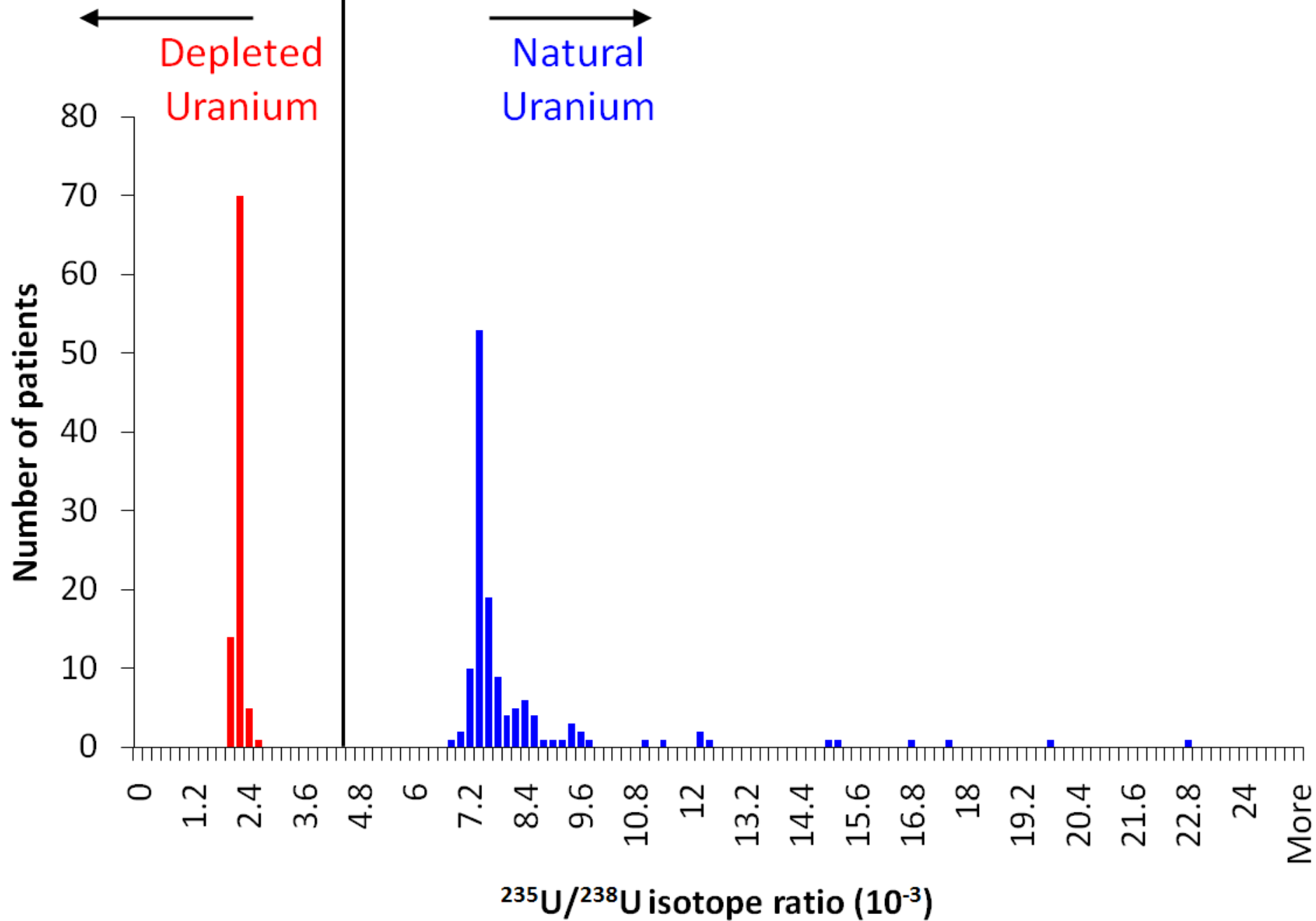
(ex. Case 1 – embedded fragments for 3 years)

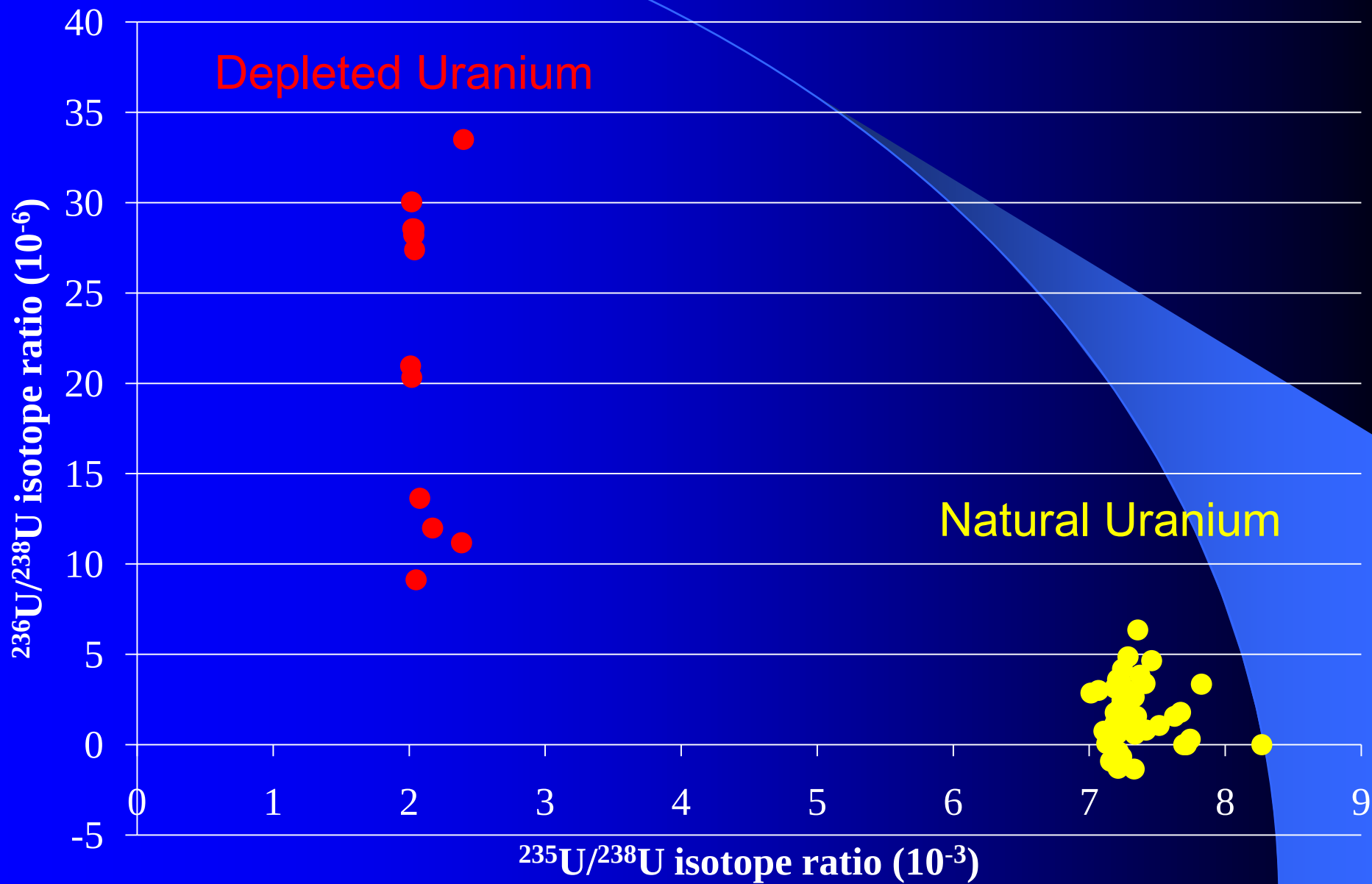
LATE: Fibrous tissue capsule → wall-off DU

Little to no inflammation

(ex. Cases 3 & 4 - 12 years later)

DU monitored periodically by 24 h urine; rising DU levels → compromise of capsule

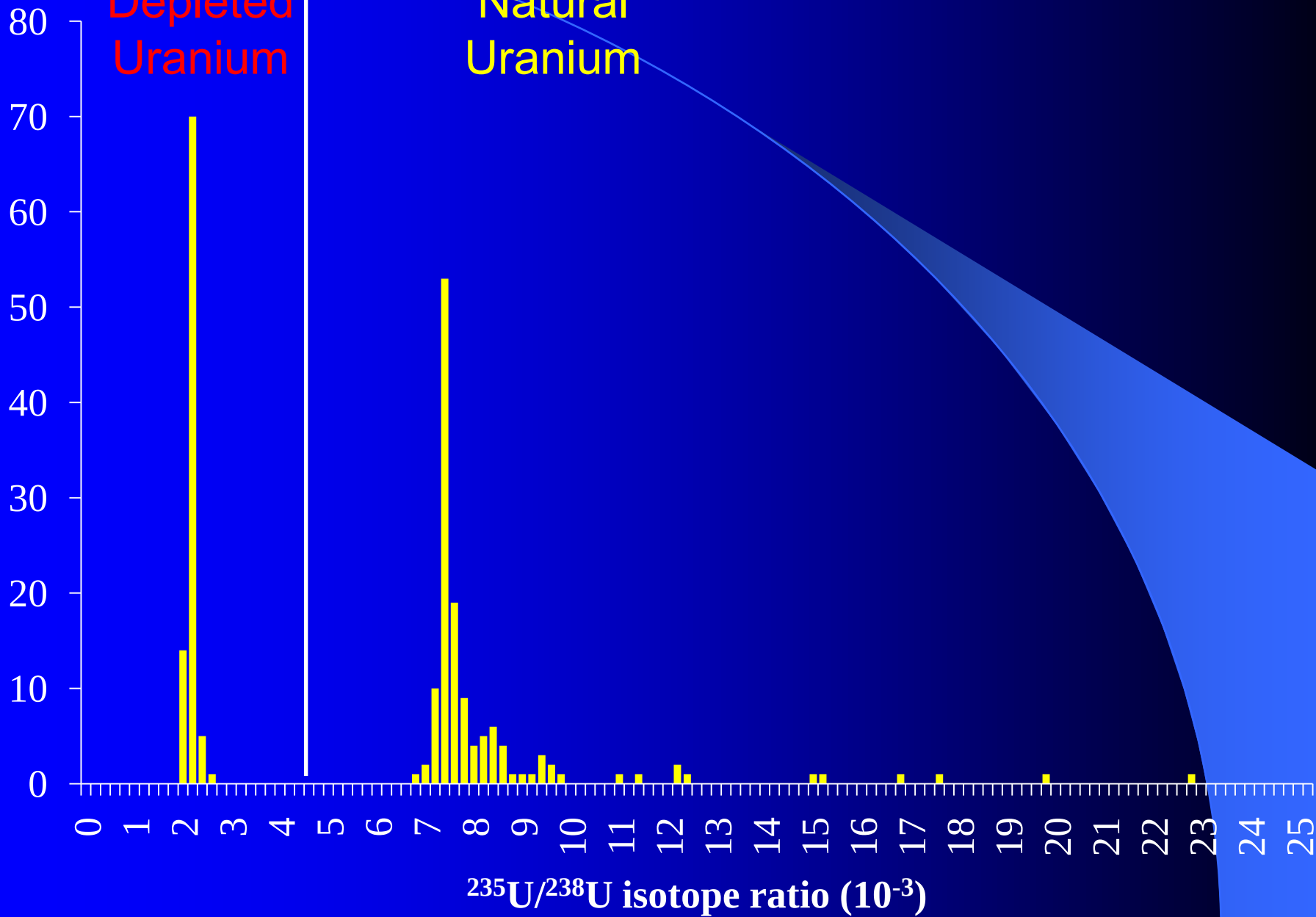




Number of patients

Depleted
Uranium

Natural
Uranium



LASER ABLATION & INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

