The Abdus Salam
International Centre for Theoretical Physics

United Nations
Educational, Scientific and
Cultural Organization

IAEA
International Atomic Energy Agency

310/1828 310/15

Workshop on Biomedical Applications of High Energy Ion Beams

Co-sponsored by: ICGEB and University of Surrey

12-16 February 2007

Venue:
Adriatico Guest House Giambiagi Lecture Hall
ICTP, Trieste, Italy

Biomedical Applications of AMS: Introduction

Claudio TUNIZ
ICTP, Italy

Biomolecular tracing through accelerator mass spectrometry

Claudio Tuniz

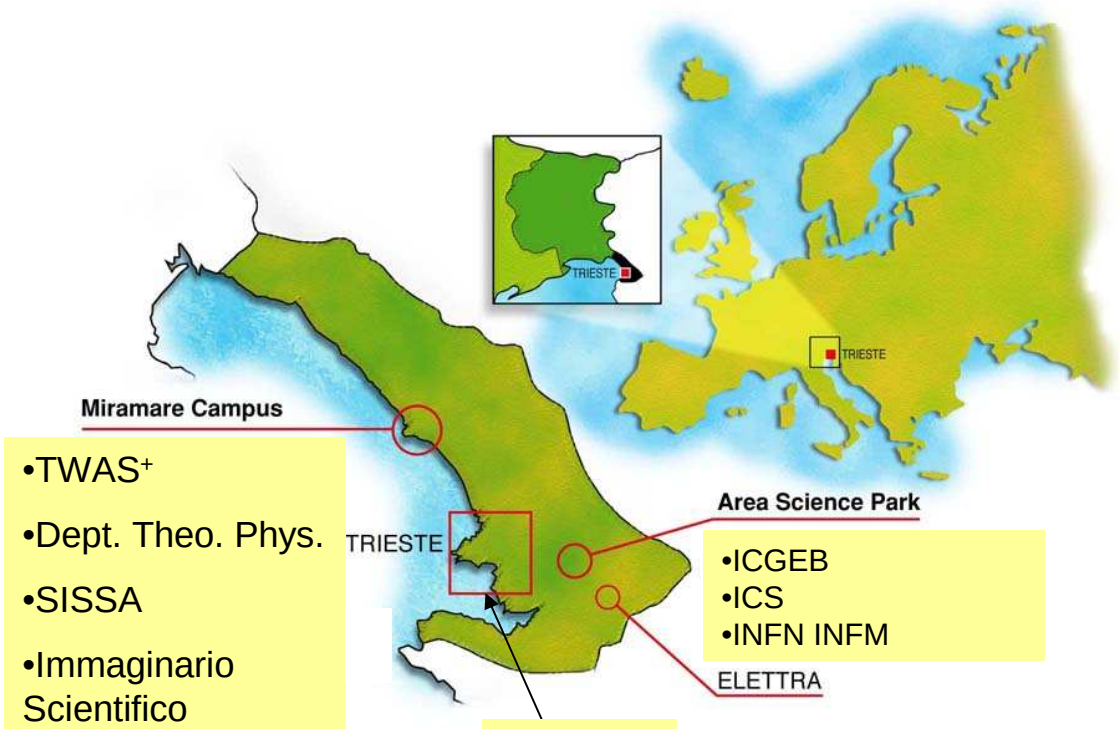
ICTP

Biomedical Applications of High Energy Ion Beams

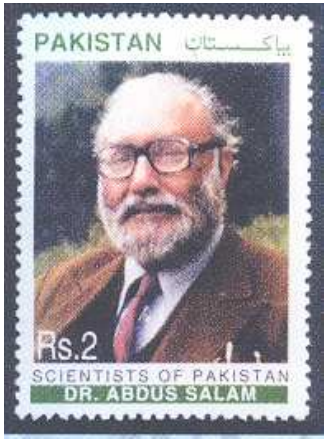
Trieste, 12-16 February 2007


The Abdus Salam International Centre for Theoretical Physics

Major scientific Institutions in Trieste



ICTP



- Founded* in 1964, ICTP operates under a tripartite agreement between two United Nations Agencies—UNESCO and IAEA—and the Government of Italy.
- ICTP's mission is **to foster the growth of advanced studies and research in developing countries**.
- Some base funding is provided by UNESCO and IAEA, some programmatic funding by SIDA, the Kuweit Foundation and others, but the largest (~82%) of the Center's budget comes from Italy.
- ICTP's working principle is **that creating scientific knowledge is important and sharing it with others is at least as important.**

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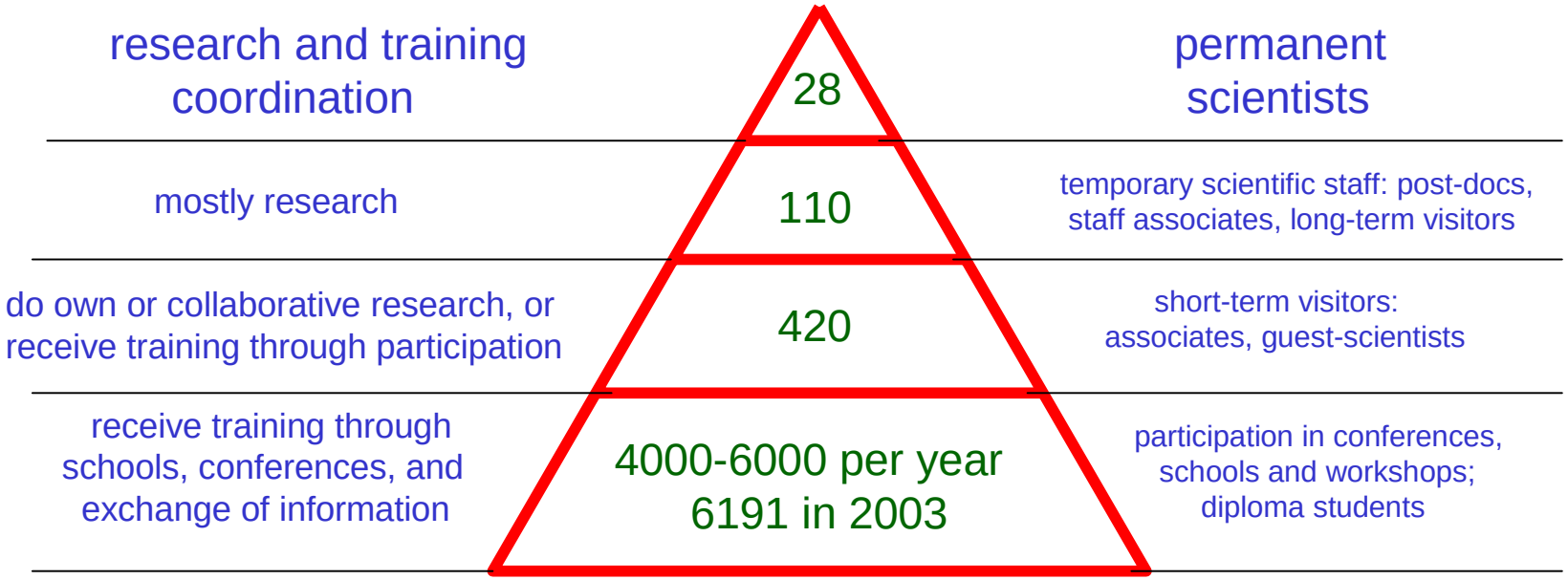
*by Abdus Salam, 1979 Nobel Laureate in Physics.

ICTP is an institution run by a few scientists for the benefit of many



ICTP Scientists, Visitors and their Functions

ICTP Scientists, Visitors and their Functions

+ about 125 general staff

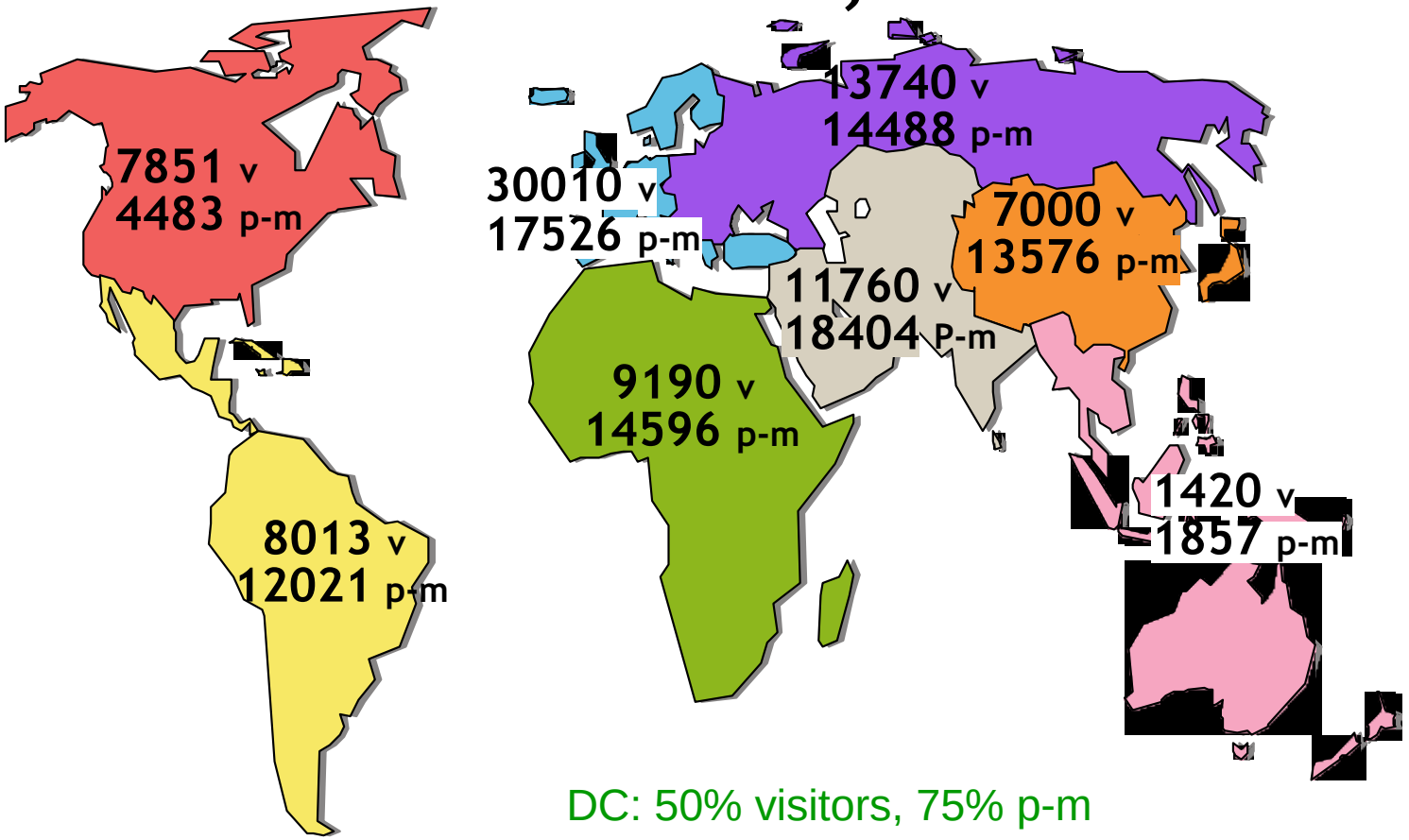
Scientific groups

1. **High energy, cosmology and astroparticle physics**
2. **Condensed matter, statistical physics**
3. **Mathematics**
4. **Earth System Physics**
5. **Applied Physics**

   optics and lasers; medical physics; energy

6. **Laboratory activities** Mlab; scientific computing; information and communication technology

ICTP visitors statistics, 1970-2003



DC: 50% visitors, 75% p-m

area	visitors	p-m
North America	7851	4483
Latin America	8013	12021
Western America	30010	17526
Eastern Europe	13740	14488

area	visitors	p-m
Africa	9190	14596
Middle East and South Asia	11760	18404
South East Asia and the Pacific	1420	1857
Far East	7000	13576

v=visitors p-m=person-months

Quality and diversity are NOT incompatible

The new ICTP multidisciplinary laboratory

- ICTP-INFN Microprocessor Laboratory
- Plasma Focus
- Advanced X-Ray Imaging
- Accelerator mass spectrometry
- Remote access

Plasma Focus Laboratory

Source of neutron and x-rays
- Training
- Biomedicine
- Materials science
- Cultural heritage

(with ENEA, Pirelli, Poland, Chile, Russian Federation)

Outline

- AMS: basic concepts
- biomed applications of long-lived radio-nuclides
- future prospects

Isotopic tracers in biomedicine

- 1910 existance of isotopes demonstrated (F. Soddy)
- 1930's nutrition studies (^{15}N, ^{13}C, ^{18}O, …)
- 1930's cyclotrons (radiotracers)
- I970's ICPMS
- 1990s AMS in biomedicine

Toxicology, Pharmacology, Nutrition

- Are data obtained from high-dose experiments relevant?
  - Is it possible to determine the impact of low, environmentally-relevant exposures?
- Are animal models valid?
  - Are studies with human subjects feasible?

AMS provides the sensitivity and precision needed to address these questions

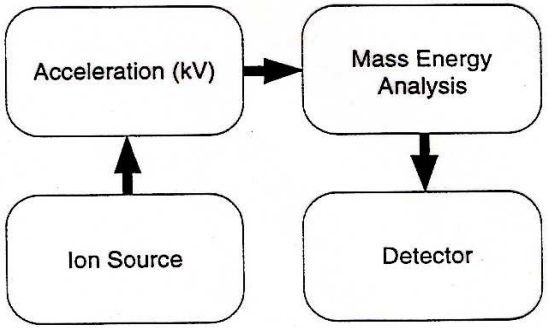
Accelerator Mass Spectrometry

Accelerator mass spectrometry (AMS) is an analytical technique that uses an ion accelerator to measure very small quantities of rare, long-lived isotopes (e.g. ^{14}C).

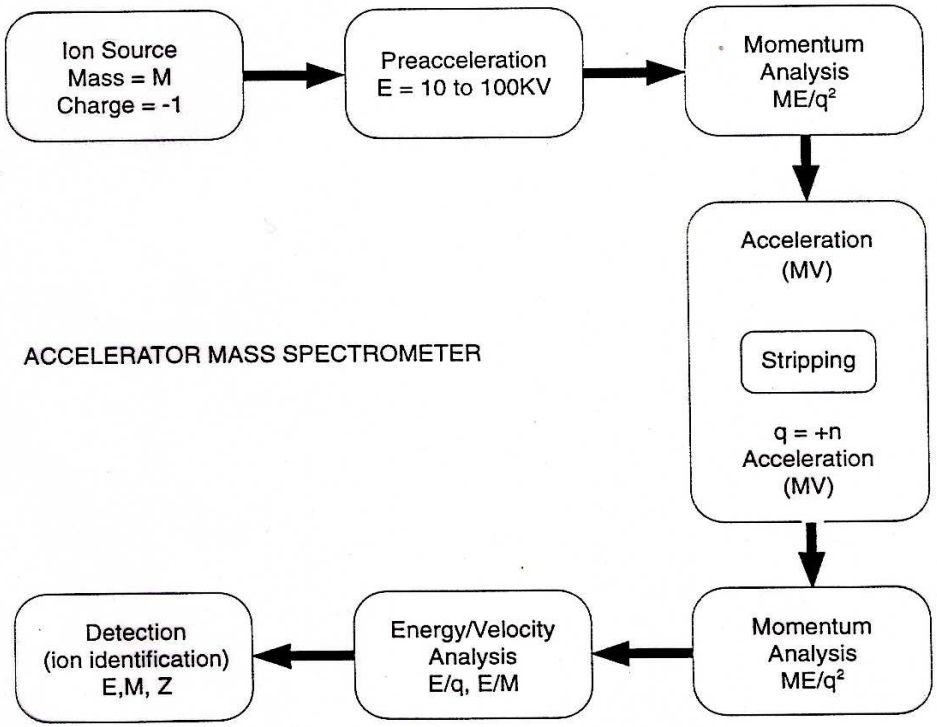
AMS was first applied to geochemical, climatological and archaeological areas, such as for radiocarbon dating, but more recently this technology has been used in biomedicine.

Accelerator Mass Spectrometry, **C. Tuniz, et al., CRC , 1998**

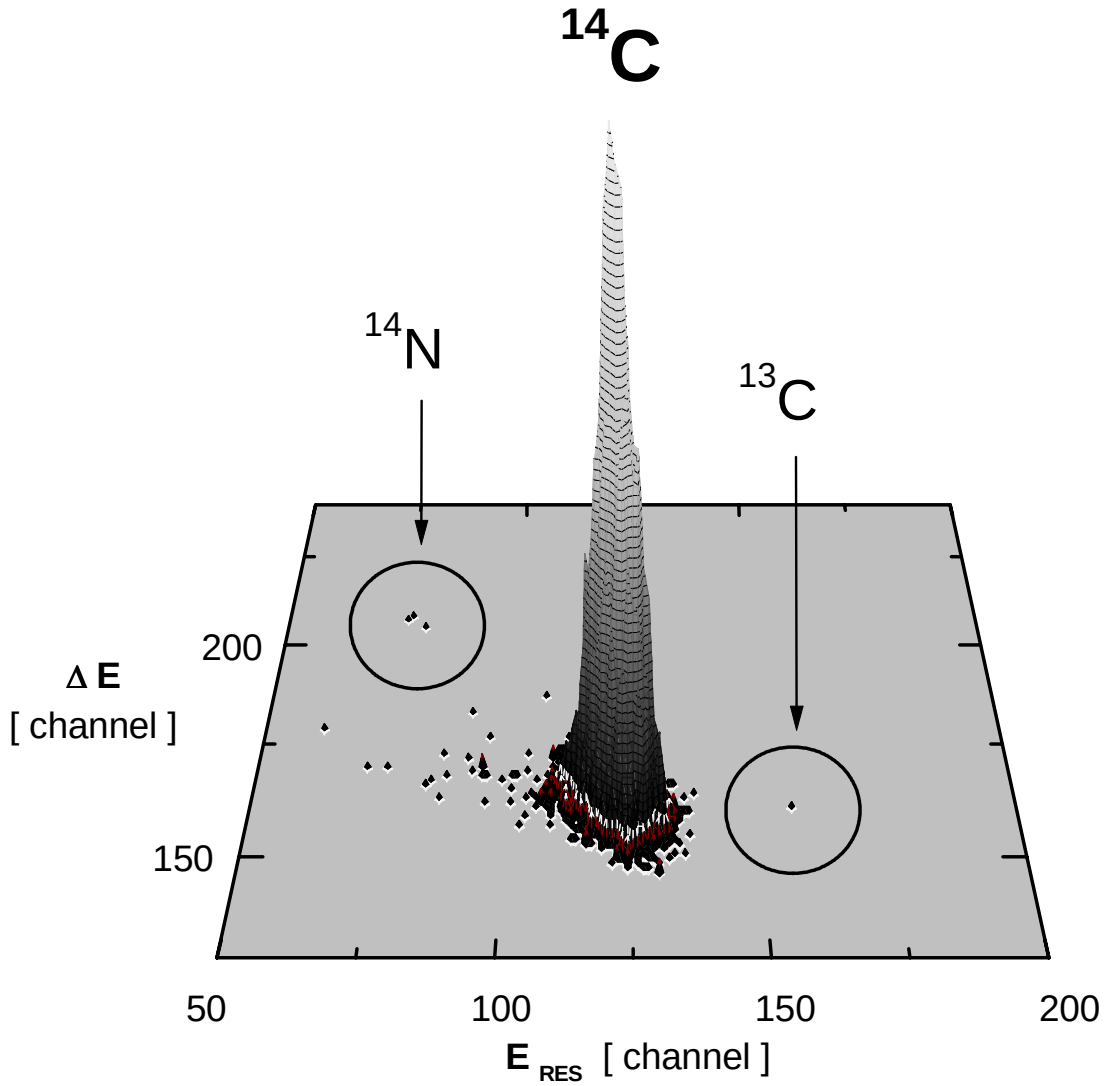
MASS SPECTROMETER



ACCELERATOR MASS SPECTROMETER

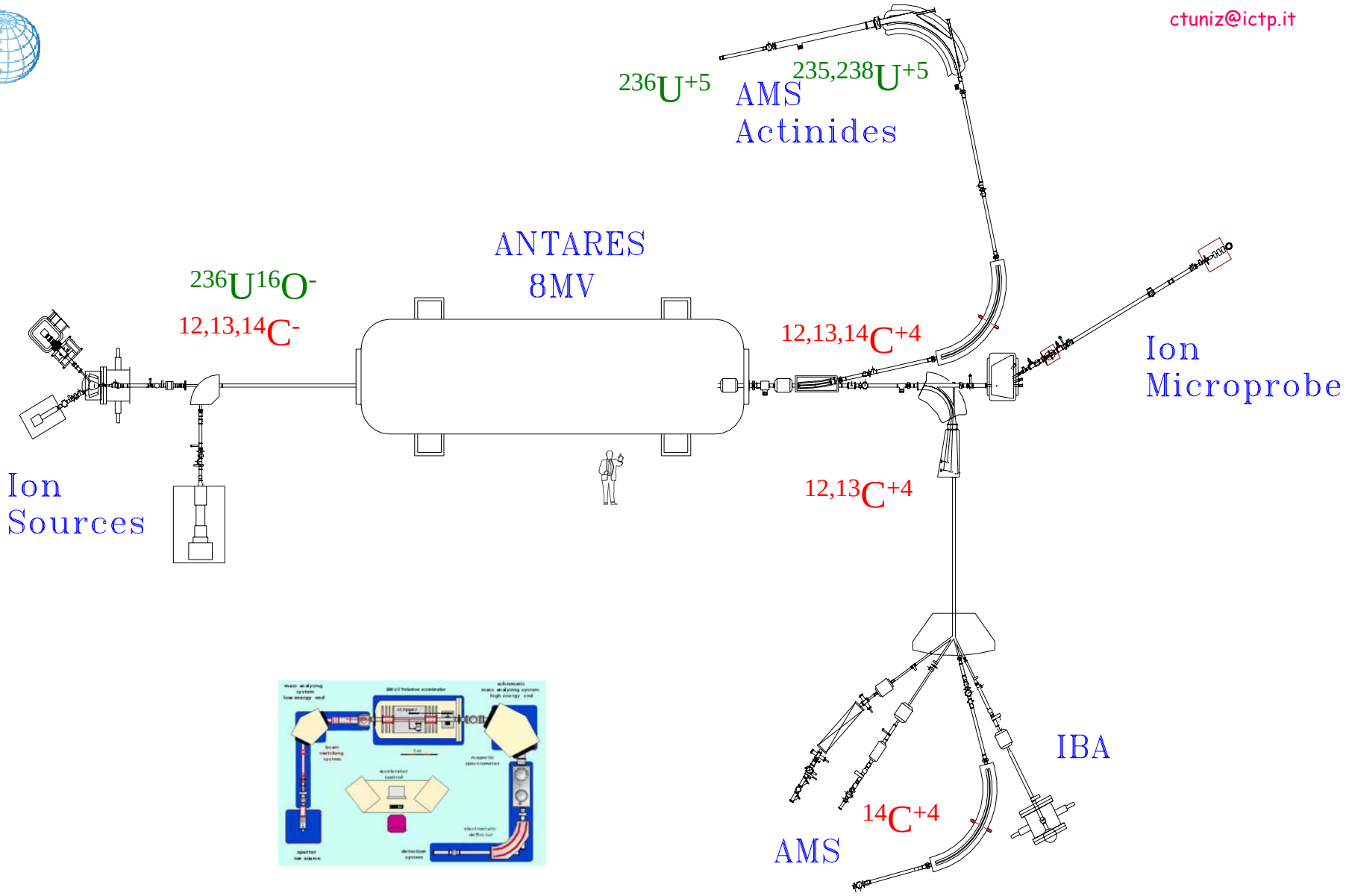


Counting atoms



Atom counters

ctuniz@ictp.it



Accelerator Mass Spectrometry, **C. Tuniz, et al., CRC , 1998**

Table-top AMS systems

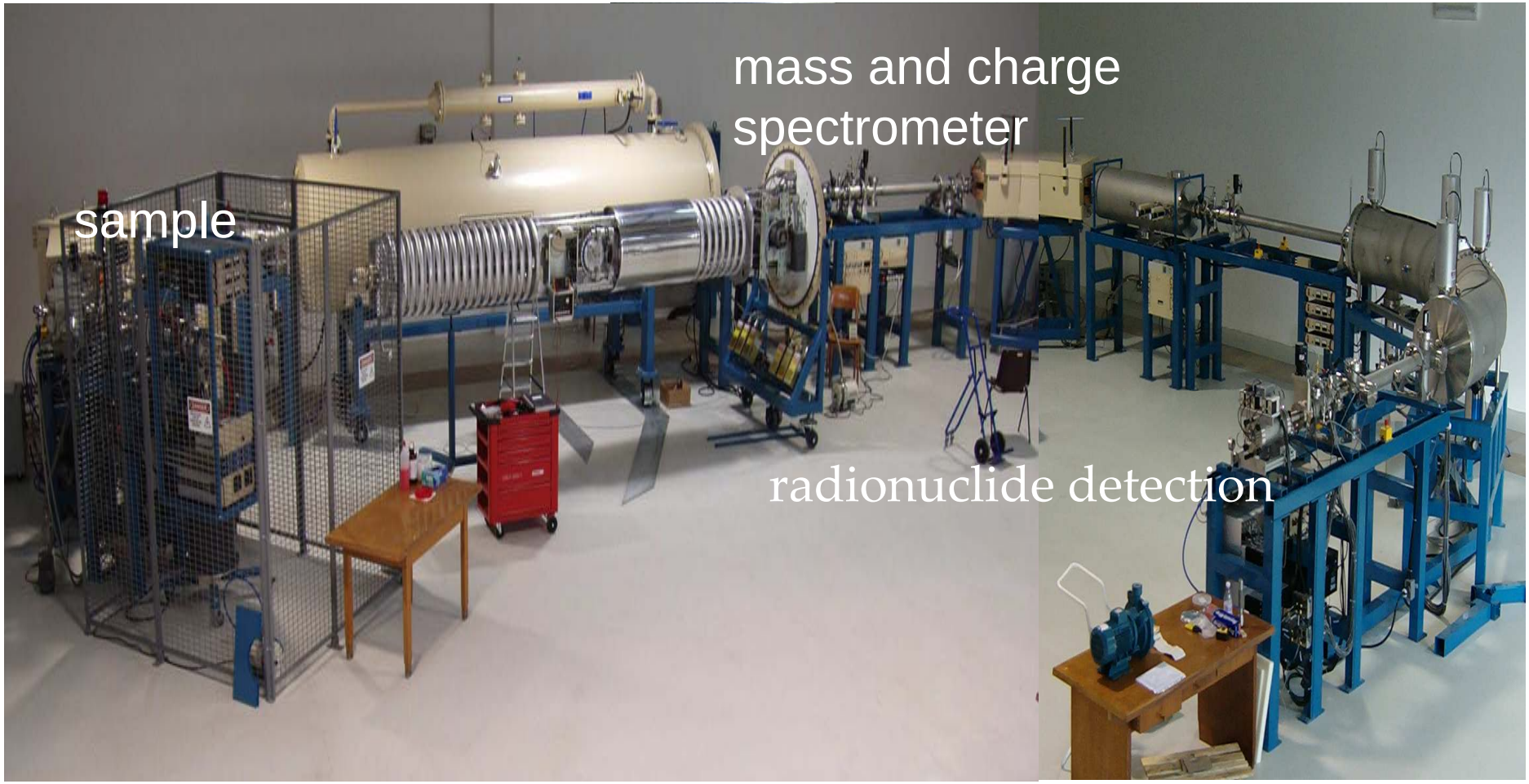


Table 1.1 Long-Lived Radioisotopes Routinely Measured by AMS

Radioisotope	^{10}Be	^{14}C	^{26}Al	^{36}Cl	^{41}Ca	^{129}I
Half-life (a)	1.51 M	5.73 k	720 k	301 k	103 k	15.7 M
Stable isotopes	^{9}Be	^{12,13}C	^{27}Al	^{35,37}Cl	^{40,42,43,44}Ca	^{127}I
Stable isobars	^{10}B	^{14}N[a]	^{26}Mg[a]	^{36}Ar,[a] ^{36}S	^{41}K	^{129}Xe[a]
Chemical form	BeO	C	Al_2O_3	AgCl	CaH_2 /CaF_2	AgI
Sample size (mg)	0.5	0.02–1	2	1	10	2
Sensitivity (atom ratio)	2×10^{-15}	1×10^{-15}	2×10^{-15}	1×10^{-15}	5×10^{-15}	5×10^{-14}

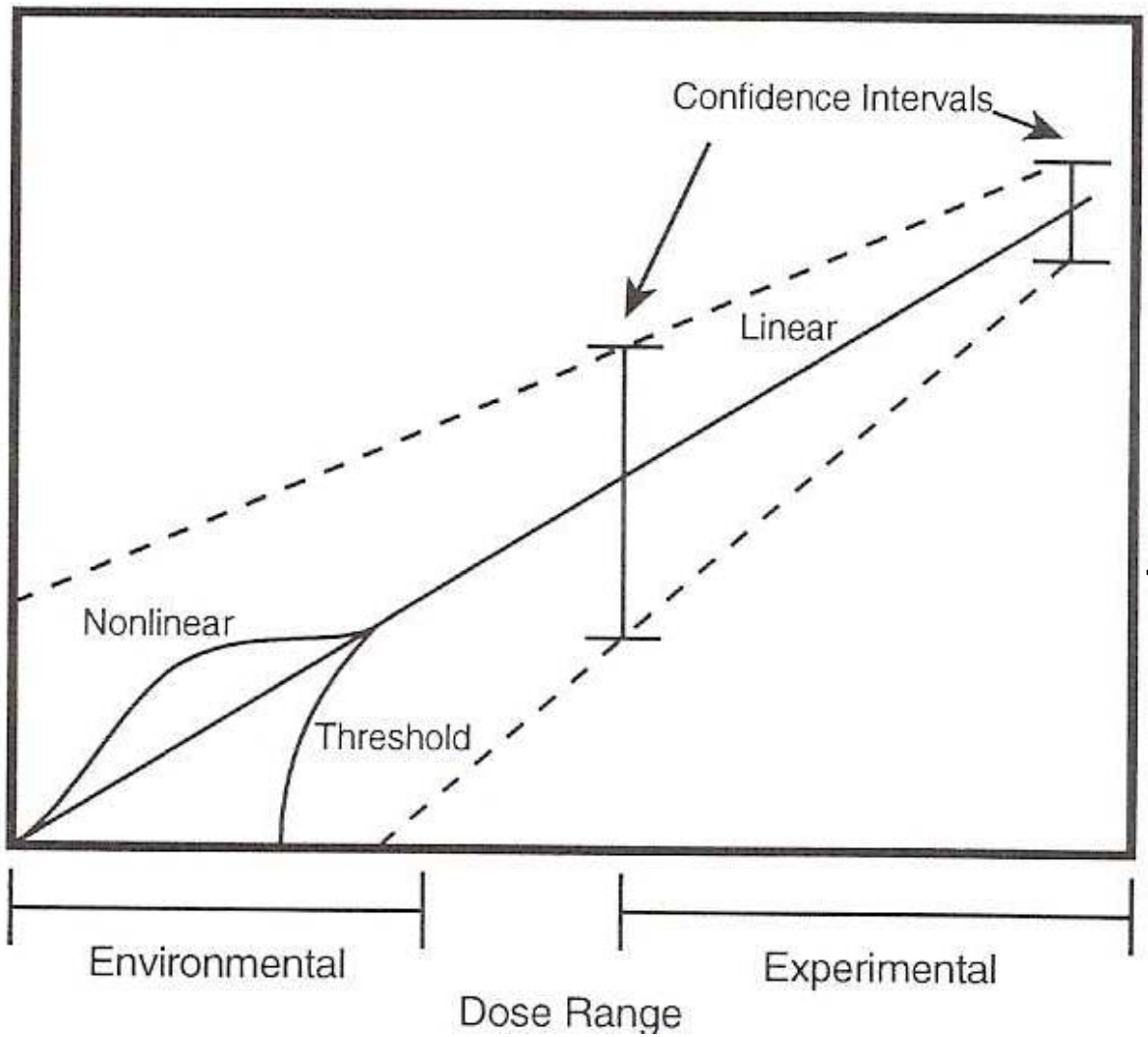
[a] Do not form negative ions.

AMS in molecular biomedicine

- Precisely quantifies zeptomoles (10^{-21}) to attomoles (10^{-18}) of long lived isotopes, such as ^{14}C
  - molecular labels in metabolic studies of nutrients, toxins, hormones and therapeutics (animals, human volunteers, cells).

[DNA binding traditionally measured by tagging the chemical of interest with carbon-14 or hydrogen-3, counting the isotope label still covalently bound to DNA]

Biological response vs dose



Immunoassay
1 adduct/10^8 nucleotides

^{32}P-postlabelling
1 adduct /10^{10} nucleotides

AMS
< 1 adduct/ 10^{12} nucleotides

Advantages

- Exposure at environmental concentrations.
- Radiologic exposures much lower than normal background
- Long-term studies (tracing metabolites for months after a single dose of retained compound)

Drug metabolism

- Adsorption, distribution, metabolism and excretion study using only 10 nanoCurie (37 Bq or ca. 0.9 μSv) of ^{14}C-labelled drugs.
  - no need of regulatory approval
  - reduce waste disposal problems
  - conduct "first into man" studies (without the need for widespread use of animals)
  - high throughput and relatively low costs

Toxicology of carcinogens

- Techniques: mass spectrometry, liquid chromatography, electrophoresis, fluorescence spectroscopy, radiotracer methods and accelerator mass spectrometry.
  - bioavailability of carcinogens;
  - carcinogens bound to DNA and protein;
- Objective: accurately estimate risk
  - understand specific molecular modifications caused by exposure to carcinogens
  - Identify specific dietary factors (e.g. heterocyclic amines that form in meat through cooking, solvents and common pollutants such as benzene, etc.)

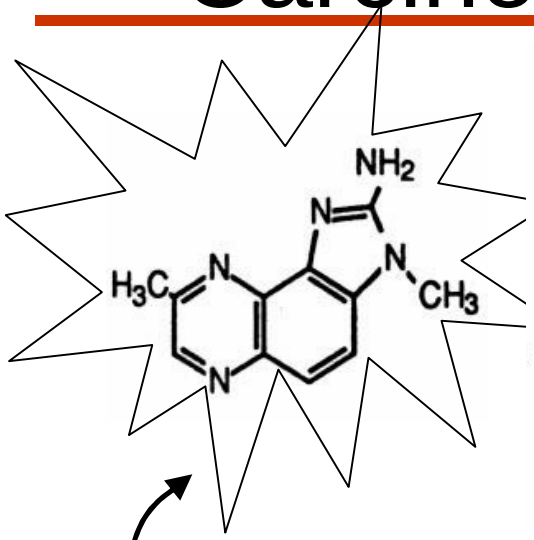
Carcinogen studies by ^{14}C-AMS

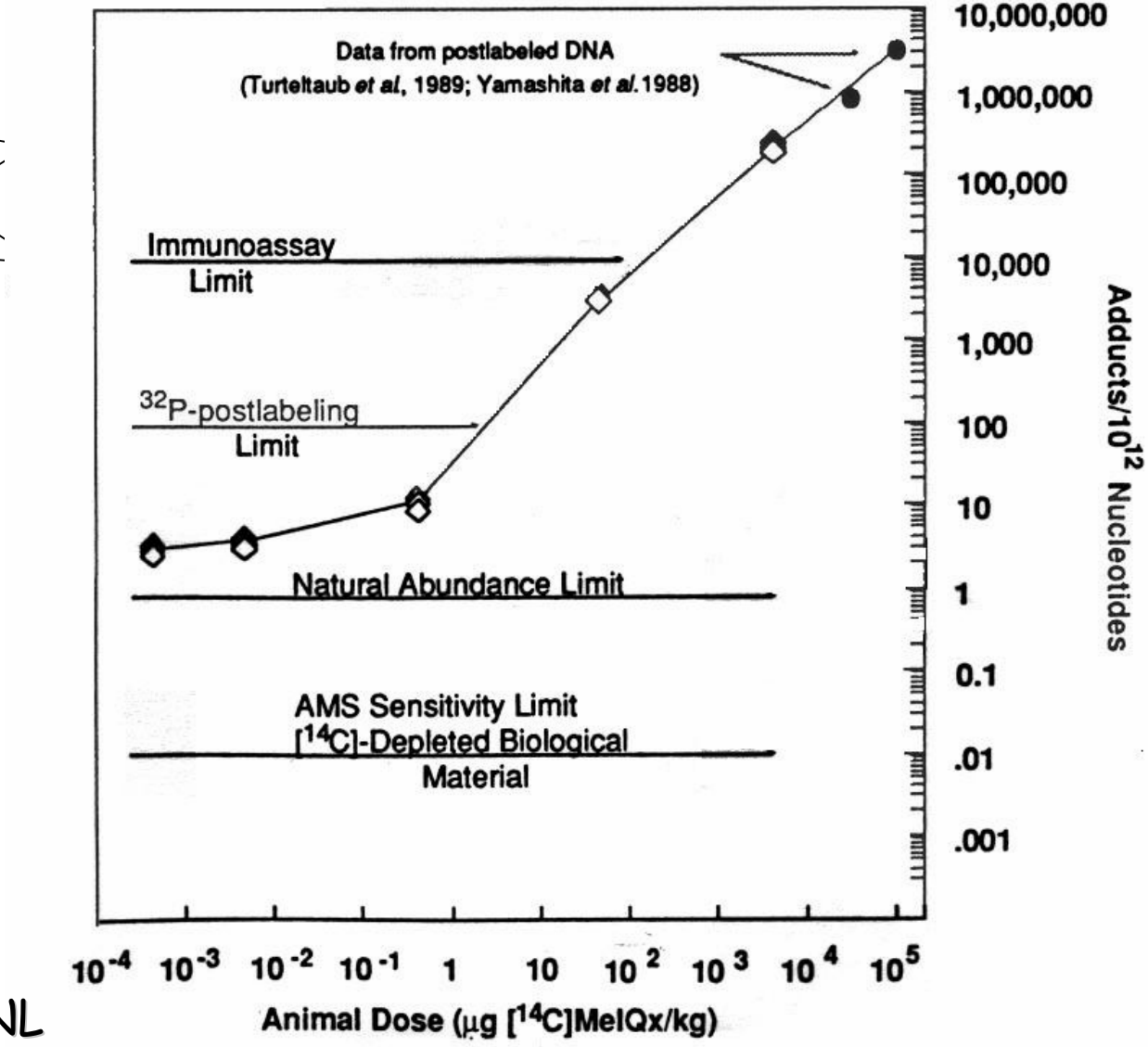
TABLE 1: Carcinogens Studied by ^{14}C-AMS

Carcinogen	Biomolecular research
MeIQx, 2-amino-3, 8 -dimethylimidazo[4,5-f] quinoxaline	DNA adducts in mouse liver[3] DNA adducts in rat liver and distribution[9] Hemoglobin (Hb), albumin adducts in rat and human[10] Metabolism in human urine[11] Distribution and DNA adducts in human and rodent colon[12] DNA adducts in rodent and human colon[13]
TCDD, 2,3,7,8-tetra-Chlorodibenzo-p-dioxin	DNA adducts in mouse liver, not observed[6]
PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine	DNA adducts in mouse liver, lung, heart and metabolites; fate and distribution in mouse[14] Distribution and metabolism in female rats and their pups[15] DNA and protein adducts in human colon and blood[16] Colon DNA adducts and metabolites in urine of human and rat[17]
Benzene	DNA and protein adducts in mouse mallow chromosome; distribution and liver DNA adducts in mouse[18] DNA adducts in liver and mallow of rat and mouse[19]
Trichloroethylene	DNA and protein adducts in mouse liver[20] Initial uptake kinetics in human skin[21]
NNK, 4-(methylnitrosamino) -1-(3-pyridyl)-1-butanone	DNA adduct in mouse liver[7] Decay kinetics of adducts[8]

Journal of Nuclear and Radiochemical Sciences, Vol. 2, No. 1-2, pp. R9-R12, 2001

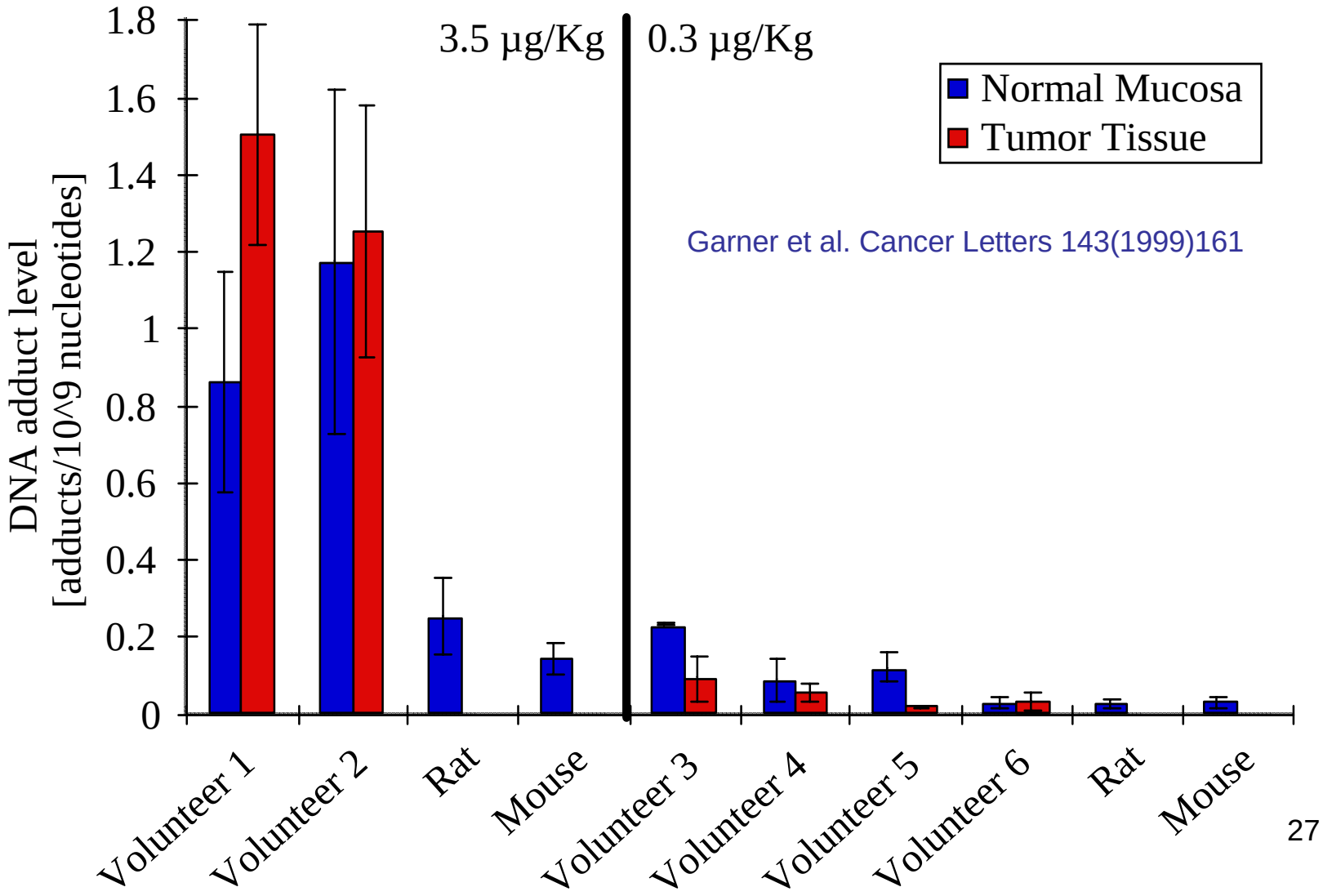
MeIQX: Carcinogen from cooked meat





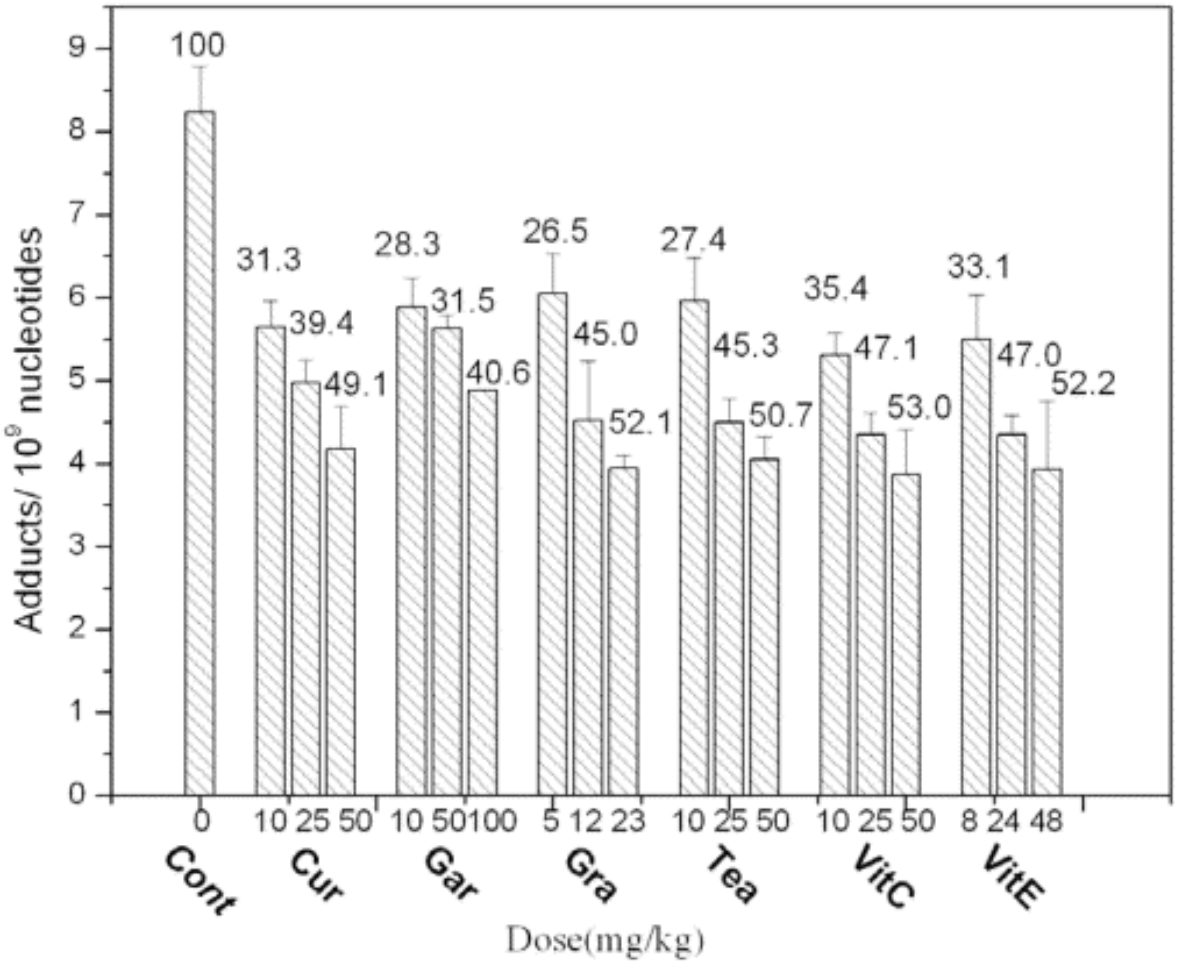
Turteltaub et al, LLNL

Different responses to low and high doses of MeIQx, from cooked meat [*men are note mice!*]



Garner et al. Cancer Letters 143(1999)161

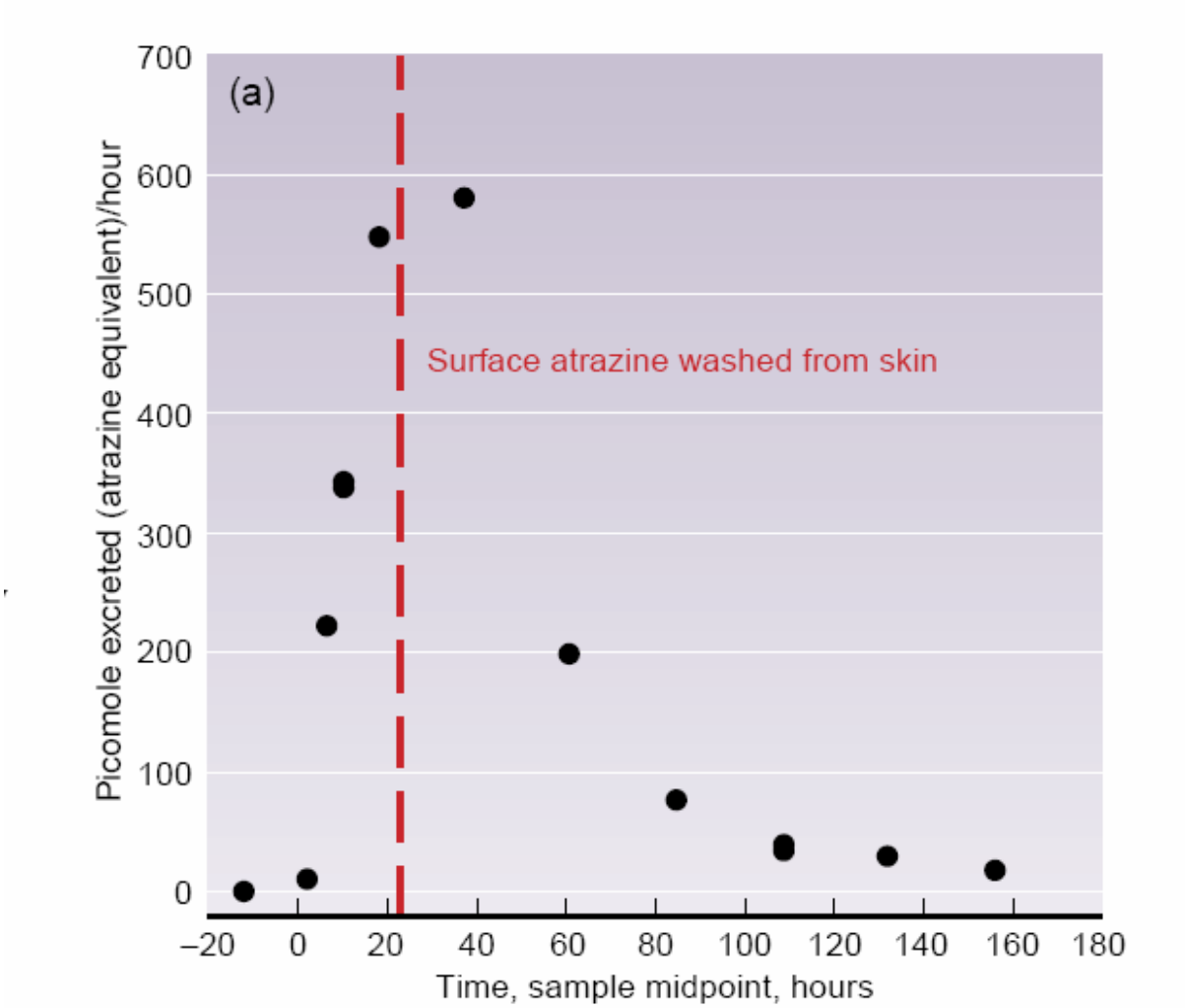
Inhibitory effects



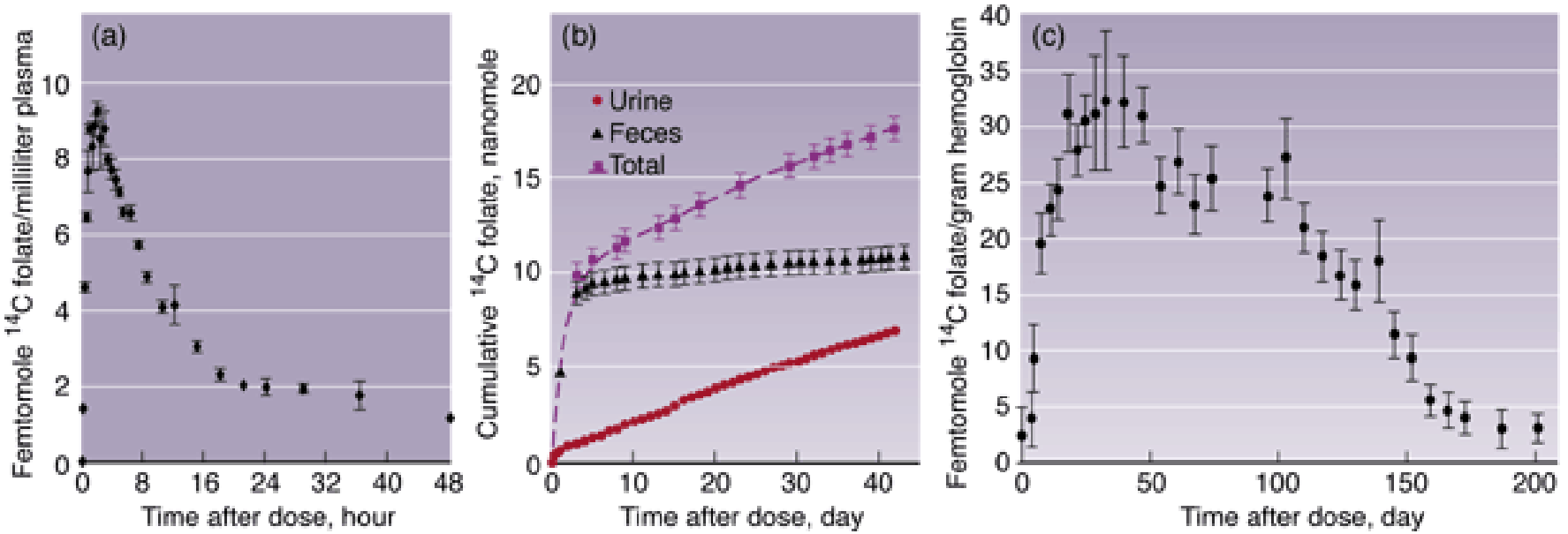
Inhibitory effects of pretreatment with curcumin (Cur), garlic squeeze (Gar), grapeseed extract (Gra), tea polyphenols (Tea), vitamin C (VitC) and vitamin E (VitE) on nicotine (Cont)-DNA adduct formation in mice. The values at the top of each bar indicate the percent of inhibitory effects (% of elimination) of dietary constituents on nicotine-DNA adduct formation, compared with the control (as 100%). Agent injections, each day x 5 days. Nicotine 6th day, 18.2 μg/kg. [~ 1 cigarette]

Cheng et al, Food and Chemical Toxicology 41(2003)1045

Atrazine


(a)
Surface atrazine washed from skin
Picomole excreted (atrazine equivalent)/hour
700
600
500
400
300
200
100
0
–20
0
20
40
60
80
100
120
140
160
180
Time, sample midpoint, hours

Tracking 35 micrograms of folic acid through a human for 200 days



A single dose of carbon-14-tagged folic acid was traced for 200 days. (a) The tagged folic acid appears very quickly in plasma (the liquid part of blood) and tapers off in about two days. (b) The amounts of tagged folate being eliminated in feces and urine were followed for 40 days. (c) Folate begins to be incorporated into hemoglobin at day 5. (Hemoglobin is the iron-containing, oxygen-carrying molecule in red blood cells.) The level of folate in hemoglobin peaks at about the 30th day and disappears only after 200 days.

Isotopes for biomedical applications

Isotope	$t_{1/2}$	Sensitivity	Applications
^{3}H	12.3 a	10^{-14}	General
^{7}Be	53.3 d	10^{-15}	Metabolism, toxicology
^{10}Be	1.51×10^{6} a	2×10^{-15}	Metabolism, toxicology
^{14}C	5730 a	1×10^{-15}	General
^{26}Al	7.20×10^{5} a	2×10^{-15}	Metabolism
^{32}Si	~140 a	10^{-13}	Bone
^{36}Cl	3.01×10^{5} a	1×10^{-15}	Metabolism
^{41}Ca	1.03×10^{5} a	5×10^{-15}	Bone
^{53}Mn	3.7×10^{6} a	10^{-10}	Metabolism
^{60}Fe	1.5×10^{6} a	10^{-12}	Metabolism
^{79}Se	~1.1×10^{6} a	—	Metabolism
^{129}I	1.57×10^{7} a	5×10^{-14}	Metabolism

Source: From Vogel, J.S. and Turteltaub K.W., *Trends Analyt. Chem.*, 11(4), 142, 1992. With permission.

Aluminium metabolism

- Humans evolved with aluminium oxide in the environment, but not elemental aluminium which was introduced by refining techniques during 1800s.
  - Antiperspirants and deodorants
  - Water treatment

→ anaemia, dementia, bone disease, Alzheimer (?)

Uptake of trace amounts of aluminium into the brain from drinking water

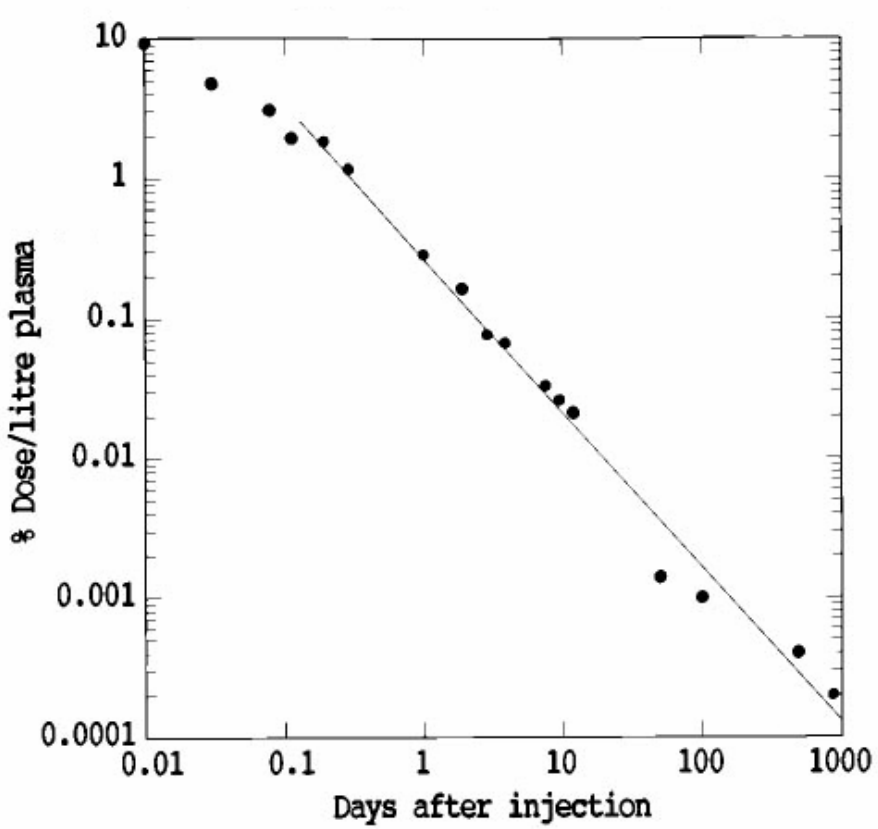
Gavaged simulated tap water, containing 100 nanogram ^{26}Al (700 kyr) into the stomach of rats.

AMS analysis of brain samples shows that trace amounts of ^{26}Al from this single exposure directly entered the brain tissue, with fractions 30-300 ppb of input dose

*(**Neurotoxicology,** Walton, Tuniz, Fink, Jacobsen, Wilcox, 1995)*

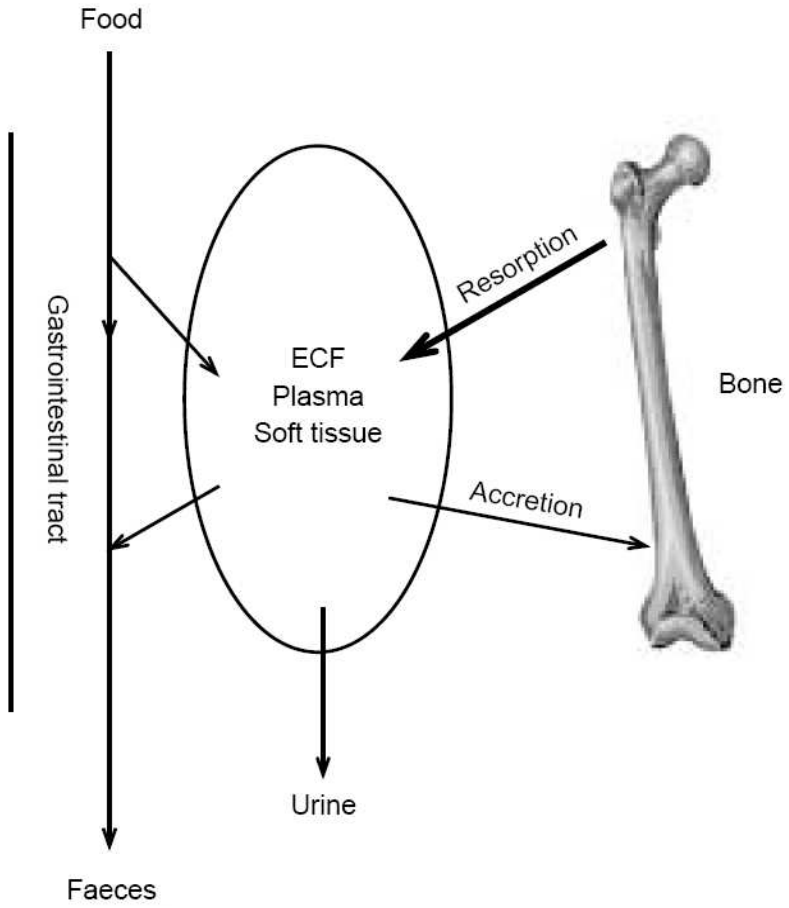
Toxicity of aluminium in humans

- 10^{15} ^{26}Al atoms (50 ng) → human volunteer
- 10^{6} atoms detected by AMS (blood, urine, …)



Priest et al, Human Experimental Toxicology, 1995

Bone metabolism



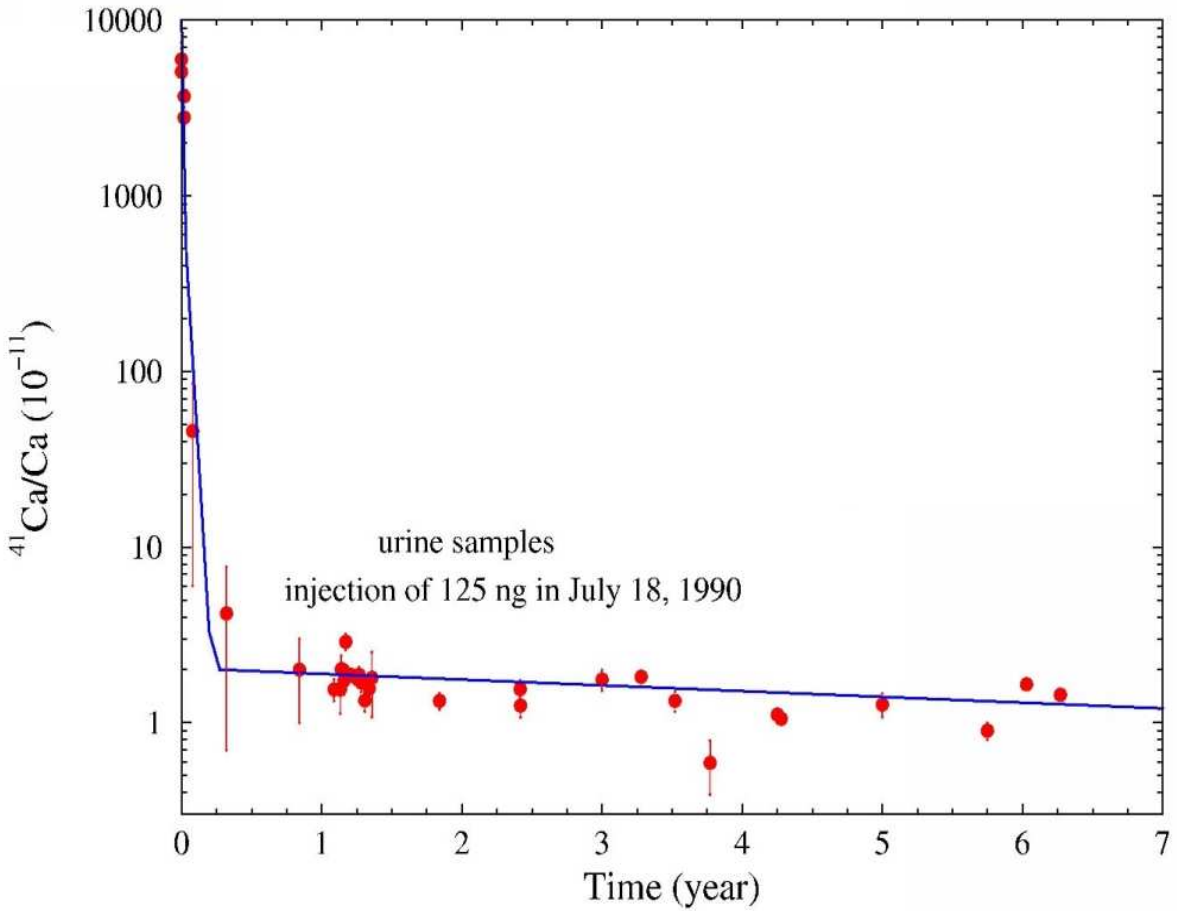
Nutrition Research Reviews (2001), **14**, 317–334

^{41}Ca for bone loss studies

Volunteer injested
125 ng ^{41}Ca

↓

Dose commitment
0.42 mSv in 50 yr



Racah Institute, Triumf Laboratory and
University of British Columbia

Absorption of inhaled Pu from the lung

[Fiefield et al. Australian National University]

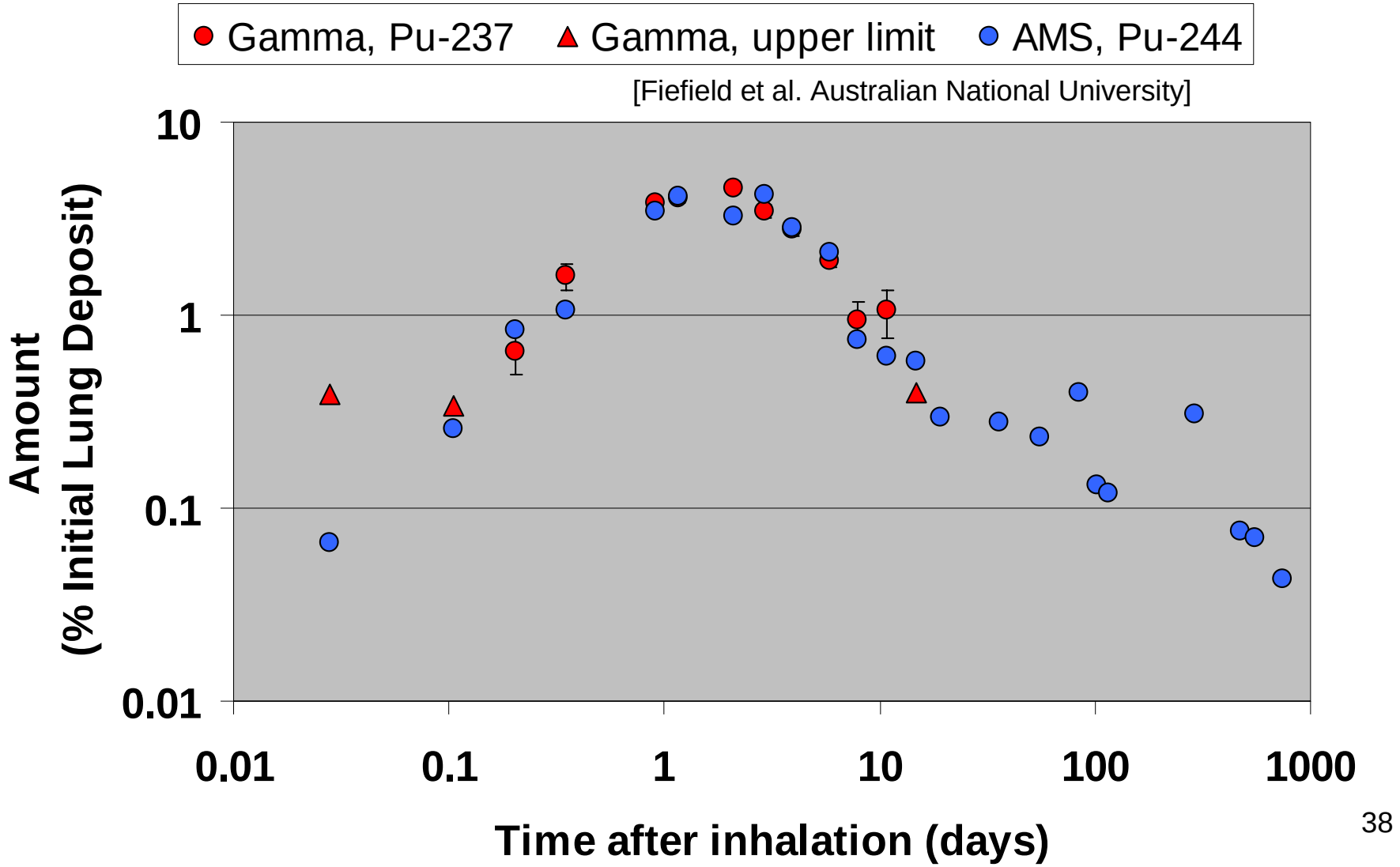
Inhalation is the main route of intake of radioactive materials for workers in nuclear industries.

How quickly, and how much is absorbed into the blood from the lungs?

NRPB (UK) study - two volunteers inhaled an aerosol with ^{237}Pu (46 days) and ^{244}Pu (80 Ma).

Measure Pu in blood and urine: ^{237}Pu - K X-rays for 10 days ^{244}Pu – by AMS for 3 years

Pu IN BLOOD AFTER INHALATION, Subject D



Active AMS biomed labs

US: Dedicated system at LLNL

UK: Dedicated system in YORK

Part-time centres in Sweden, France, Germany, China, Japan, Israel and Australia

Xceleron accelerating drug development

Concluding ….

- AMS has the sensitivity and precision to address a multitude of biomedical questions
- Merging the fields of physics, chemistry and biology (as well as others) will continue to create new insights and opportunities
- Newer, smaller spectrometers will proliferate over the next ten years

Thanks!