

LABORATORIO PER L'INSEGNAMENTO DELLE SCIENZE DI BASE

TEMA

**“Metodi alternativi e complementari alla sperimentazione animale:
sappiamo davvero di cosa si tratta e chi li utilizza?
Parliamone in modo scientifico”.**

Università degli Studi di Milano – Bicocca, in collaborazione con la Commissione Europea
(European Union Reference Laboratory for the Alternatives to Animal Testing,
EURL ECVAM, Joint Research Center)

Chiara Urani (UniMIB) e Laura Gribaldo (European Commission)



Piano Nazionale
Lauree Scientifiche
Scienze Naturali e Ambientali

STRUTTURA DELLE ATTIVITA'

1. Somministrazione di domande (pre-conoscenze) alla classe/studenti
2. Discussione e confronto sulle risposte date
3. Parte **teorica** e **parte pratica** con attività di gruppo.

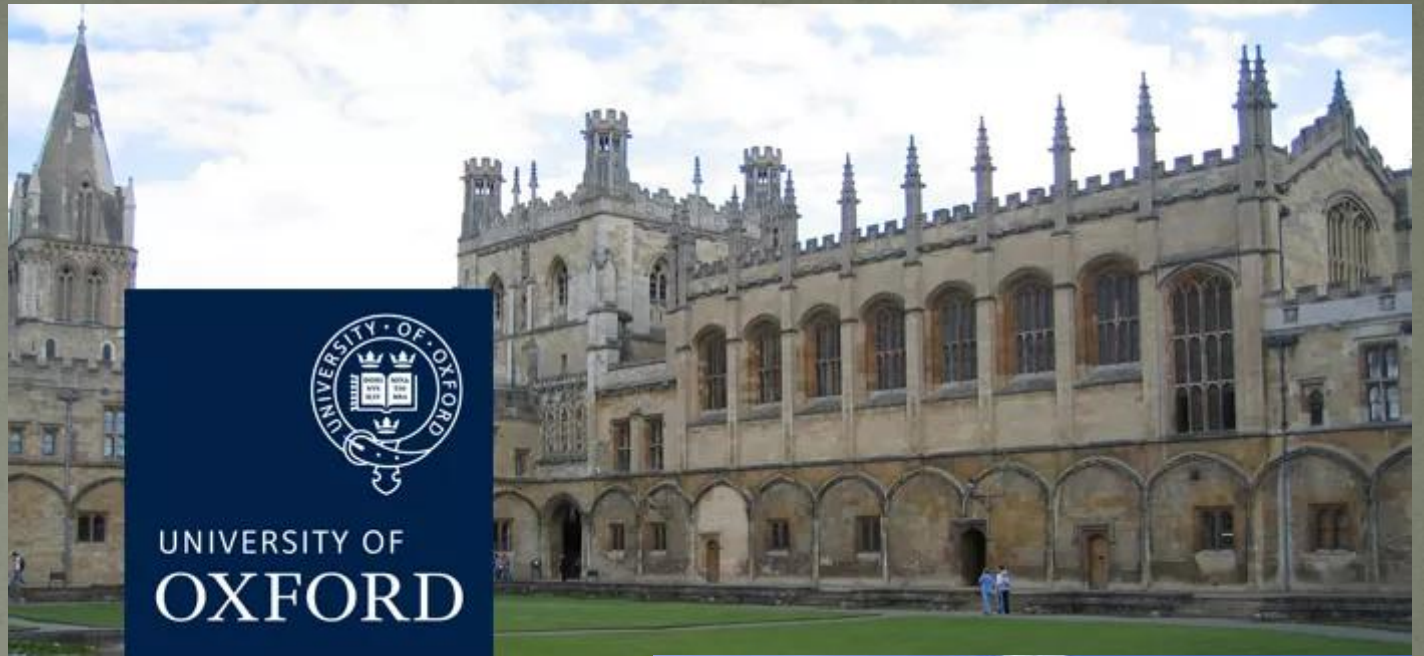
OBIETTIVI

a) descrivere i principi delle 3Rs e dei metodi alternativi alla sperimentazione animale; b) introdurre al metodo scientifico; b) ragionare su una problematica di interesse ambientale; c) insegnare con metodo cooperativo la pianificazione e stesura di un breve progetto scientifico su una tematica proposta; d) presentazione da parte degli studenti dei progetti elaborati e discussione finale.



Tutto ebbe inizio da Russel e Burch

il concetto delle 3Rs

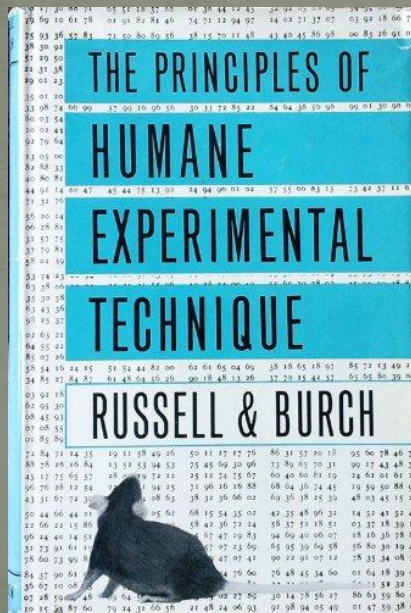


UK, 1959





Rex Burch



William Russell



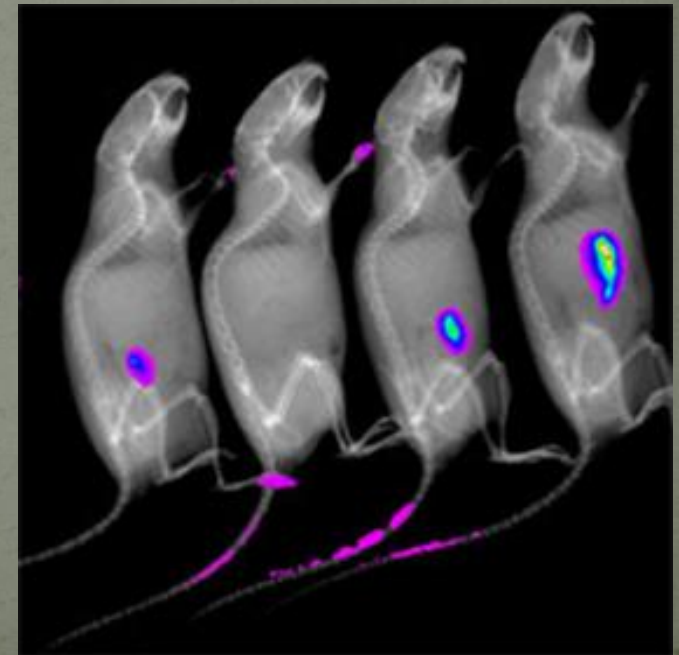
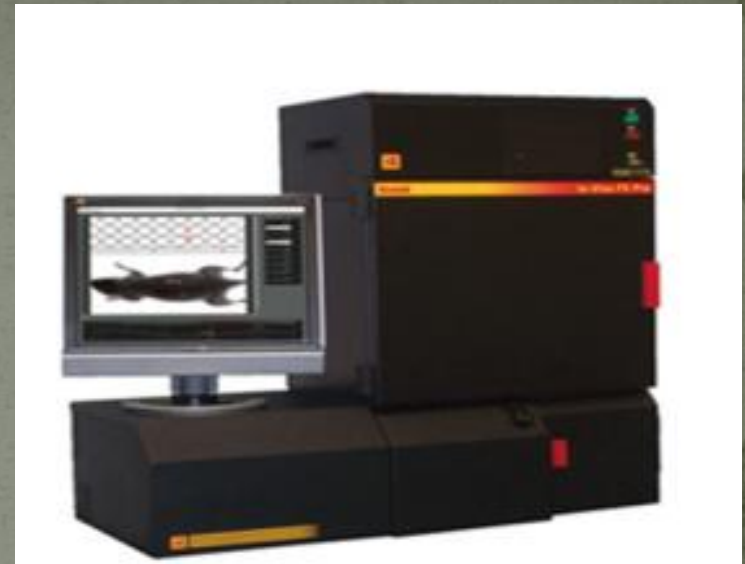
Rifinire

- *"simply to reduce to an absolute minimum the amount of stress imposed on those animals that are still used".*



- Migliore controllo del dolore
- Capacità tecniche migliorate
- Strumentazione migliorata
- Meno stress: telemetria
- Procedure meno invasive

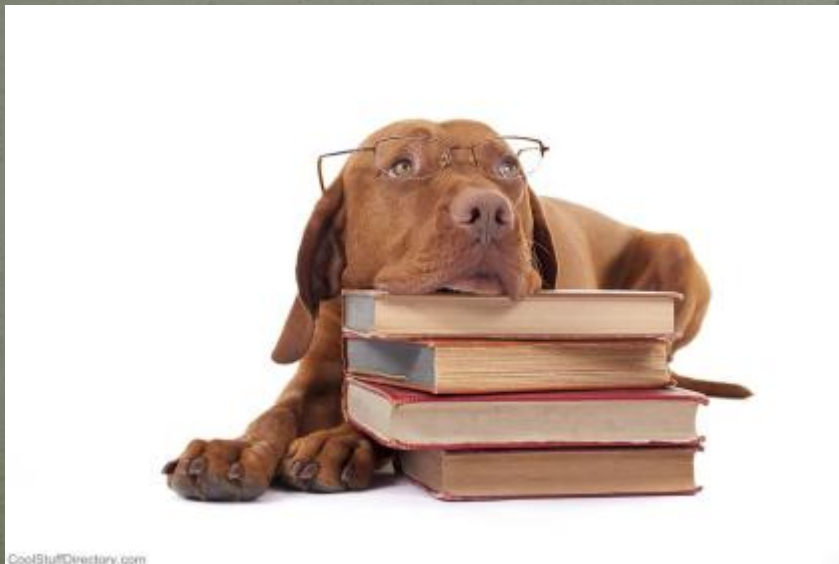
Endoscopia / laparoscopia
Microscopia confocale
Risonanza magnetica
biofotonica
Sistema MicroCAT



Arricchimento ambientale



Tecnica del rinforzo positivo.



CoolStuffDirectory.com



Ridurre

- *“reduction in the number of animals used to obtain information of a given amount and precision”.*



Strumenti di riduzione



Il principio delle 3R e la legislazione



Direttiva 1986 (86/609/EEC)

Direttiva 2010/63/EU:

Decreto Legislativo n. 26 del 4 marzo 2014

Direttiva 2003/15/ce (VII emendamento alla direttiva 76/768 cee)

Regolamento REACH (2006/1907/EC)

CLP (2008/1272/EU), il Regolamento Cosmetico (2009/1223/EU) e il regolamento per le sostanze ad attività biocida (2012/528/EU)

EURL ECVAM



Il Centro Comune di Ricerca della Commissione Europea



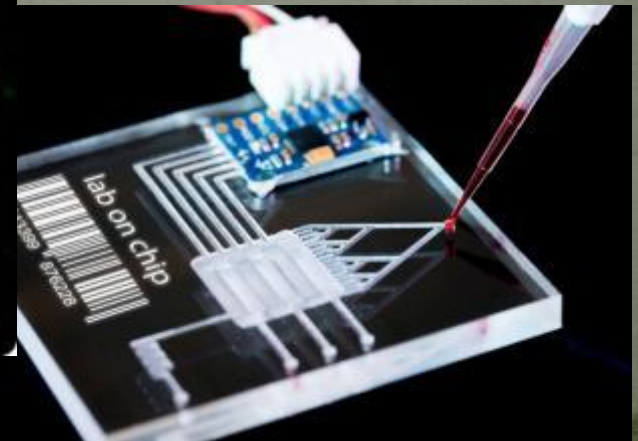
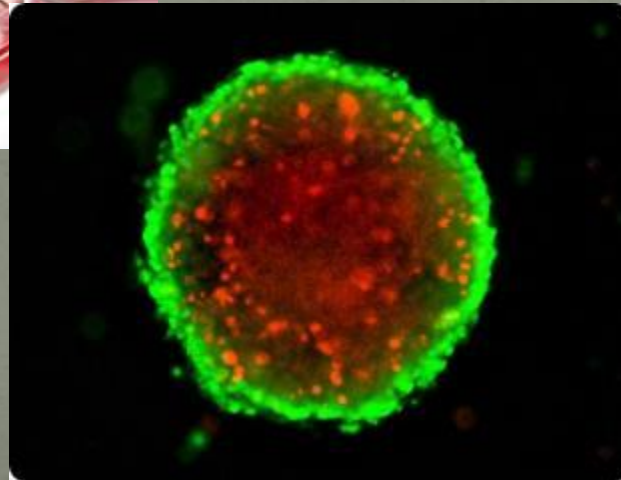
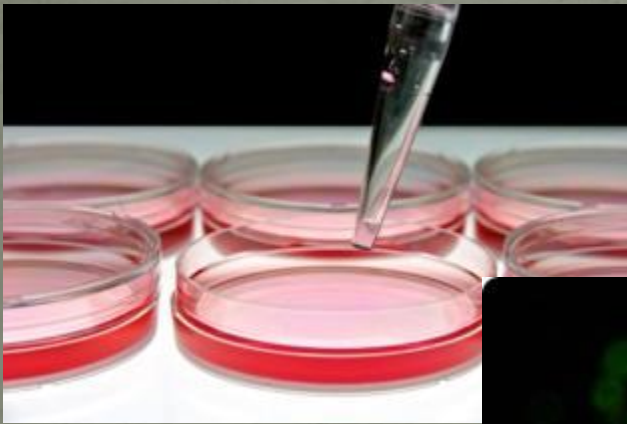
JRC Role: facts & figures



- 6 locations in 5 Member States: Italy, Belgium, Germany, The Netherlands, Spain
- Policy neutral: has no policy agenda of its own
- 42 large scale research facilities, more than 110 online databases
- 1500 core research staff, 3000 total staff
- 83% of core research staff having a PhD
- Over 1,400 scientific publications per year

Rimpiazzare

- “any scientific method employing non-sentient material which may replace methods which use conscious living vertebrates”.



POPULATION

ORGANISM /
BODY

ORGAN /
TISSUE

CELL

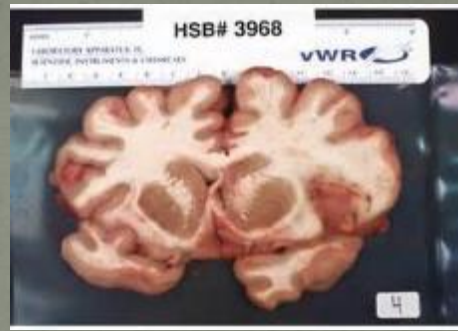
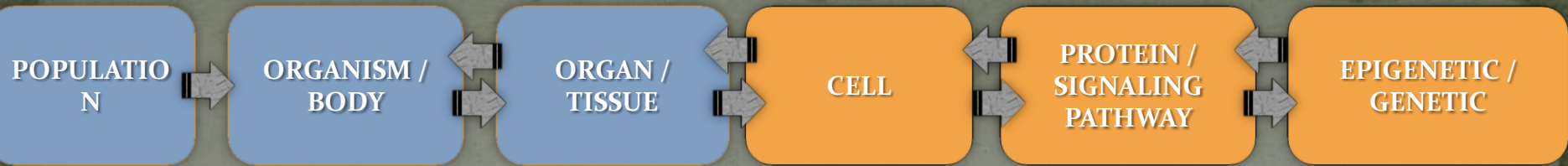
PROTEIN /
SIGNALING
PATHWAY

EPIGENETIC /
GENETIC

Quali sono gli strumenti e i
modelli disponibili per studiare i
vari livelli di complessita'
biologica?



Studi epidemiologici
di popolazione, studi
clinici



Tessuti umani ex-vivo
(sani e malati, e.g.
cervello, liquido
cerebro spinale,
sangue)



Imaging: MRI, PET...

Alzheimer Disease Assessment Scale—Cognitive Subscale (ADAS-cog) 11-Item

	Score range
Memory and new learning	0 - 25
Word recall (mean number of words not recalled)	0 - 10
Orientation (one point for each incorrect response)	0 - 8
Word recognition (mean number of incorrect responses)	0 - 12
Remembering test instructions	0 - 5
Language	0 - 25
Commands	0 - 5
Spoken language ability	0 - 5
Naming objects/fingers	0 - 5
Word-finding difficulty	0 - 5
Comprehension	0 - 5
Praxis	0 - 10
Constructional praxis	0 - 5
Ideational praxis	0 - 5
Total	0 - 70

Increasing scores indicate worsening cognitive function.
Rosen WG, et al. Am J Psychiatry. 1994;151:159-164.



Tests cognitivi: MMSE,
HVLT

POPULATION

ORGANISM /
BODY

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(A. Muotri, www.ucsd.edu)

Cellule staminali umane
indotte pluripotenti (hiPSCs)
& differenziate neuronali
(normal & patient)

Modelli 3D: migliore
vitalita', proprieta' cellulari e
tissutali conservate in vitro

Nature. 2013 Sep 19;501(7467):373-9.

POPULATION

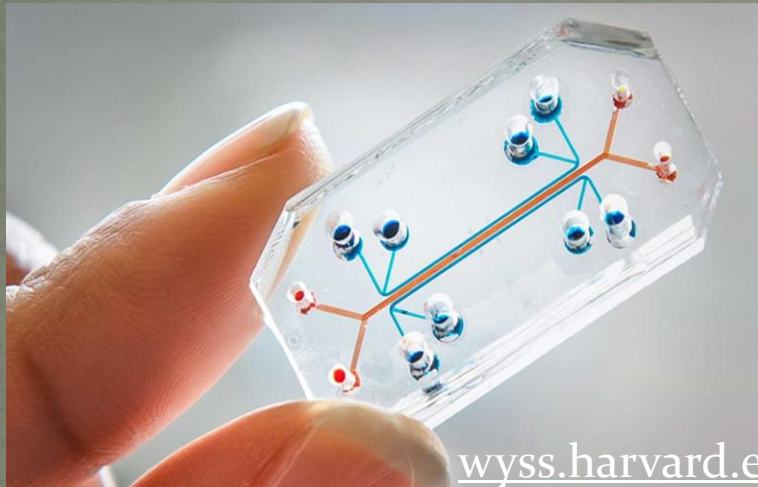
ORGANISM /
BODY

ORGAN /
TISSUE

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PROTEIN /
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GENETIC



wyss.harvard.edu/

Microfluidics, organ-on-chip:
Rapido, riproducibile, sensibile



...disponibile anche in formato
high-throughput per drug testing...

POPULATION

ORGANISM /
BODY

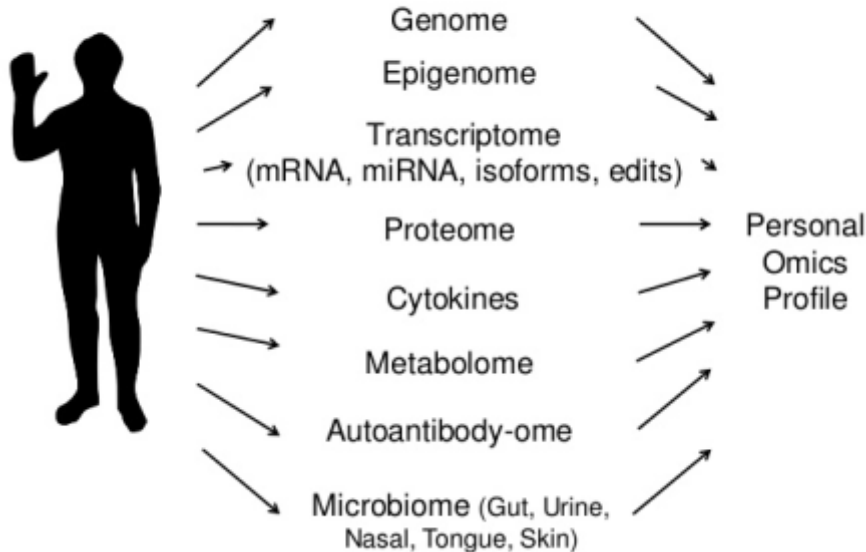
ORGAN /
TISSUE

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EPIGENETIC /
GENETIC

Personal “Omics” Profiling (POP)



Next-generation
sequencing
(‘omics’)

Integrated
computer modeling
Systems biology &
pharmacology



Modelli computazionali

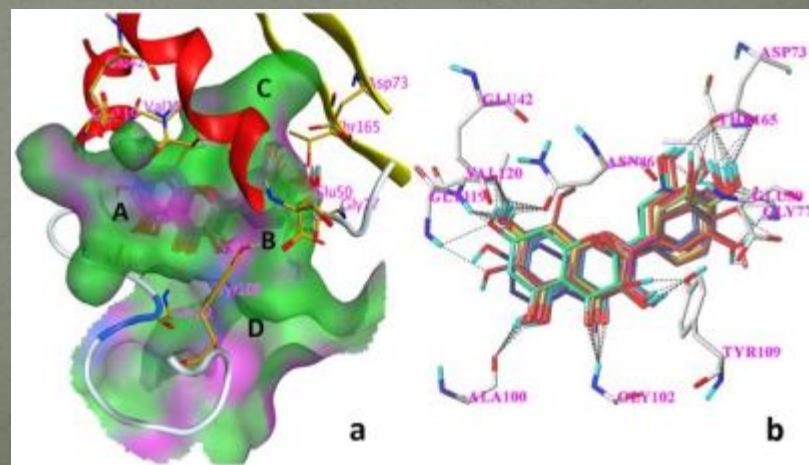
- *In chemico*

- *In silico*



Modelli Q(S)AR (Quantitative Structure-Activity Relationship)

Sviluppo di algoritmi e simulazioni al computer di assorbimento, distribuzione, metabolismo ed escrezione (ADME)

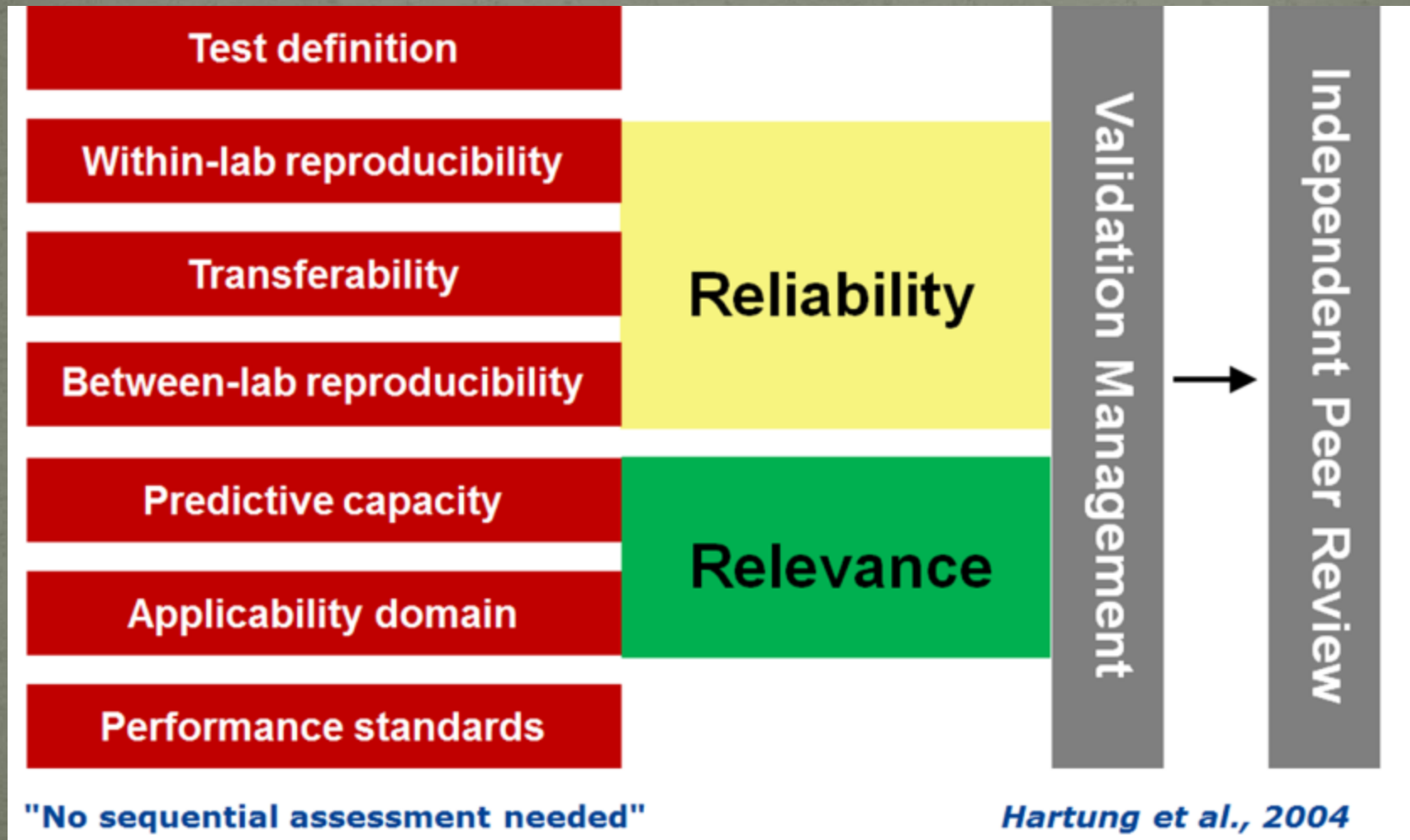


Interazioni tra le 3 R: analisi costi/benefici

- Maggiore attenzione verso lo stato di benessere (Refinement)
- Minore stress in una particolare condizione sperimentale
- Minore variabilità dei risultati: possibilità di ridurre il numero degli animali utilizzati (*Reduction*).



Concetto di Validazione



Conclusioni

- *If we are to use a criterion for choosing experiments to perform, the criterion of humanity is the best we could possibly invent.*
- *The greatest scientific experiments have always been the most humane and the most aesthetically attractive, conveying that sense of beauty and elegance which is the essence of science at its most successful.*



VALUTAZIONE DI EFFETTI BIOLOGICI DI CONTAMINANTI AMBIENTALI

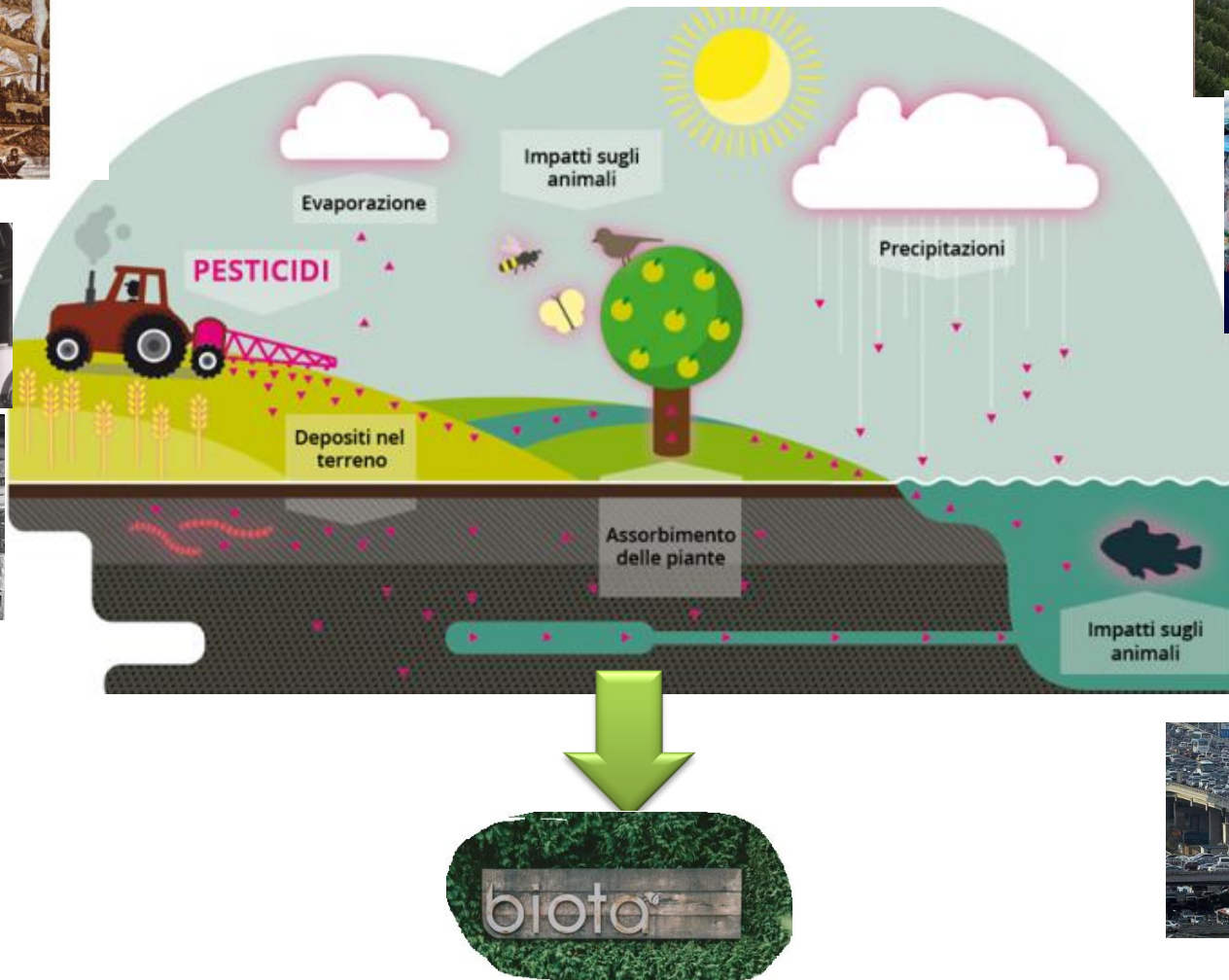
1. La contaminazione ambientale (aspetti generali)
2. Caso di studio: contaminazione da metalli ; il caso del cadmio
3. Fonti naturali e antropiche
4. Vie di esposizione e possibili cause su organismi
5. Scelta di modelli biologici di studio
6. Vantaggi e svantaggi di modelli e di metodi alternativi alla sperimentazione animale
7. Discussione e organizzazione di gruppi di lavoro
8. Elaborazione di un progetto e sua presentazione
9. Discussione generale e conclusioni



Rivoluzione Industriale: rilascio in ambiente di migliaia di sostanze chimiche.

Da un lato c'è stato un aumento della qualità della vita...

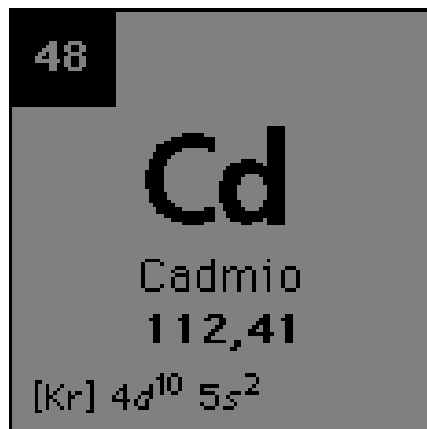
La rivoluzione industriale –
Inghilterra 1776-1851



dall'altro...aumento del rischio tossicologico per l'uomo e l'ambiente

OGGI PARLEREMO DI...CADMIO

Numero atomico



Simbolo atomico

Nome dell'elemento

Peso atomico

Configurazione
elettronica



QUALI SONO LE FONTI **NATURALI** E ANTROPICHE DI CADMIO?

FONTI NATURALI:

- attività vulcaniche
- spray marino
- incendi
- erosione di suoli
- trasporto del vento



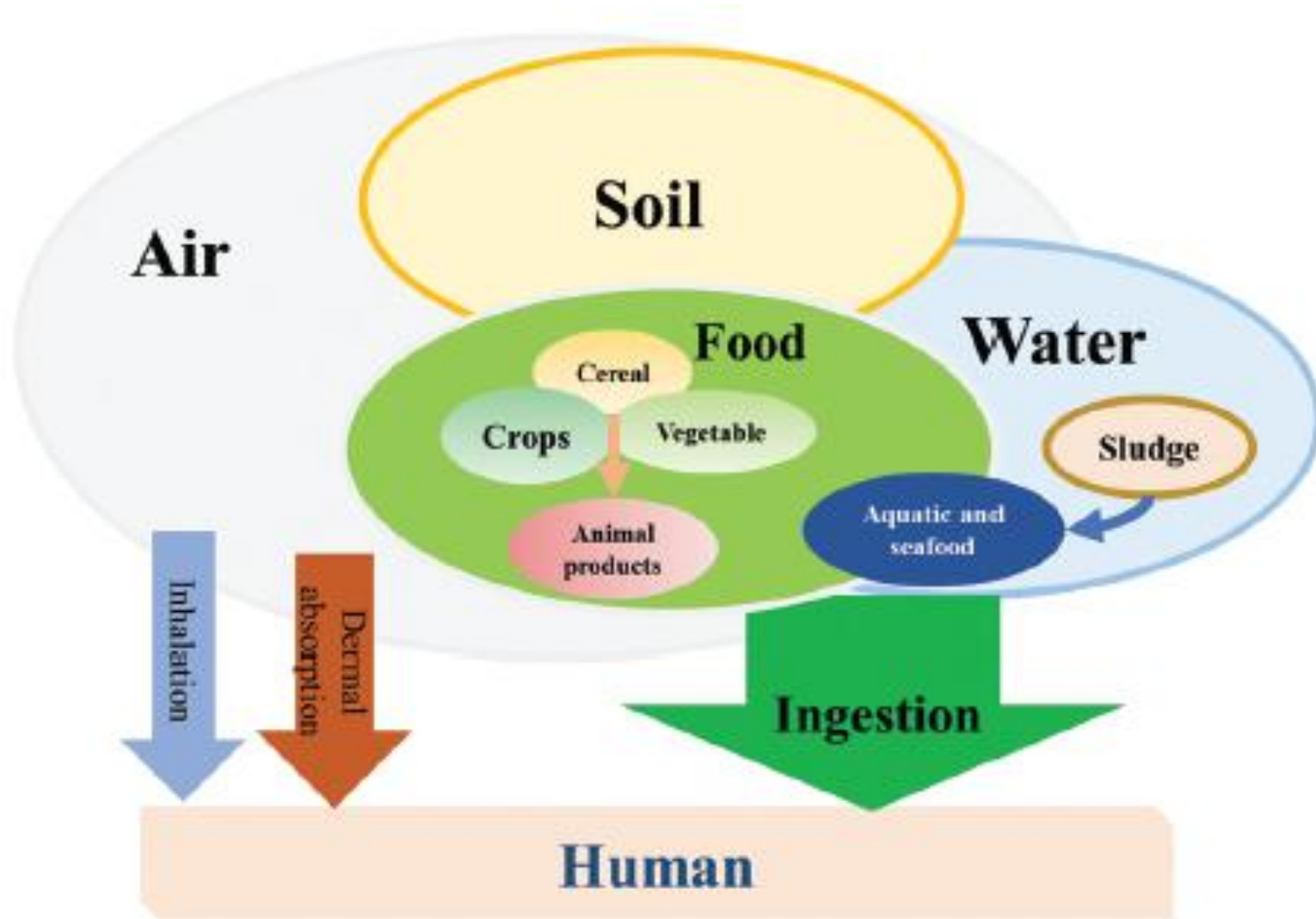
FONTI ANTROPICHE:

- utilizzo di batterie nichel-cadmio
- pigmenti
- industria galvanoplastica
- combustione di energia fossile
- pitture fluorescenti usate come stabilizzanti
- fertilizzanti
- fanghi di depurazione
- saldature
- fumo di sigaretta



circa 30000 ton/anno

PRINCIPALI VIE DI ESPOSIZIONE NON-OCCUPAZIONALE



PERCHE' IL CADMIO E' UN METALLO TOSSICO?

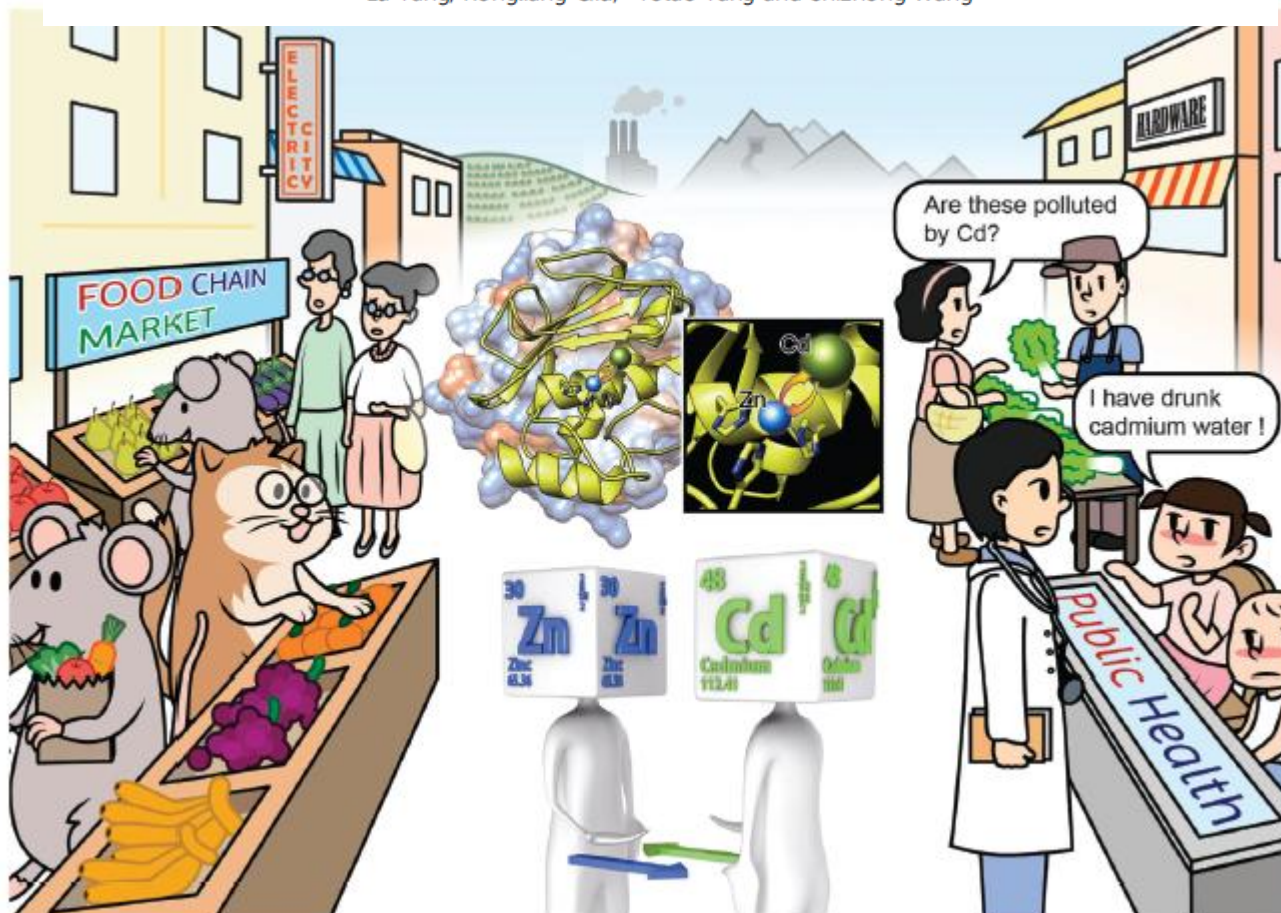
Periodic Table of Elements

H Hydrogen 1.0079 Li Lithium 6.941 Na Sodium 22.989768 K Potassium 39.0983 Rb Rubidium 85.4678 Cs Cesium 132.90543 Fr Francium 223.0197 Alkali Metals Alkaline Earth Metals Transitional Metals Other Metals Nonmetals Noble Gases Atomic Number 21 Symbol Sc Name Scandium 44.95591 Atomic Weight 2He Helium 4.00260																																																																																			
Black = Solid Blue = Liquid Red = Gas White = Synthetically Prepared																																																																																			
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Francium	Radium		Rutherfordium	Dubnium	Seaborgium	Bohrium	Hassium	Meitnerium	Darmstadtium	Roentgenium	Copernicium	Ununtrium	Ununquadium	Ununpentium	Ununhexium	Ununseptium	Ununoctium																																																																		
223.0197	226.0254		[261]	[262]	[266]	[264]	[269]	[268]	[269]	[272]	[277]	Unknown	[289]	Unknown	[288]	Unknown	Unknown																																																																		
Lanthanide Series - <table><tr><td>57La</td><td>58Ce</td><td>59Pr</td><td>60Nd</td><td>61Pm</td><td>62Sm</td><td>63Eu</td><td>64Gd</td><td>65Tb</td><td>66Dy</td><td>67Ho</td><td>68Er</td><td>69Tm</td><td>70Yb</td><td>71Lu</td></tr><tr><td>Lanthanum</td><td>Cerium</td><td>Praseodymium</td><td>Neodymium</td><td>Promethium</td><td>Samarium</td><td>Europium</td><td>Gadolinium</td><td>Terbium</td><td>Dysprosium</td><td>Holmium</td><td>Erbium</td><td>Thulium</td><td>Ytterbium</td><td>Lutetium</td></tr><tr><td>138.9055</td><td>140.115</td><td>140.90765</td><td>144.24</td><td>144.9127</td><td>150.36</td><td>151.9655</td><td>157.25</td><td>158.92534</td><td>162.50</td><td>164.93032</td><td>167.26</td><td>168.93421</td><td>173.04</td><td>174.967</td></tr></table>																		57La	58Ce	59Pr	60Nd	61Pm	62Sm	63Eu	64Gd	65Tb	66Dy	67Ho	68Er	69Tm	70Yb	71Lu	Lanthanum	Cerium	Praseodymium	Neodymium	Promethium	Samarium	Europium	Gadolinium	Terbium	Dysprosium	Holmium	Erbium	Thulium	Ytterbium	Lutetium	138.9055	140.115	140.90765	144.24	144.9127	150.36	151.9655	157.25	158.92534	162.50	164.93032	167.26	168.93421	173.04	174.967																					
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Actinide Series - <table><tr><td>89Ac</td><td>90Th</td><td>91Pa</td><td>92U</td><td>93Np</td><td>94Pu</td><td>95Am</td><td>96Cm</td><td>97Bk</td><td>98Cf</td><td>99Es</td><td>100Fm</td><td>101Md</td><td>102No</td><td>103Lr</td></tr><tr><td>Actinium</td><td>Thorium</td><td>Protactinium</td><td>Uranium</td><td>Neptunium</td><td>Plutonium</td><td>Americium</td><td>Curium</td><td>Berkelium</td><td>Californium</td><td>Einsteinium</td><td>Fermium</td><td>Mendelevium</td><td>Nobelium</td><td>Lawrencium</td></tr><tr><td>227.0278</td><td>232.0381</td><td>231.03688</td><td>238.0289</td><td>237.0482</td><td>244.0642</td><td>243.0614</td><td>247.0703</td><td>247.0703</td><td>251.0776</td><td>[254]</td><td>257.0951</td><td>258.1</td><td>259.1009</td><td>[262]</td></tr></table>																		89Ac	90Th	91Pa	92U	93Np	94Pu	95Am	96Cm	97Bk	98Cf	99Es	100Fm	101Md	102No	103Lr	Actinium	Thorium	Protactinium	Uranium	Neptunium	Plutonium	Americium	Curium	Berkelium	Californium	Einsteinium	Fermium	Mendelevium	Nobelium	Lawrencium	227.0278	232.0381	231.03688	238.0289	237.0482	244.0642	243.0614	247.0703	247.0703	251.0776	[254]	257.0951	258.1	259.1009	[262]																					
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Cadmium–zinc exchange and their binary relationship in the structure of Zn-related proteins: a mini review

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POSSIBILI EFFETTI SULL'UOMO

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Tossicità renale

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IL METODO SCIENTIFICO



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Sviluppare ipotesi (affermazioni verificabili)



Realizzare esperimenti



Raccogliere dati



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Replicare gli esperimenti



OBserve
ASk
HYPOTHEsize
EXPERIMENT
ANALYZE
CONCLUDE
REPLICATE

E ADESSO PROVATECI VOI

Scrivete, anche in modo schematico, un
PROGETTO
che descrive come affrontereste il seguente
problema ambientale
o caso di studio

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Contaminazione da
cadmio e salute umana

Alcuni spunti di lavoro...

PREMESSE

- Vie di esposizione
- Organi/tessuti bersaglio
- Potenziali effetti

PROCEDURE

- Scelta del modello di studio che tenga conto dei principi delle 3Rs
- Vantaggi e limitazioni