From AOPs (Adverse Outcome Pathways) to the next generation risk assessment (NGRA) for the final goal of full animal replacement in toxicology

Costanza Rovida, CAAT-EU (European Centre for Alternatives to Animal Tests)

costanza.rovida@chimici.it

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Principle of Risk Assessment

RISK = HAZARD x EXPOSURE

Definition of the threshold for the hazard

Assessment of the exposure

RCR (Risk Characterisation Ration) = $\frac{\text{Exposure}}{\text{No effect Level}}$

RCR < 1 **SAFE**

RCR > 1 **RISK**

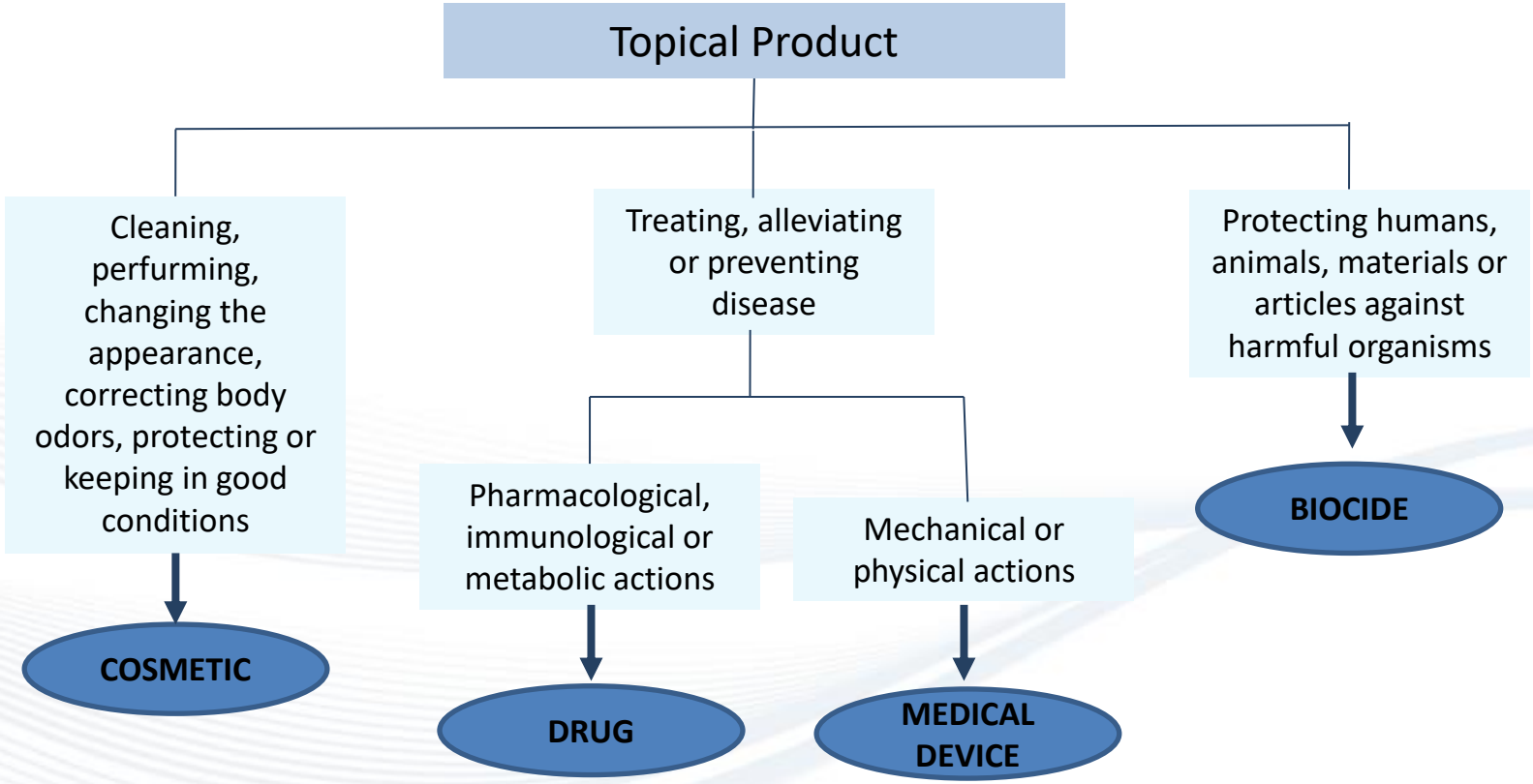
Each Regulation has its own conditions for Risk Assessment

Regulation EC 1107/2009 on Plant Protection Products

Regulation EC 1935/2004 on food contact material

Regulation EC 528/2012 on Biocidal Products

Cosmetic, medical device, drug,…?



Cosmetic, medical device, drug,…?

Topical Product

- Cleaning, perfurming, changing the appearance, correcting body odors, protecting or keeping in good conditions → **COSMETIC**
- Treating, alleviating or preventing disease
  - Pharmacological, immunological or metabolic actions → **DRUG**
  - Mechanical or physical actions → **MEDICAL DEVICE**
- Protecting humans, animals, materials or articles against harmful organisms → **BIOCIDE**

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DRUG

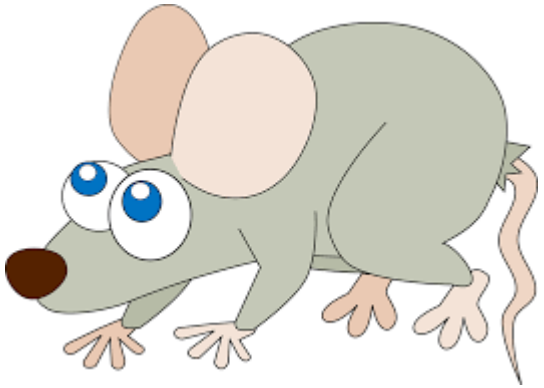
MEDICAL DEVICE

Pr… s,
ani… or
ha… s

BIOCIDE

At the moment, the official way to do Risk assessment is from in vivo studies

In vivo tests: administration



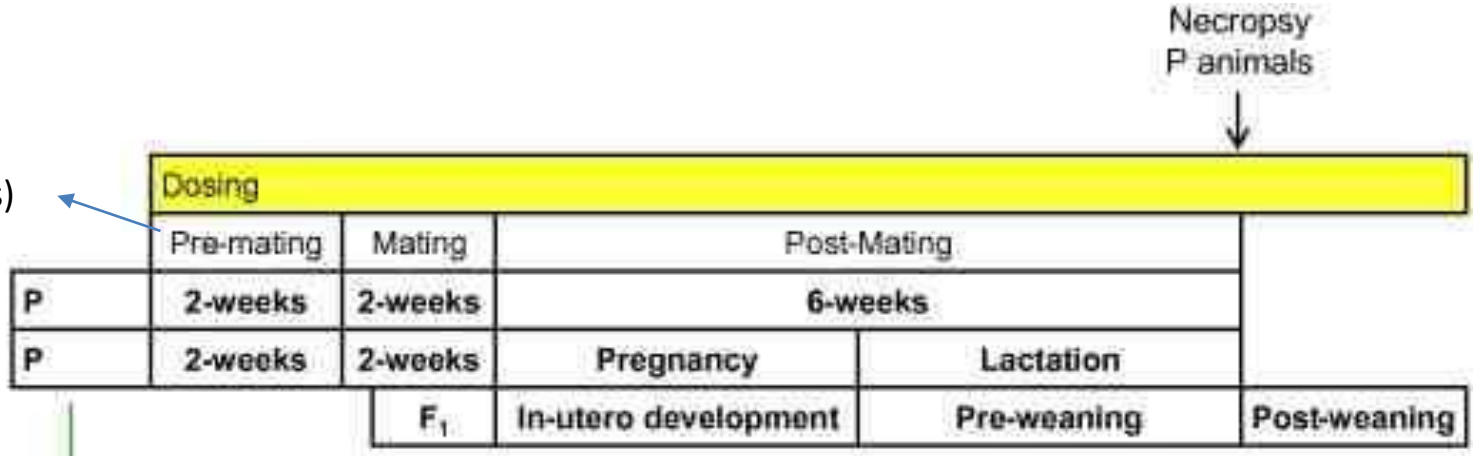
- Diet: feed
- Drinking water
- Gavage

Some examples:
NaCl was tested at 4% w/w in feed and 2% w/w in drinking water
EtOH, a biocide active substance, was tested at 15% w/w in drinking water – more than wine!

If gavage does not enter the stomach, the substance is breath and goes into the lungs. If it is too long, it can damage the stomach.
Volume:
1 ml / 100 g bw → **600 ml**

In vivo tests: protocol (example of the OECD TG 443, EOGRTS)

often longer (10 weeks)



Parental generation	Cohort	Designation	Animals/Cohort	Sexual maturation	Approximate age at necropsy (weeks)
Target is 20 litters per group	1A	Reproductive	20M + 20F	Yes	13
	1B	Reproductive	20M + 20F	Yes	14 or 20-25 if triggered
	2A	Neurotoxicity	10M + 10F @	Yes	11-12
	2B	Neurotoxicity	10M + 10F @	No	3
	3	Immunotoxicity	10M + 10F @	Yes	8
	Surplus	Spares		No	3

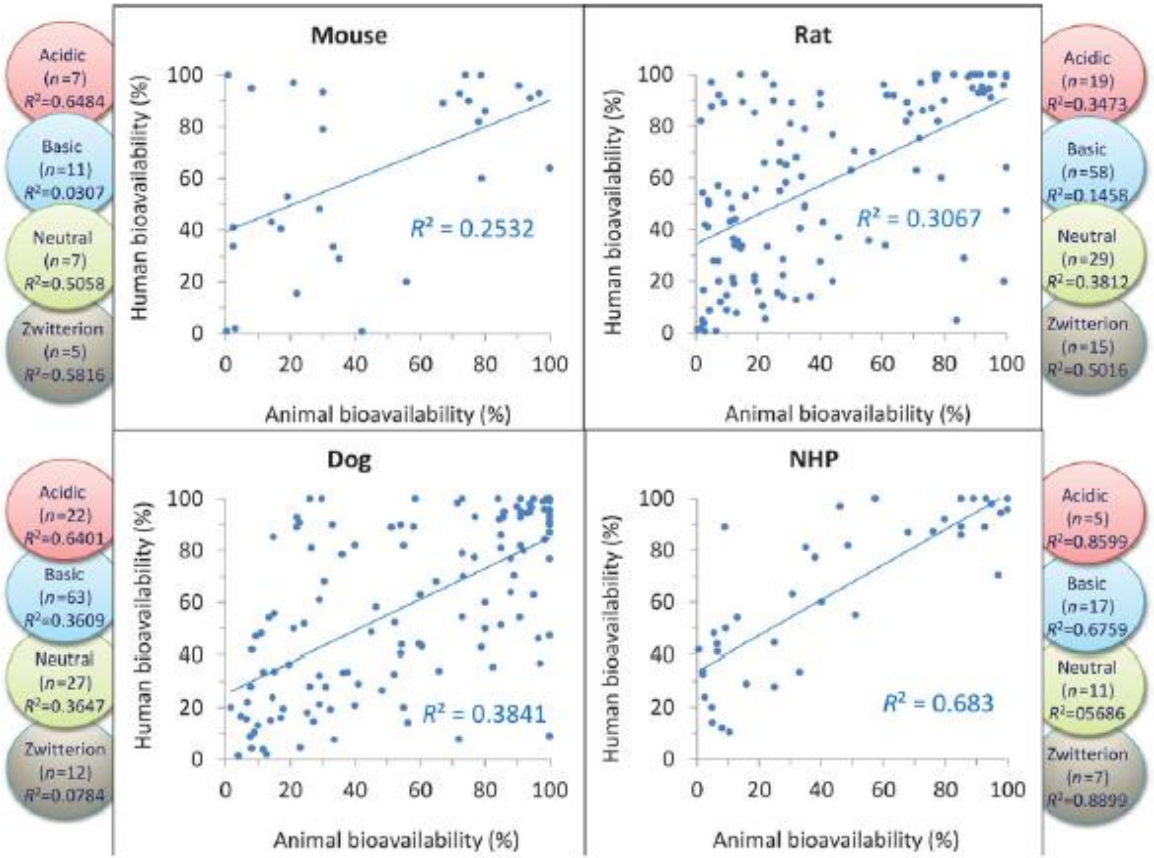
@ one per litter and representative of 20 litters in total where possible

Rat – human comparison: gastrointestinal tract

Gastrointestinal Region	Mouse	Rat	Rabbit	Human
Stomach - fasten	4.04	3.9	1.9	0.4 - 4
Stomach - fed	2.98	3.2		2.0 – 4.5
Duodenum	4.75	5.5	6	5.0 – 7.0
Jeiunum	5.01	6.13	6.8	6.6
Ileum	4.8	5.9	7.5	7.5
Colon	4.7	5.5		6.4
Feces	4.7	5.7		6.5

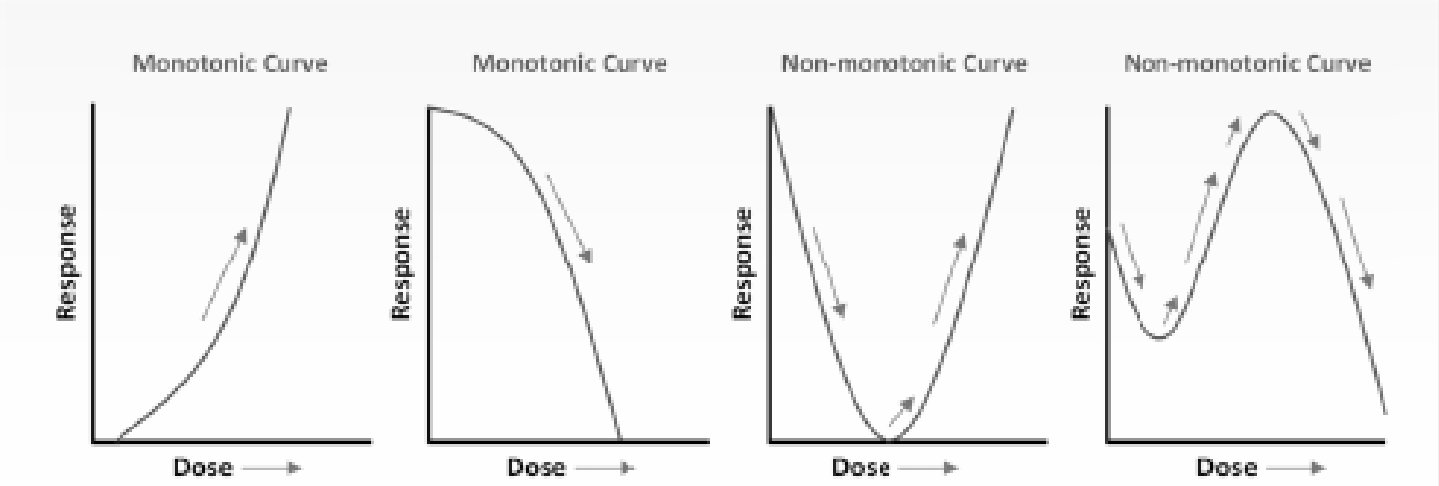
pH in the different gastrointestinal regions

Bioavailability of various drugs in human versus mouse, rat, dog, and non-human primate (NHP).



Hatton et al. (2014) *doi:10.1002/jps.24365*

Non Monotonic Dose Response of Endocrine Disruptors



- Plurality of molecular target
- Receptor desensitization
- Metabolic effect hypotheses
- Mixed-ligand hypothesis

What about mixture or multiple exposure to chemicals?

Lagarde et al. (2015) http://www.ehjournal.net/content/14/1/13

Come si calcola la dose di no effetto (DNEL)

Oral Gavage

1000 – 300 – 150 – 0 mg/Kg

- **Repeated dose toxicity**
- **Reproductive toxicity**
- **Developmental Toxicity**
- **Carcinogenicity**

Allometric scaling	**4 (rat-human)**
Oral to inhaled quantity	*70Kg/10m3/person

NOAEC = 367.5 mg/m³

Calcolo Assessment Factor (AF)

Difference in duration of exposure: 6

Other interspecies differences: 2.5

Intraspecies differences: 5

Totale AF = 75

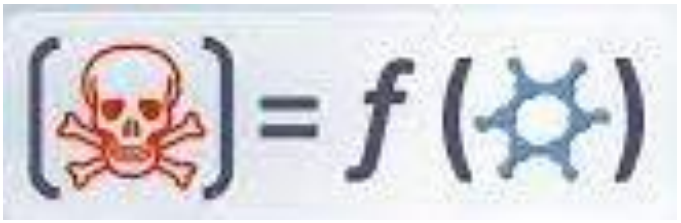
$$\text{DNEL} = \frac{367.5\ \text{mg/m}^3}{75} = 4.9\ \text{mg/m}^3$$

Derived no Effect Level, workers, inhalation

RCR

General rules for adaptation (Annex XI)

- Weight of evidence using existing data and non-animal methods
- Mathematical models/QSARs
- "Suitable" *in vitro* methods
- Grouping and Read-across



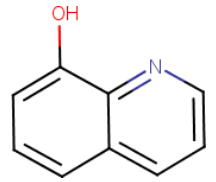
REACH pioneered use of such alternative methods to replace animal testing.

Derivation of DNEL after Annex XI application

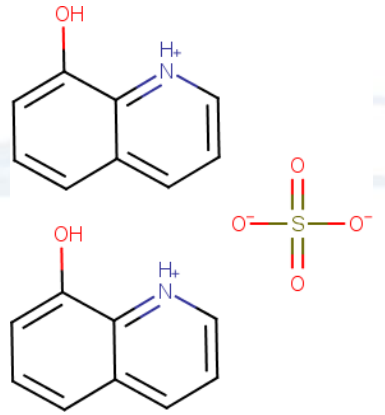
There is no precise rules. Consider:

- Bioavailability
- Additional Assessment Factor
- MW compensation

An example



Source:
8hydroxyquinoline
MW 145.16 g/mol

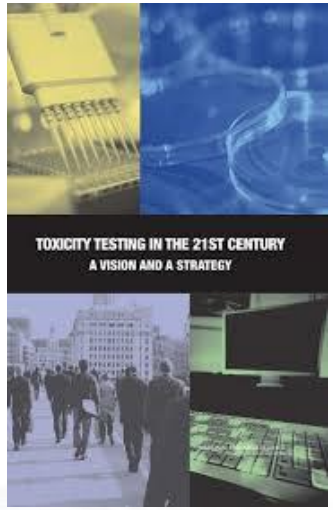


Target:
bis-8hydroxyquinoline sulphate
MW 388.4 g/mol

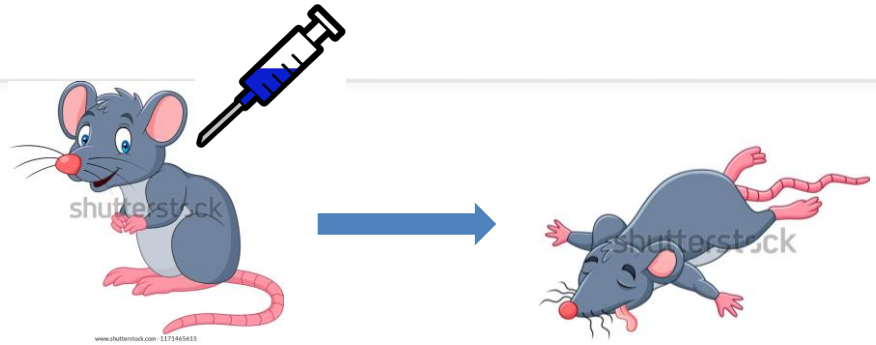
DERIVED NO EFFECT LEVEL (DNEL) – dermal consumer

$NOAEL_{8hydroxyquinoline}$ = 200 mg/kg bw/day → Moli = 200/145.16 = 1.37 mmol
$NOAEL_{solfata}$ = 1,37/2 * 388,4 = 266 mg/kg bw/day
AF = 600
$DNEL_{solfata}$ = 0.443 mg/kg bw/day

Paradigm shift



Traditional toxicology

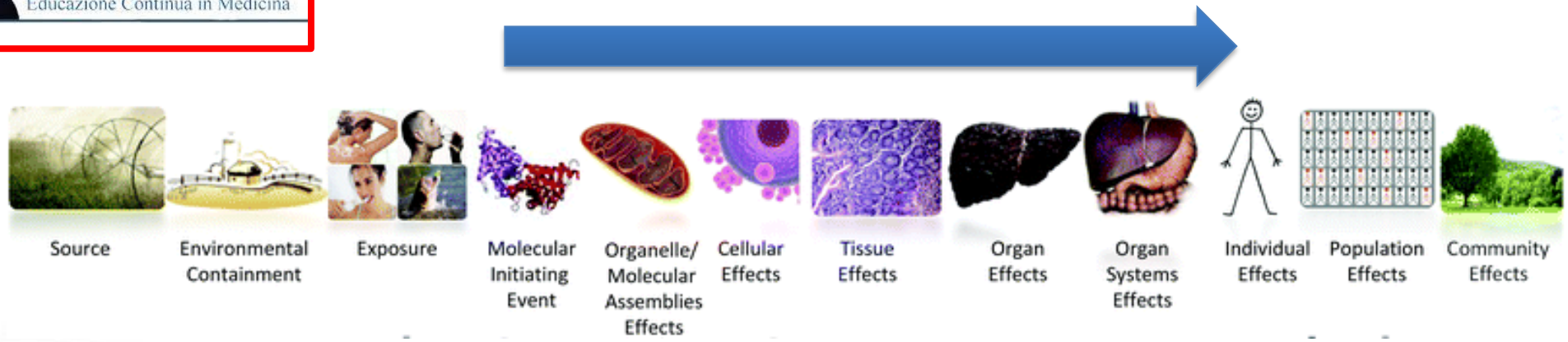


(Courtesy of Mel Andersen)

Report del National Research Council of the National Academies Published on 2007

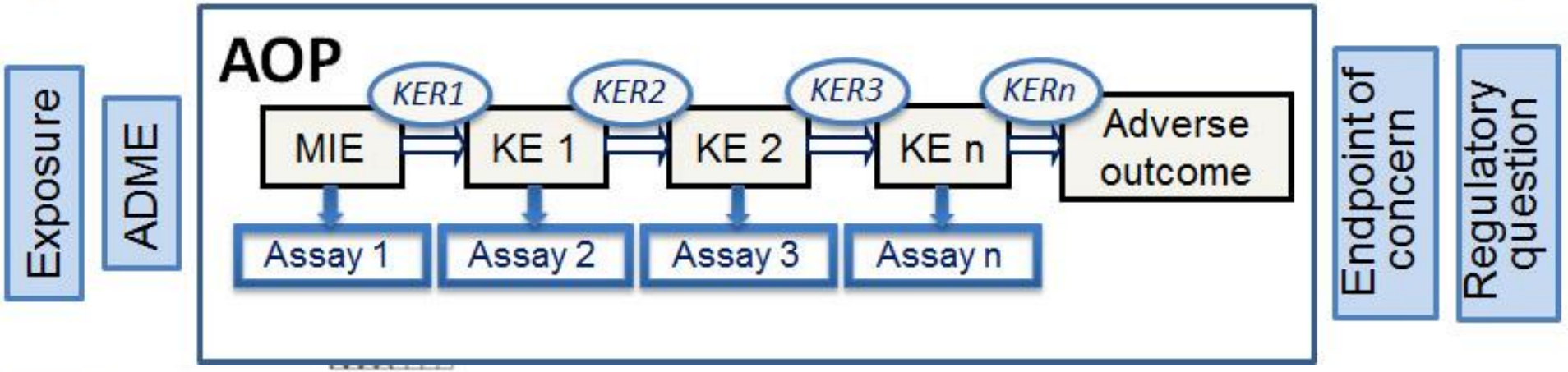
ADVERSE OUTCOME PATHWAY

E.C.M.
Educazione Continua in Medicina



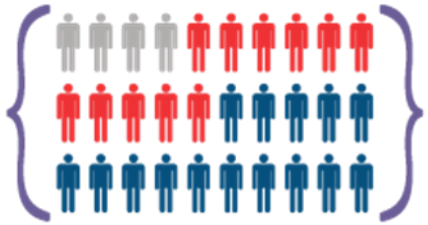
IATA

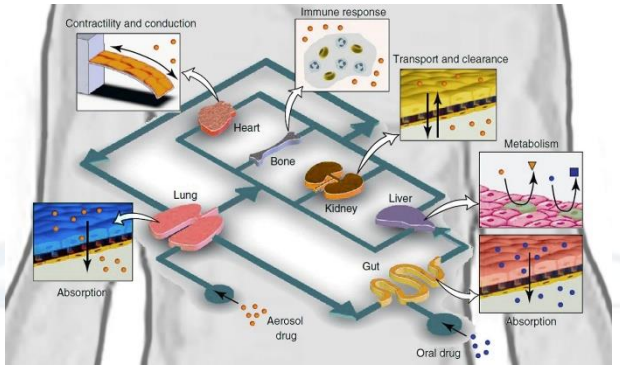
Decision Context



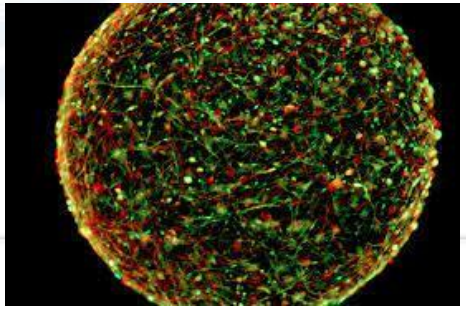
NAMs, New Approach Methodologies

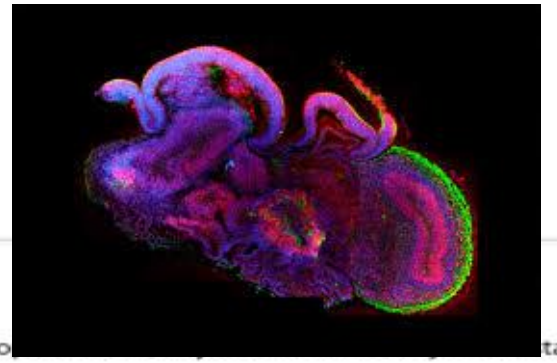
Qualunque tecnologia, metodo approccio o loro combinazione e che può essere utilizzata per fornire informazioni circa le proprietà tossicologiche delle sostanze chimiche e per effettuare valutazioni di rischio, senza ricorrere a all'uso di animali vertebrati vivi.
Questa nuova metodologia richiede approcci integrati IATA (Integrated Approaches to Testing and Assessment), e necessita di approcci definiti per l'interpretazione dei risultati oltre a una stima delle incertezze



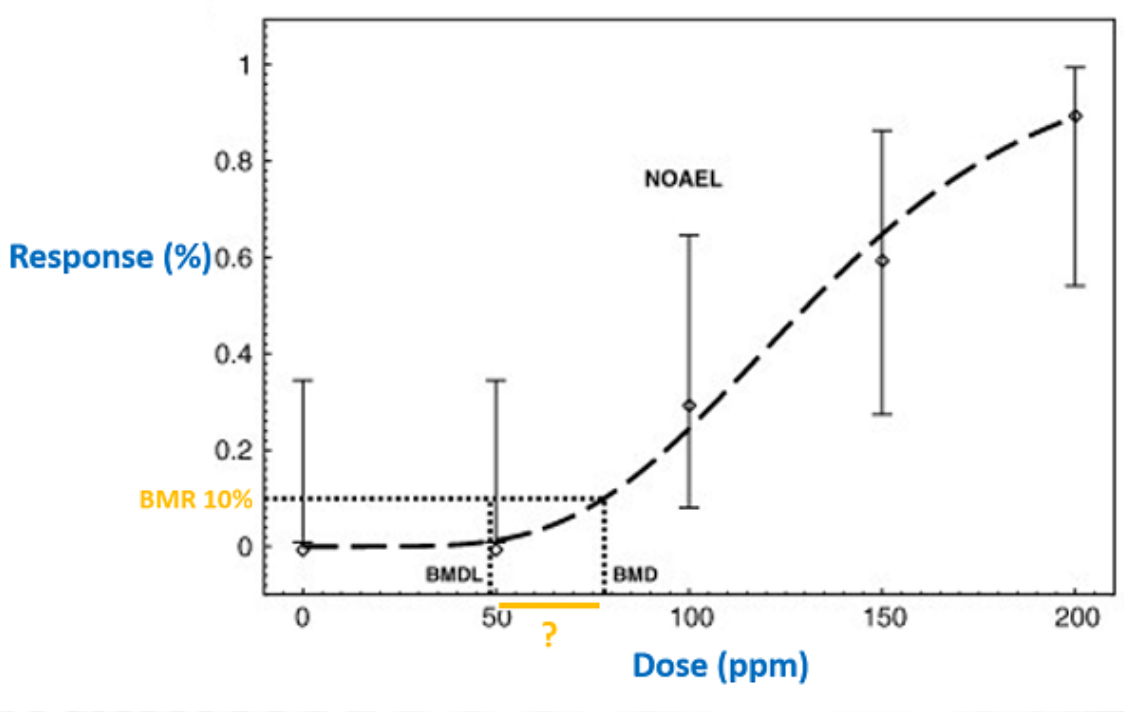


Complex integrated in vitro systems
QSARs and Artificial Intelligence (AI)
Epidemiological data
In vivo / ex vivo data





QIVIVE = Quantitative in vivo in vitro Extrapolation



Application

In vitro effect concentrations

↓ link to

PBK model-predicted internal concentrations

↓ translate to

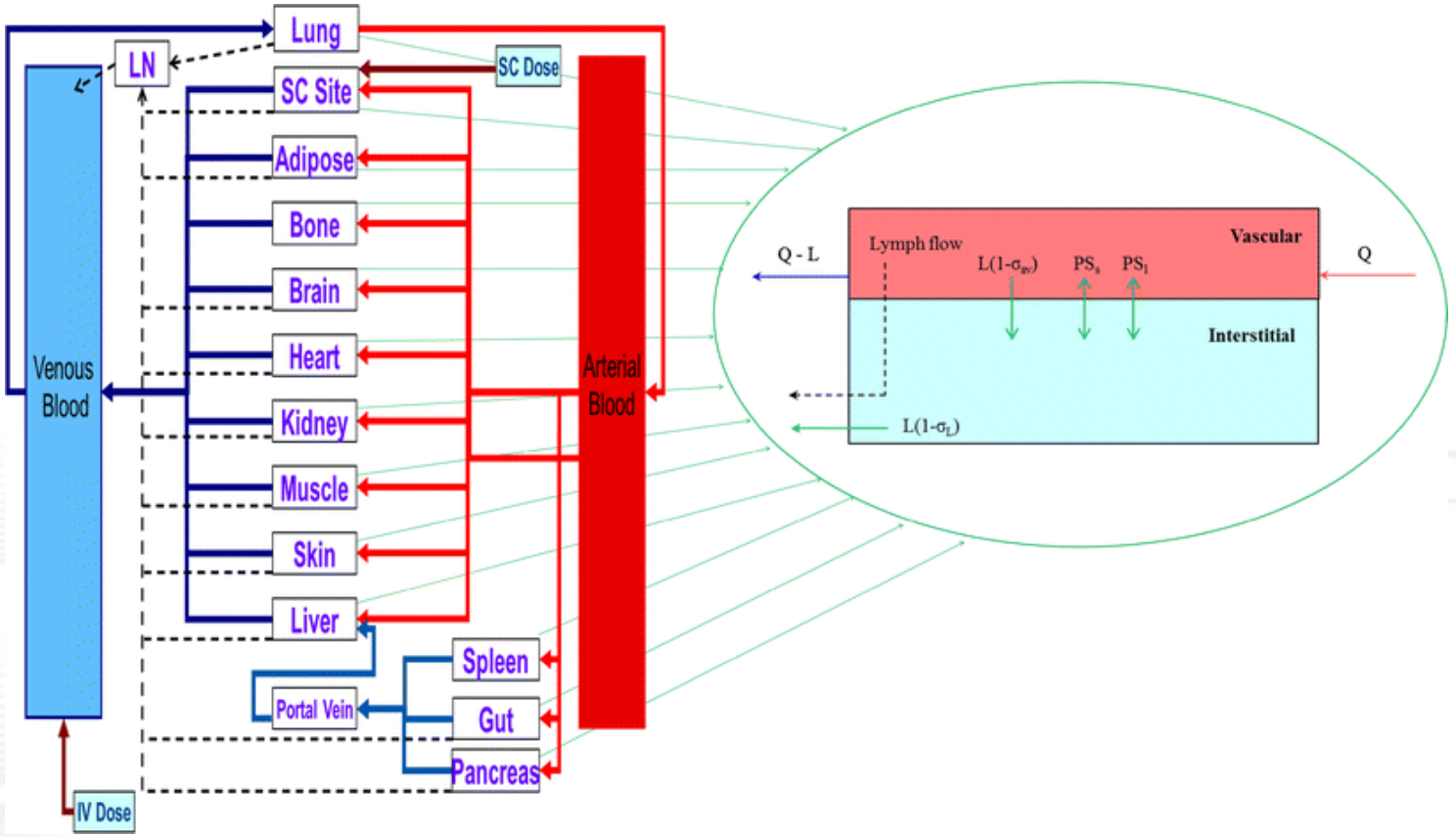
Oral dose levels

=

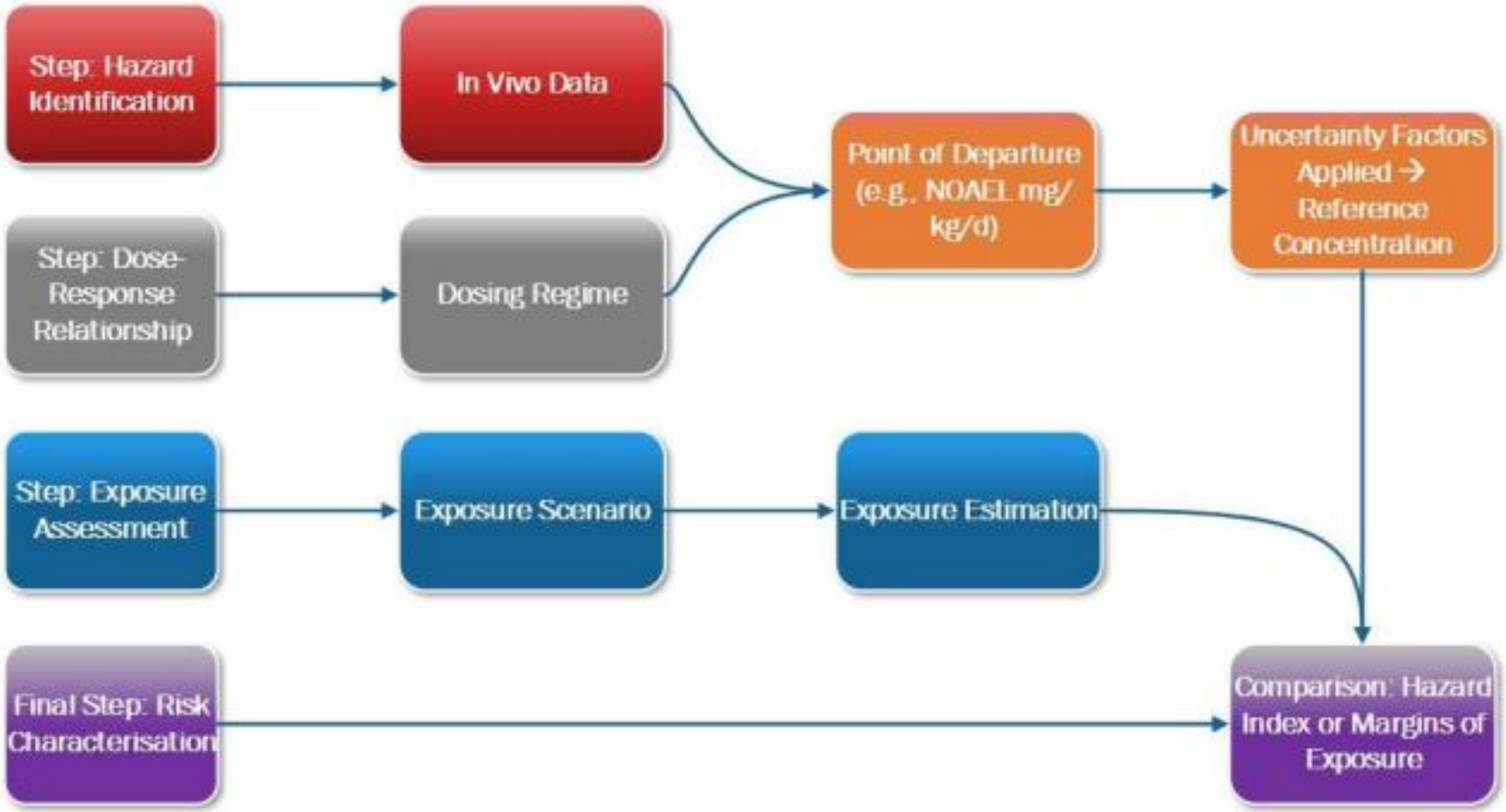
Oral equivalent doses

PBPK models (Physiologically Based Pharmacokinetic Model)

ADME
Absorption
Distribution
Metabolism
Excretion

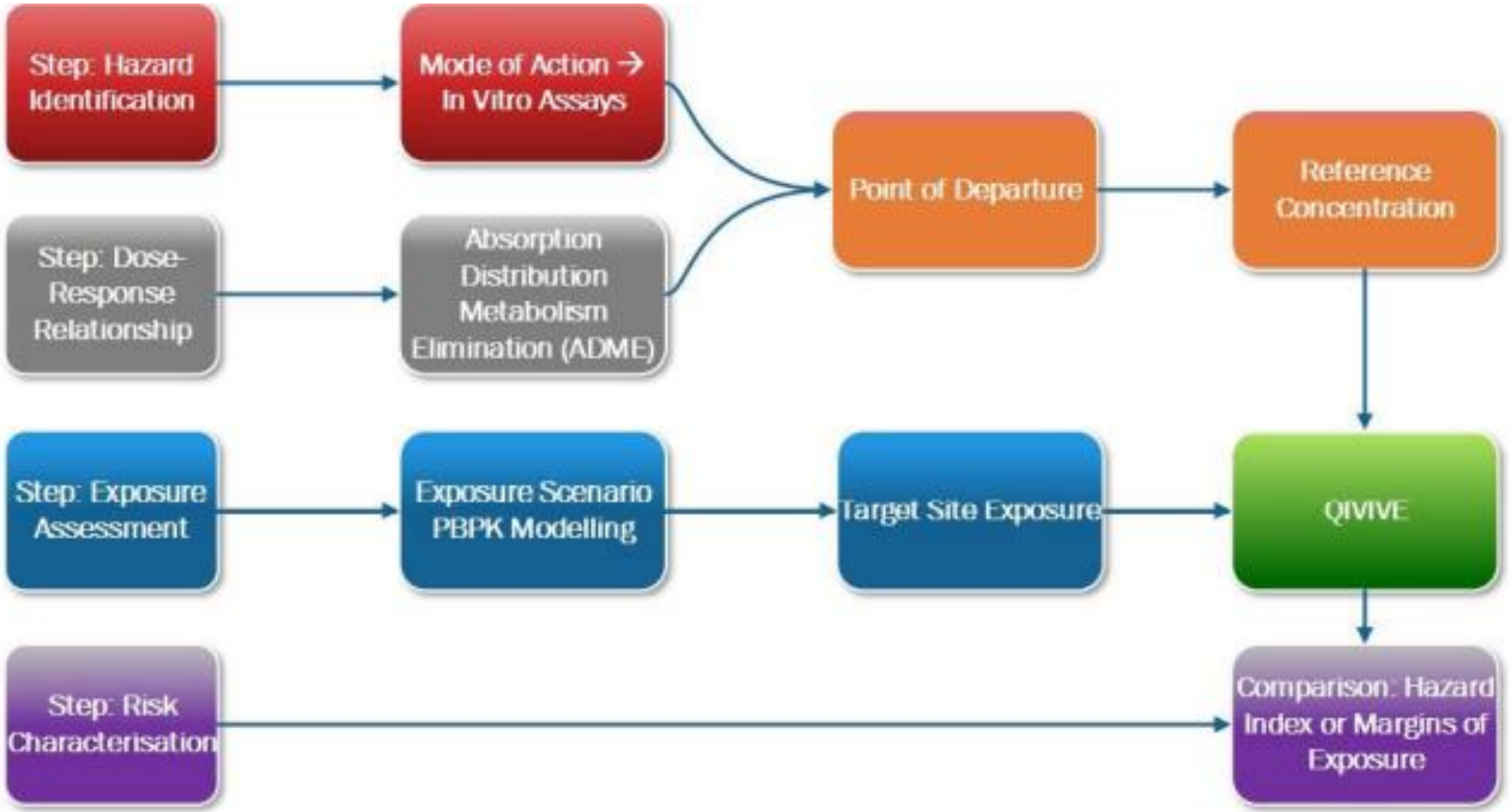


Traditional Safety Assessment



Toxicol In Vitro. 2018 Aug;50:137 146

Shifted Safety Assessment Paradigm



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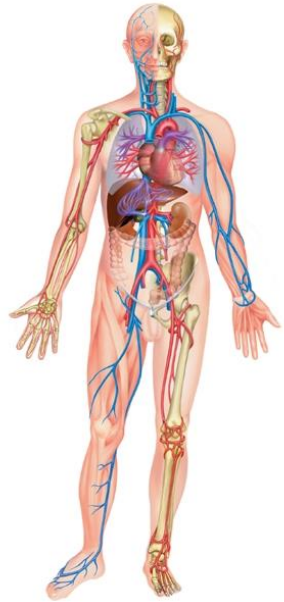
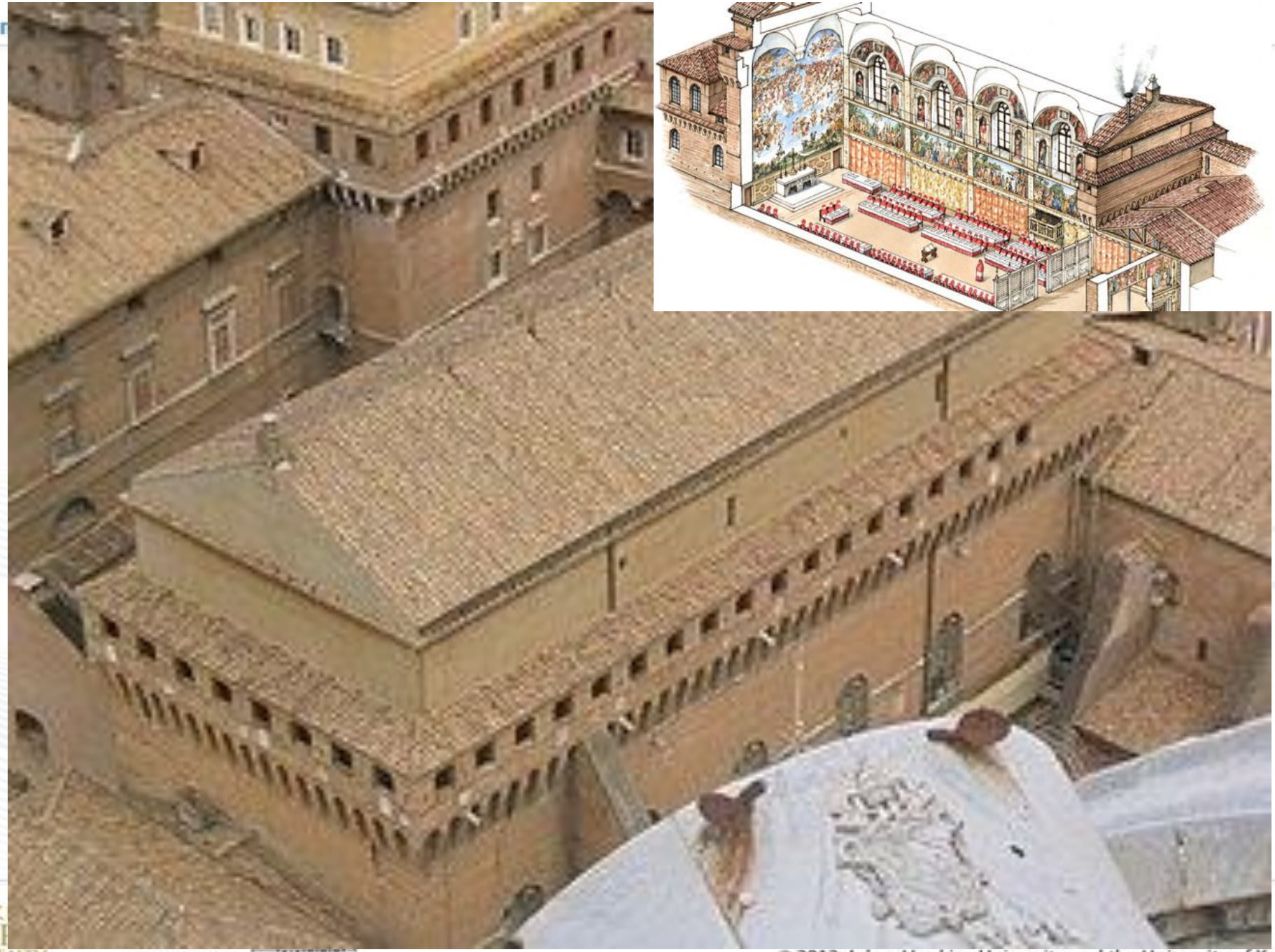
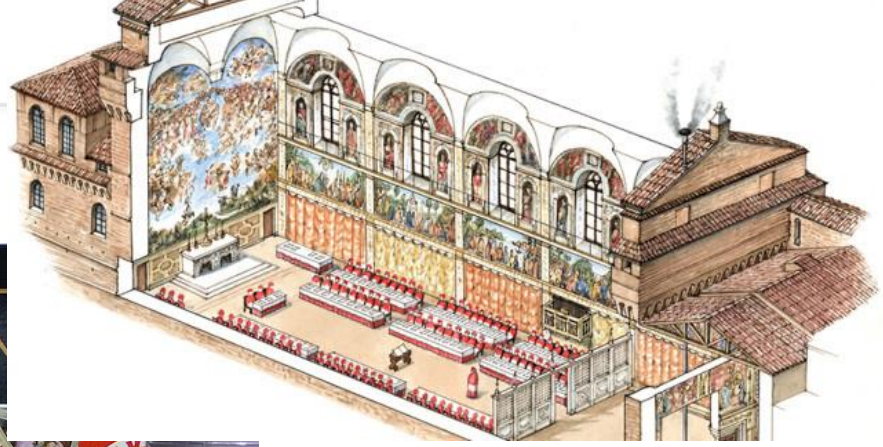




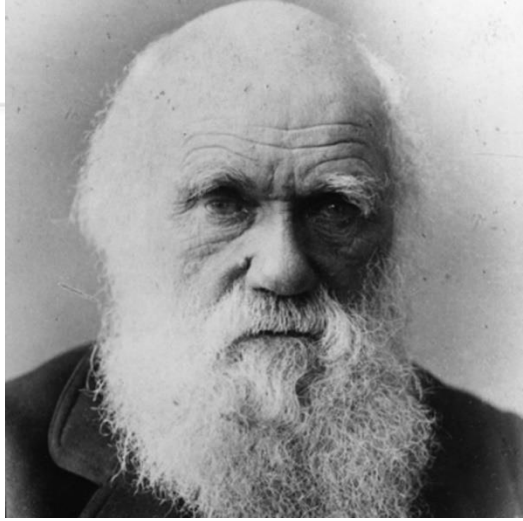
Test *in vivo*



AOP

Conclusioni

- Da un punto di vista regolatorio ci sono due pulsioni:
  - Da una parte la richiesta di andare verso I metodi in vitro è sempre più pressante
  - Dall'altra parte c'è una forte resistenza a lasciare I metodi tradizionali per abbracciare quelli nuovi
- Bisogna aprirsi verso la scienza per ottenere una maggiore salvaguardia dell'uomo e dell'ambiente
- Mai tralasciare il rigore scientifico nelle valutazioni
- Il risultato di un singolo test va inserito in una valutazione globale
- La valutazione delle sostanza deve essere complessiva e non end-point per end-point
- Attenzione a costi e semplicità



Charles Darwin, 1871

"You ask about my opinion on vivisection. I quite agree that it is justifiable for real investigations on physiology; but not for mere damnable and detestable curiosity. It is a subject which makes me sick with horror, so I will not say another word about it, else I shall not sleep tonight."

Grazie per l'attenzione!