

Nanotoxicity: from whole animals to subcellular organelles

Paolo Bigini & Luisa Diomedede

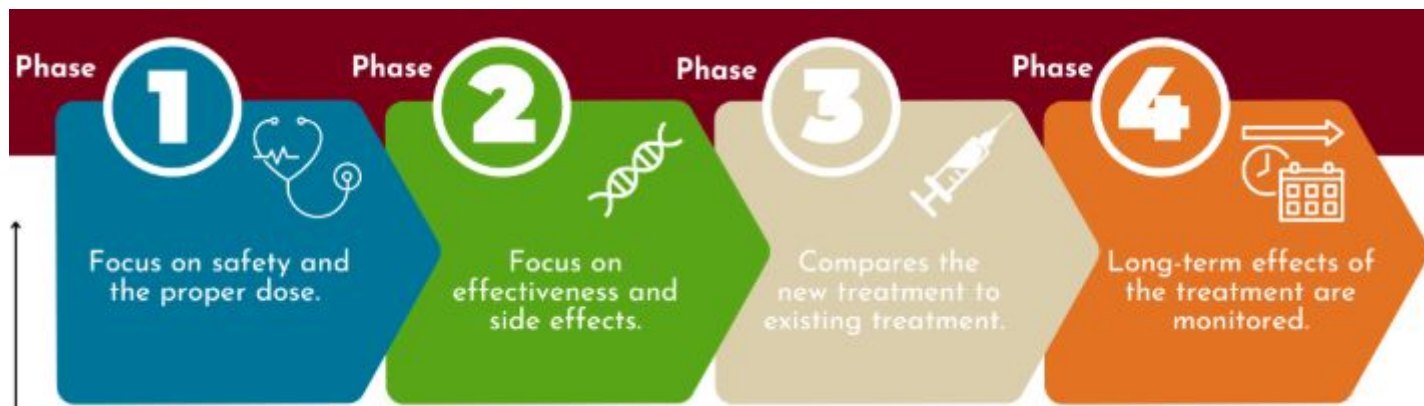
Istituto di Ricerche Farmacologiche Mario Negri IRCCS



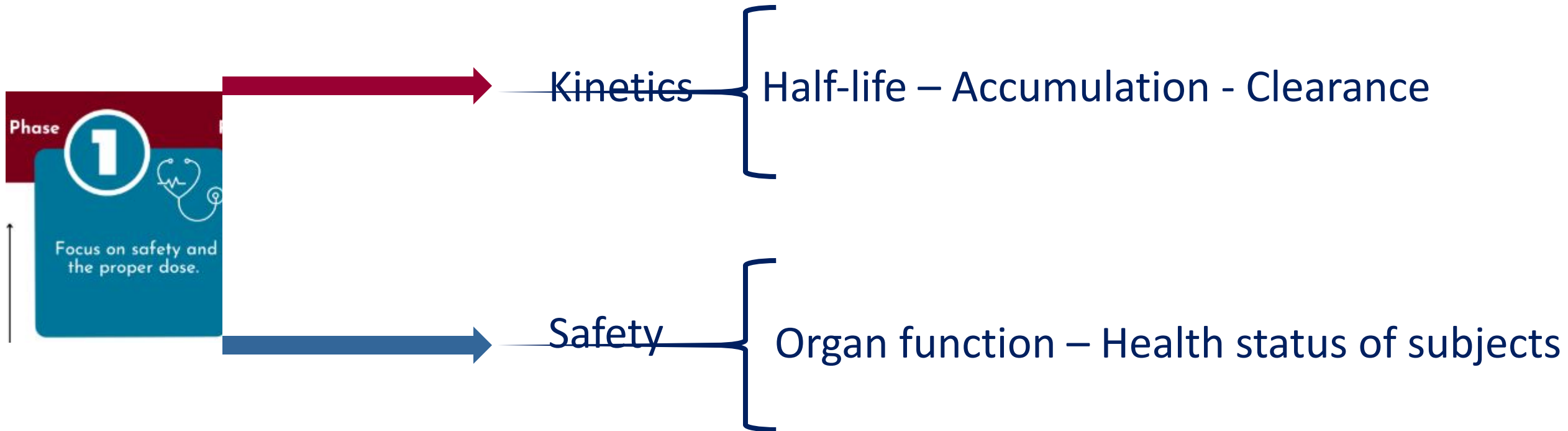
Center of Preclinical Toxicology and Biochemistry

PreToxBi

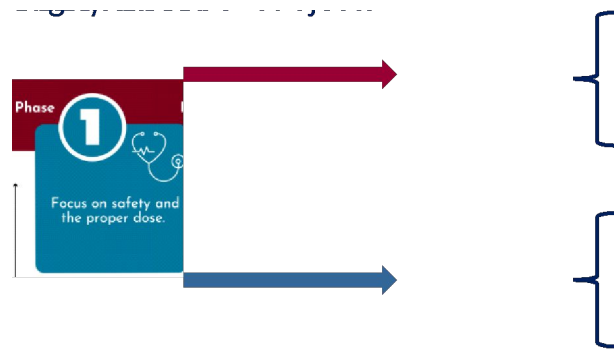
NanoSafety and Bio-Nano Interaction: learning from pharmacology



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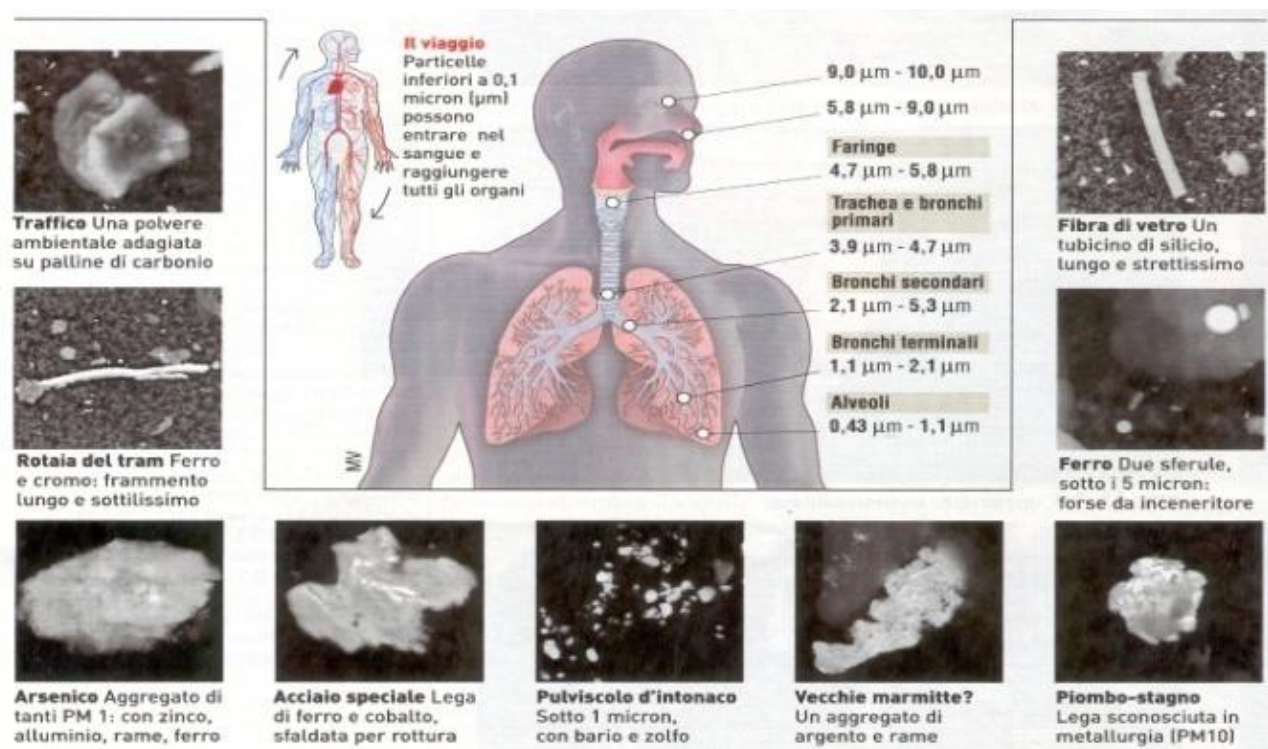


The approach to understand side-effect of drugs or nanocarriers in phase 1
should not differ from the investigation of the toxicity of nanomaterials

NanoSafety and Bio-Nano Interaction: from pharma to tox

ENTRY

NASAL
ORAL
DERMAL

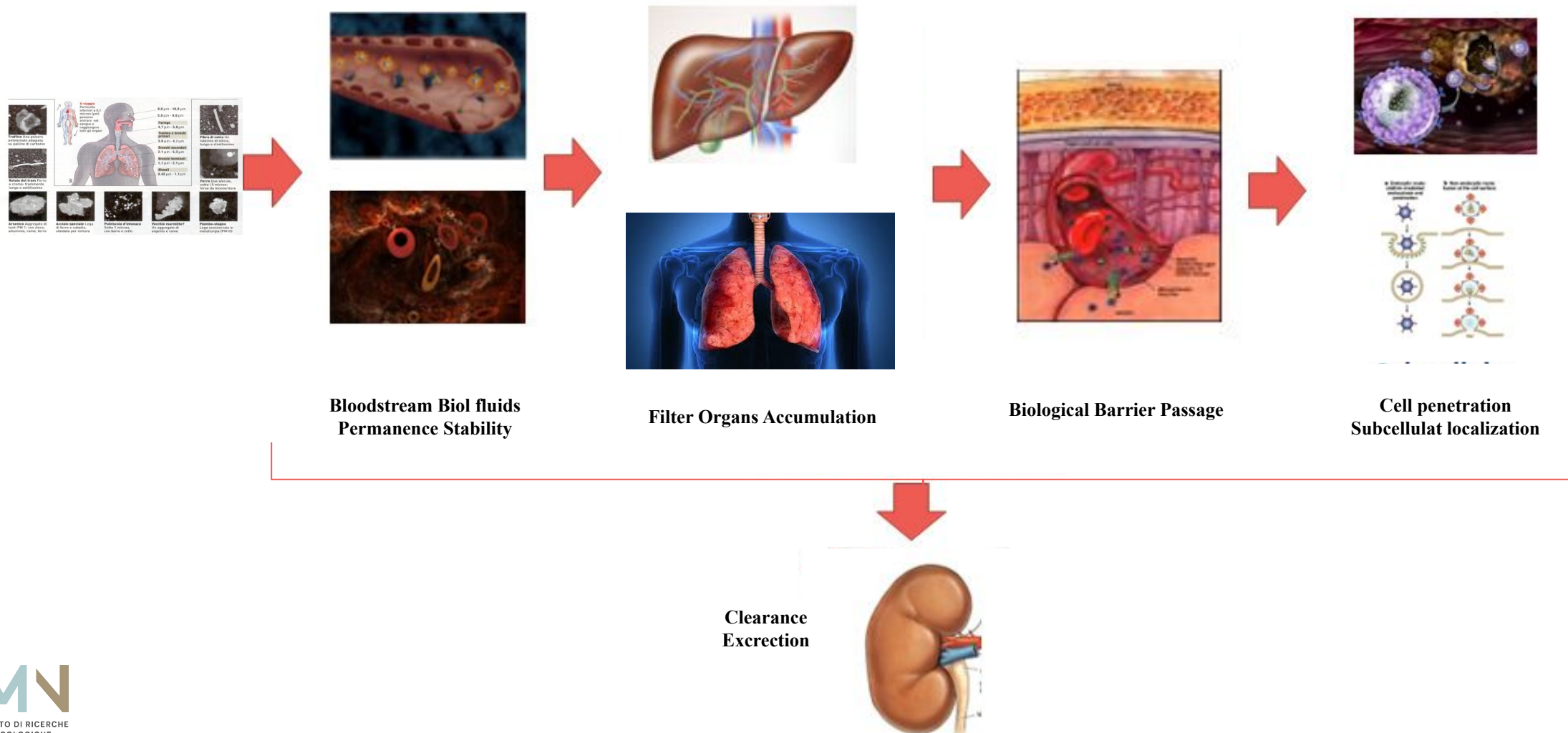
**TARGET**

HEALTHY/SICK
CHILD/ADULT/OLD
MALE/FEMALE

EXPOSURE

ACUTE
CHRONIC
PERMANENT

NanoSafety and Bio-Nano Interaction: track their fate



NanoSafety and Bio-Nano Interaction: human studies

Direct

Quick impact

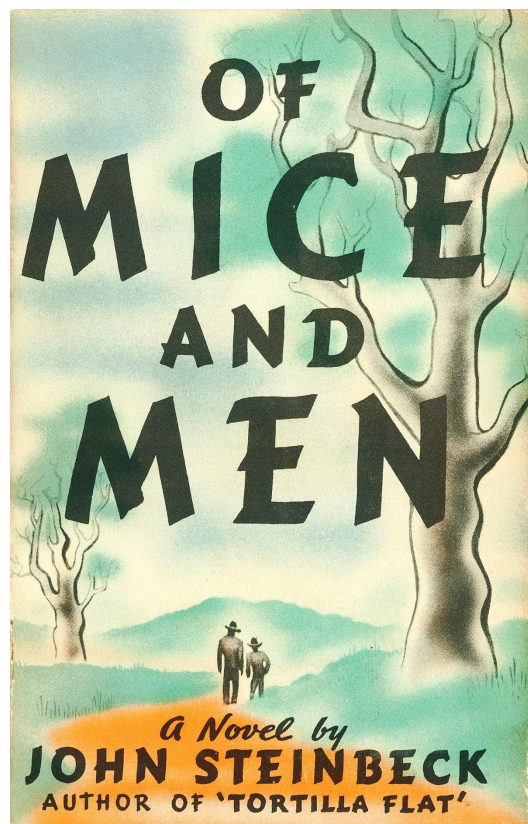


Ethical limitations

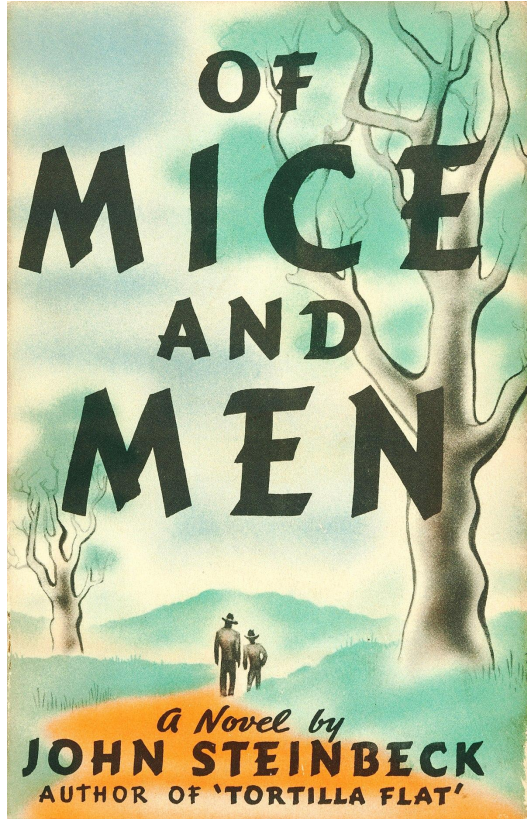
No single material

Heterogeneity cohorts

NanoSafety and Bio-Nano Interaction: from humans to mice



NanoSafety and Bio-Nano Interaction: from humans to mice



Reduction :

Minimum number of animals used, consistent with the objectives of the research project

Refinement :

Optimization of the methodology to reduce the suffering of animal during the studies

NanoSafety and Bio-Nano Interaction: from theory to practice

Reduction :

Minimum number of animals used, consistent with the objectives of the research project

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mouse and toxicology

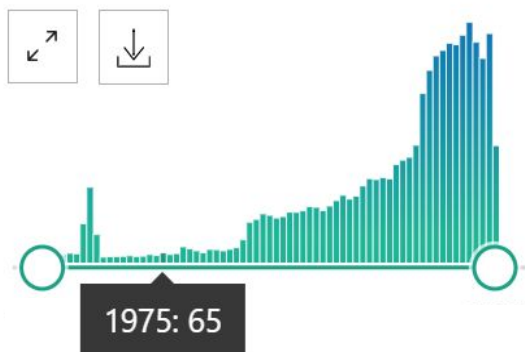
Search

Advanced Create alert Create RSS User Guide

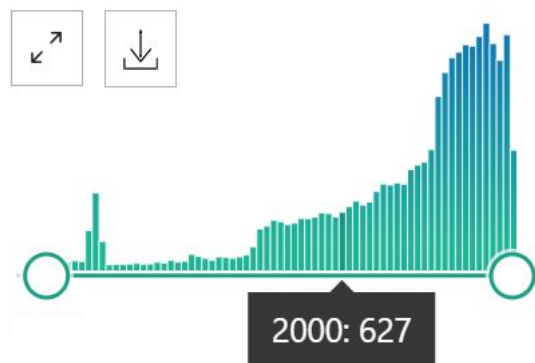
Save Email Send to Sort by: Best match Display options

46,279 results

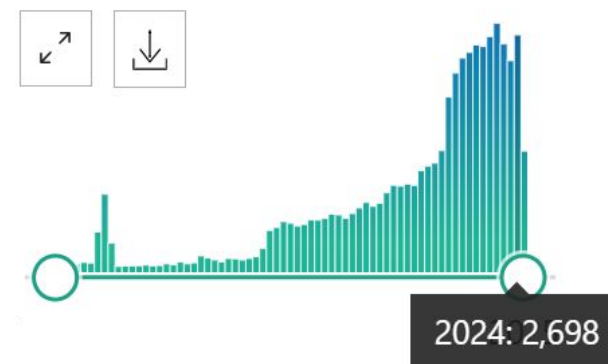
RESULTS BY YEAR



RESULTS BY YEAR



RESULTS BY YEAR



NanoSafety and Bio-Nano Interaction: our way to move

Reduction :

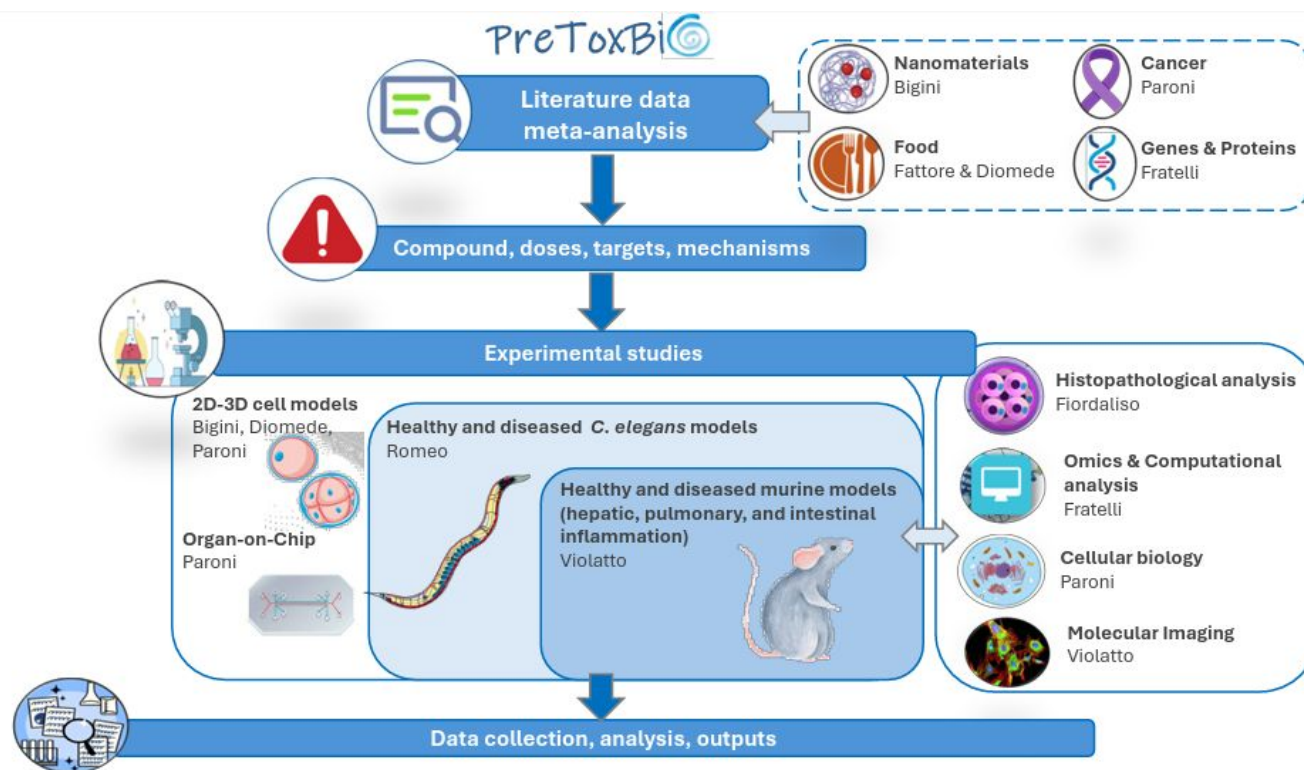
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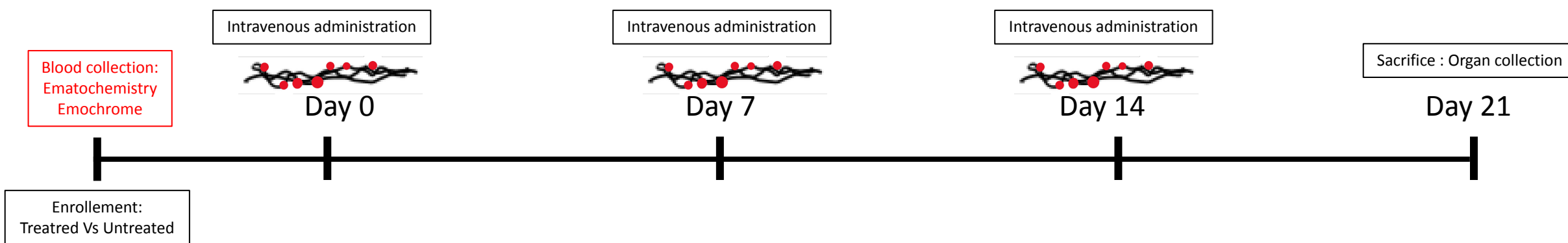


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NanoSafety and Bio-Nano Interaction: an example....

AIM: Determine the impact of circulating metal nanofibers in the liver health status



VETERINARY CHECK : Health status Discomfort

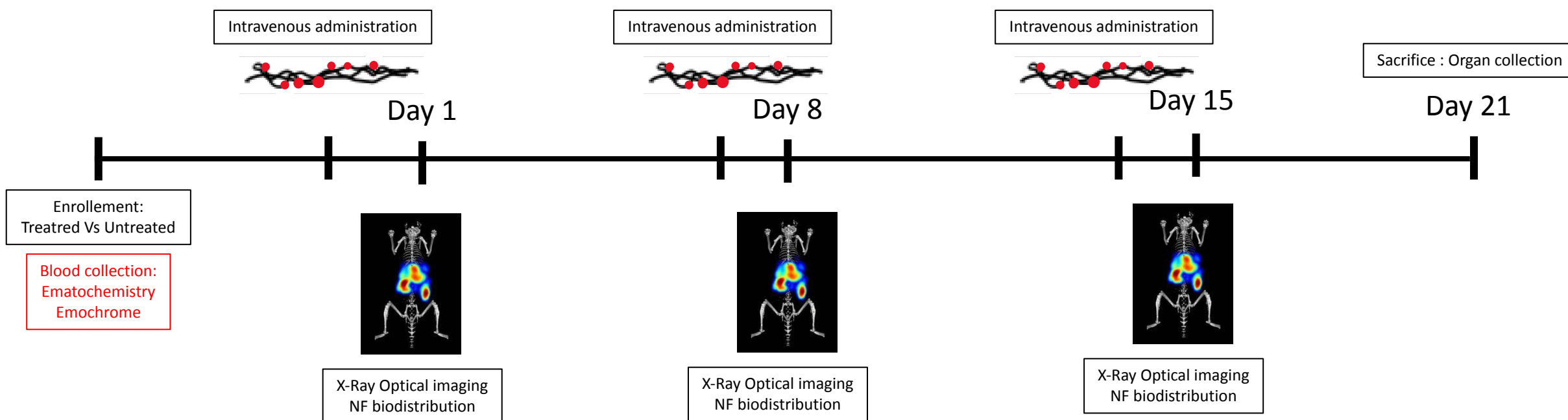
General clinical evaluation criteria	Partial score
Body weight	
No weight loss	0
Weight loss < 10%	1
Weight loss 10% < x < 20%	2
Weight loss x ≥ 20%	3
Weight loss ≥ 20% that lasts for 72 hours	HEP

Hydration	
Normal	0
Reduced	1
Appearance of the coat	
Normal	0
Mild piloerection	1
Marked piloerection	2
Nasal/eye secretions	
Present	1
Absent	0
Animal gait	
Normal	0
Reluctance to move	1
Apathy/immobility	2
Apathy/immobility for more than 12 hours	HEP

Social behavior	
Normal	0
Apathetic, aggressive	2
Respiratory function	
Normal	0
Tachypnea (rapid breathing)	1
Dyspnea (difficulty breathing) mild	2
Dyspnea severe	HEP

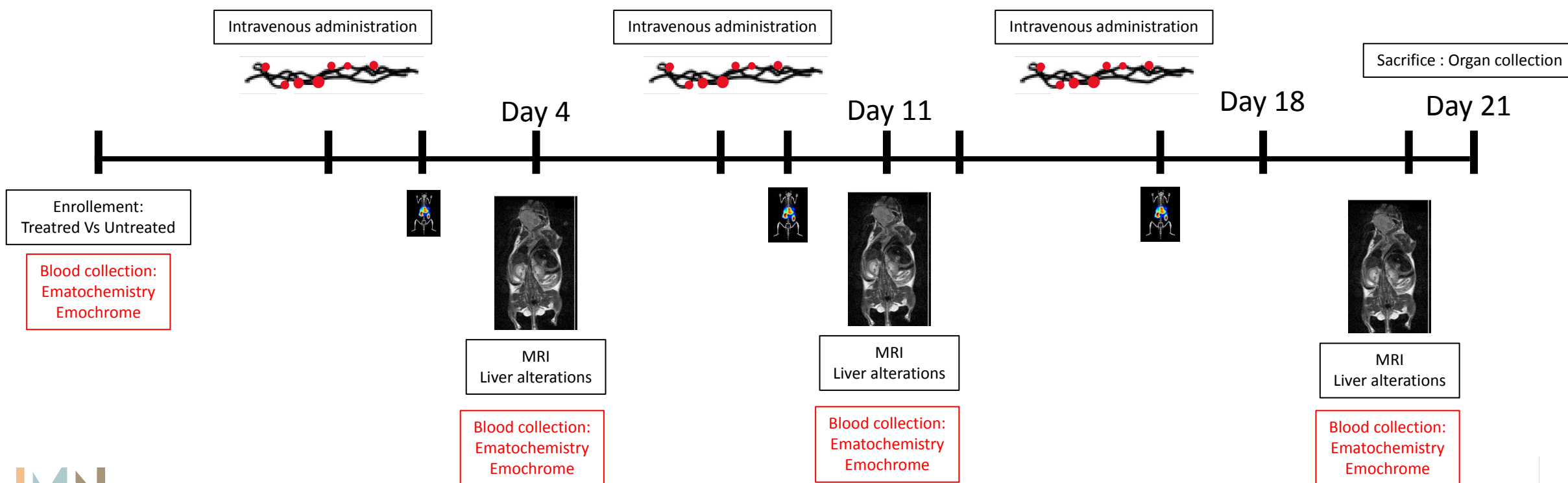
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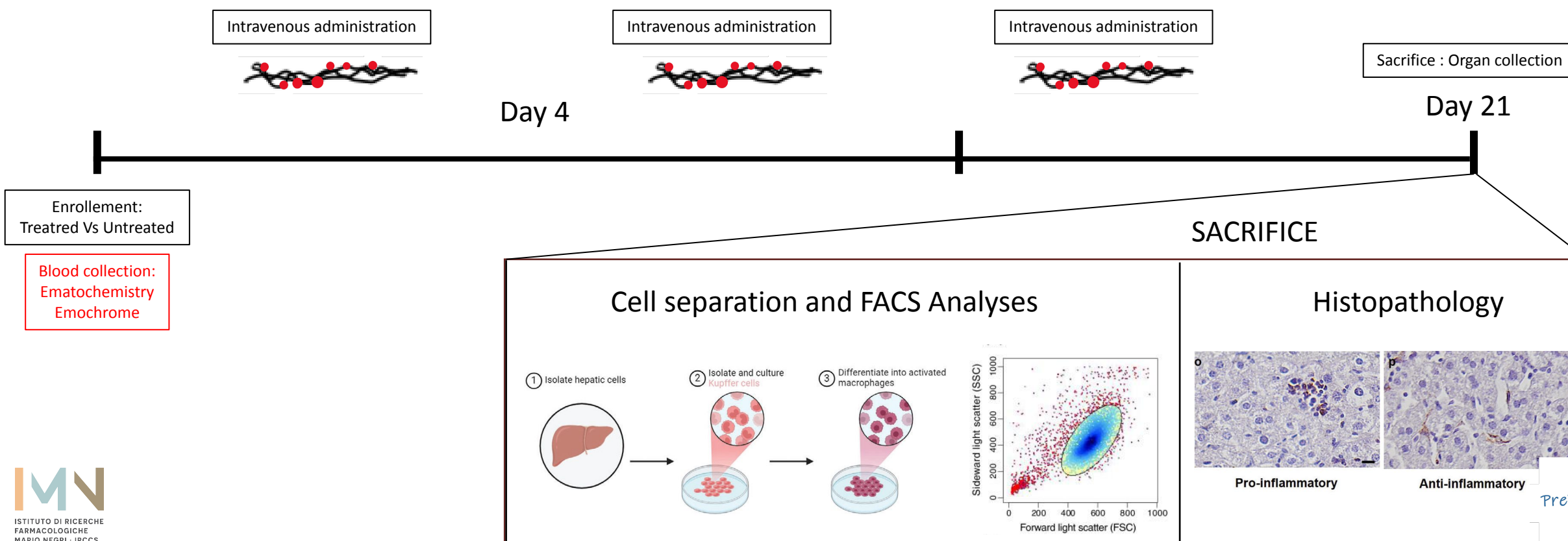
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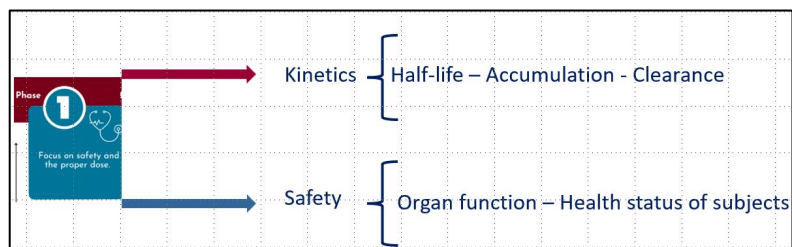
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NanoSafety and Bio-Nano Interaction: from pharma to tox

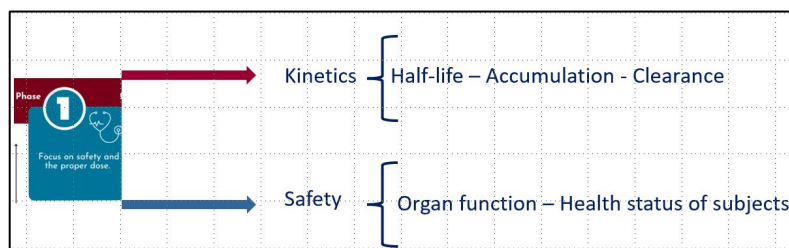
Clinical check:
No features of stress or health decay



NanoSafety and Bio-Nano Interaction: from pharma to tox

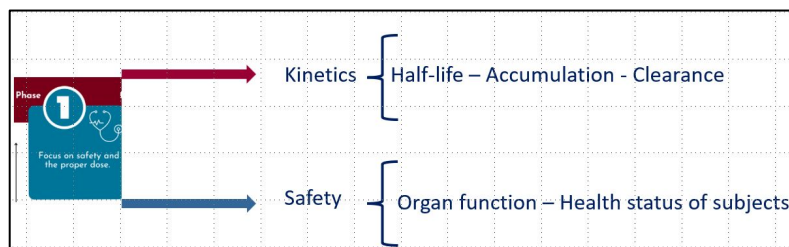
Clinical check: No features of stress and organic sufferance

In vivo Imaging (MRI-IVIS):
Progressive hepatic accumualtion mild clearance.
No morphometric alterations



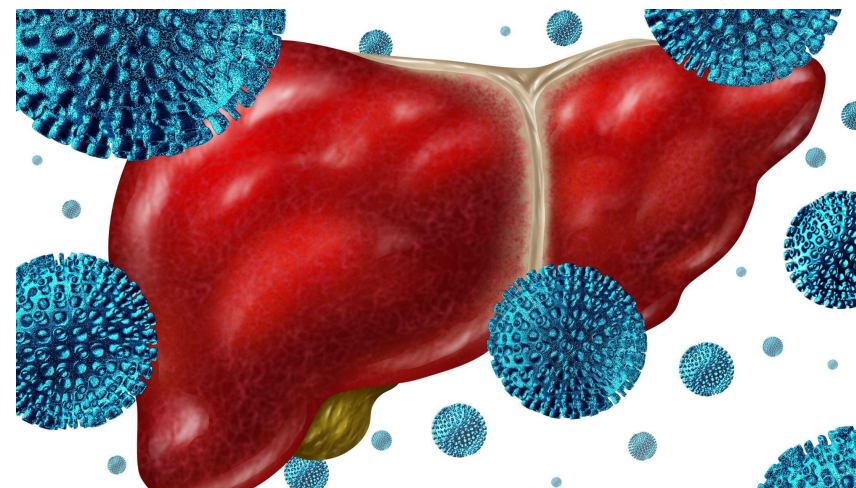
NanoSafety and Bio-Nano Interaction: from pharma to tox

Clinical check: No features of stress and organic sufferance



Ematochemistry Blood counting:
No changes in hepatic markers

Light and transient emochrome alteration



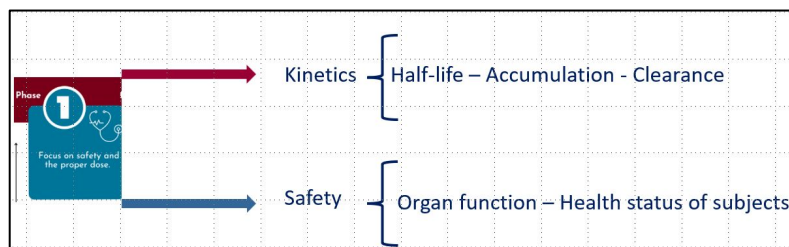
NanoSafety and Bio-Nano Interaction: from pharma to tox

Clinical check: No features of stress and organic sufferance

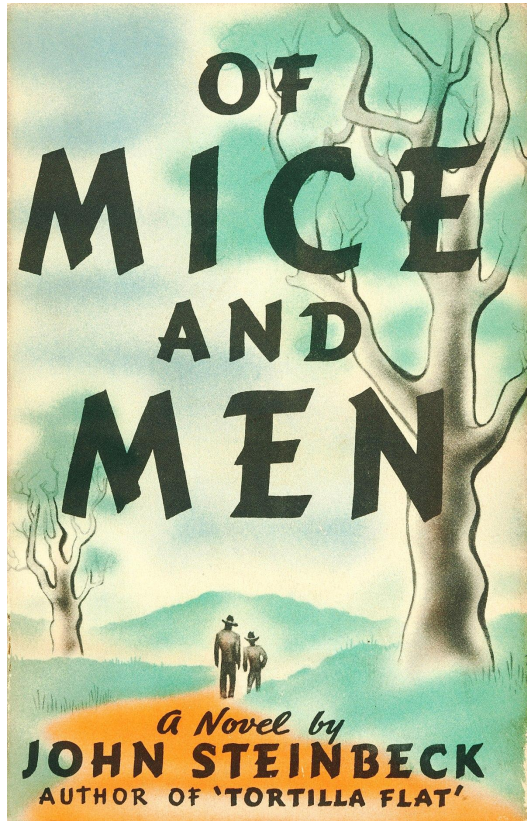
Ex vivo studies:

No necrosis or fibrosis activation

Increase of myeloid cells and immune infiltrates



NanoSafety and Bio-Nano Interaction: the third “R”



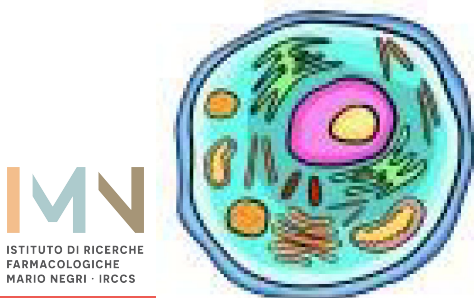
Replace :

use of alternative methods, compatible with the objective of the research project

C. elegans: a bridge between *in vitro* models and vertebrates

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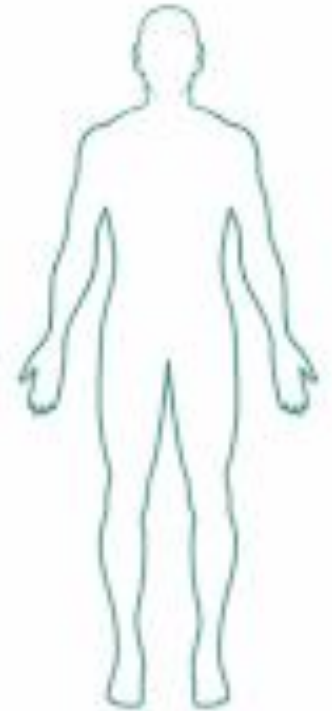
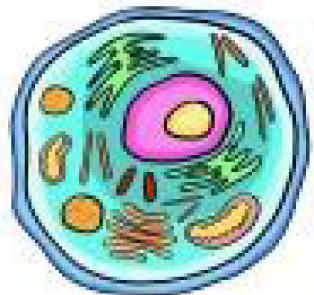
use of ~~alternative~~ methods, compatible with
the objective of the research project



C. elegans: a bridge between *in vitro* models and vertebrates

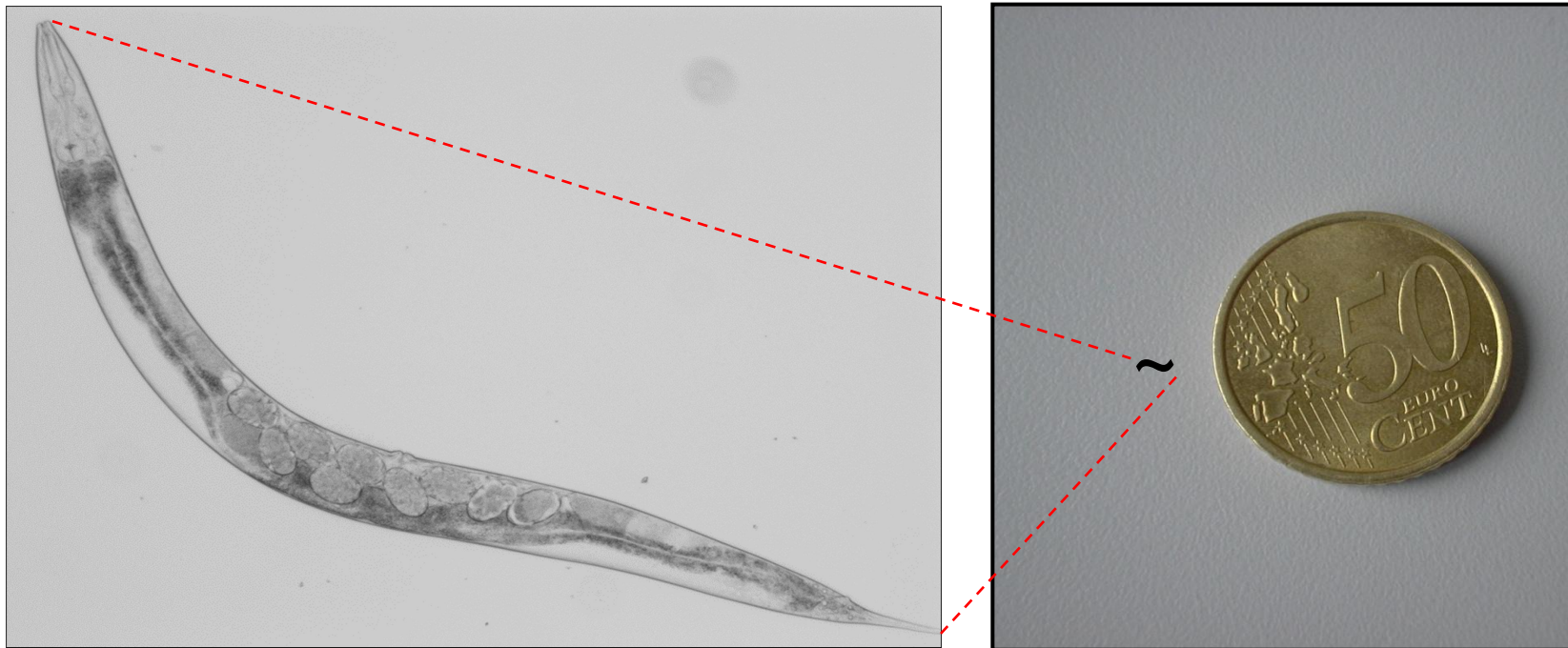
Replace :

use of **complementary** methods, compatible with the objective of the research project



The nematode *C. elegans*

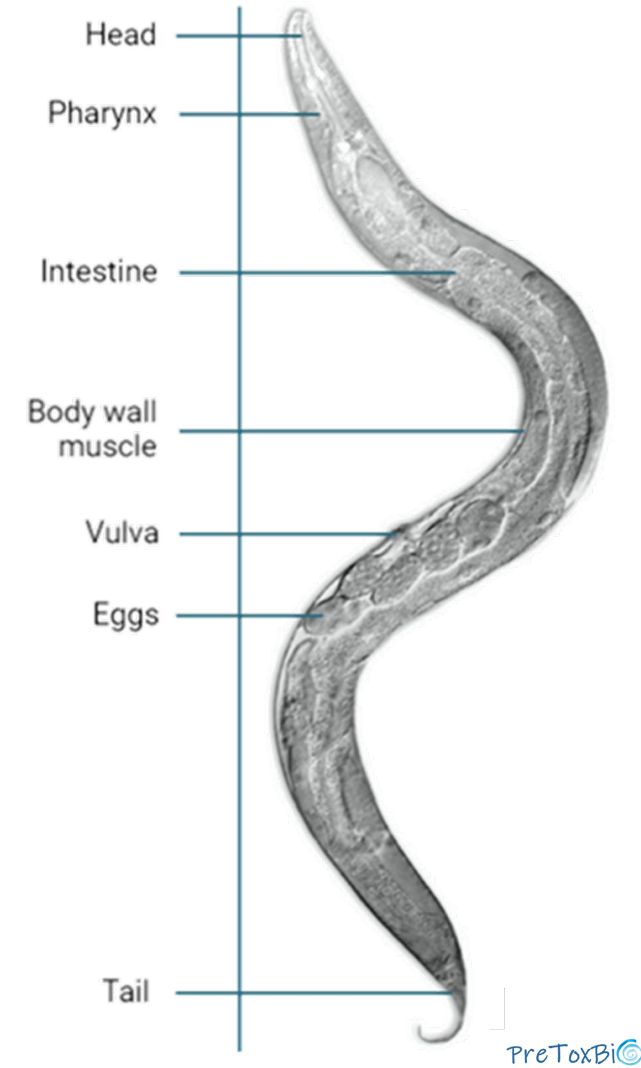
Caenorhabditis elegans, from the Greek *caeno*- (recent), *rhabditis* (rod-like), and Latin *elegans* (elegant), is a free-living, transparent, bacterivorous, nonpathogenic, and nonparasitic nematode.



It is about 1 mm in length and 80 microns thick.

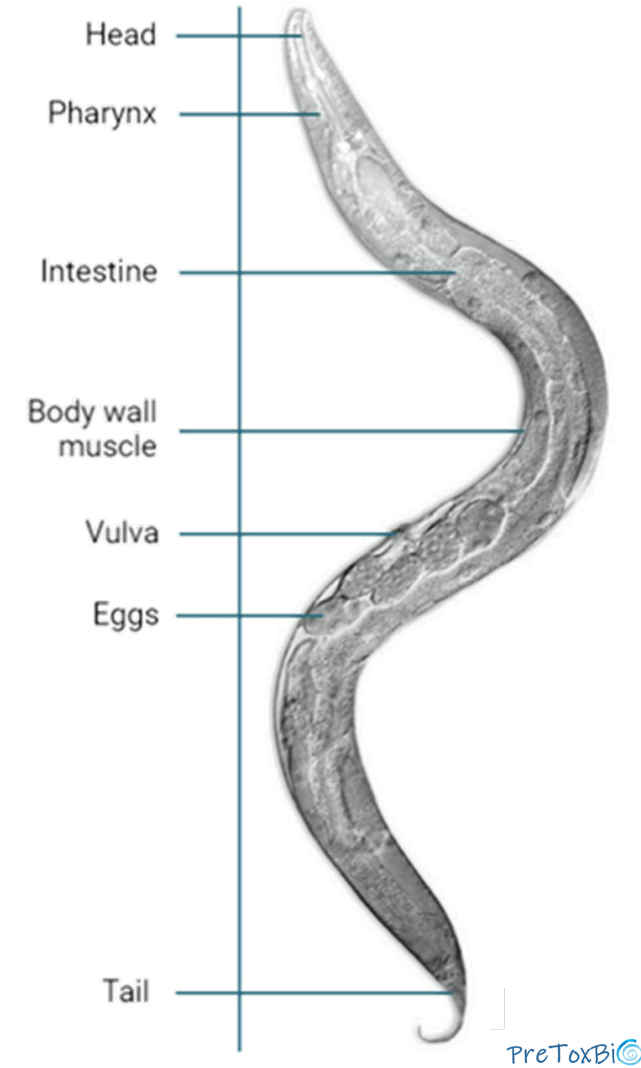
What can *C. elegans* offer?

- It is relatively simple from an anatomical point of view, but it involves specialized cells.
- ~60% of genes are homologous to humans, and about 40% of proteins are preserved in vertebrates.
- Genes responsible for disease in humans have counterparts in worms.
- Protein homeostasis involves complex biochemical and molecular pathways, most of which are highly conserved in mammals.
- Short lifespan: life expectancy can be easily measured and modified.
- It is inexpensive and devoid of legislative restrictions and ethical issues.
- A good system for high-throughput screening.



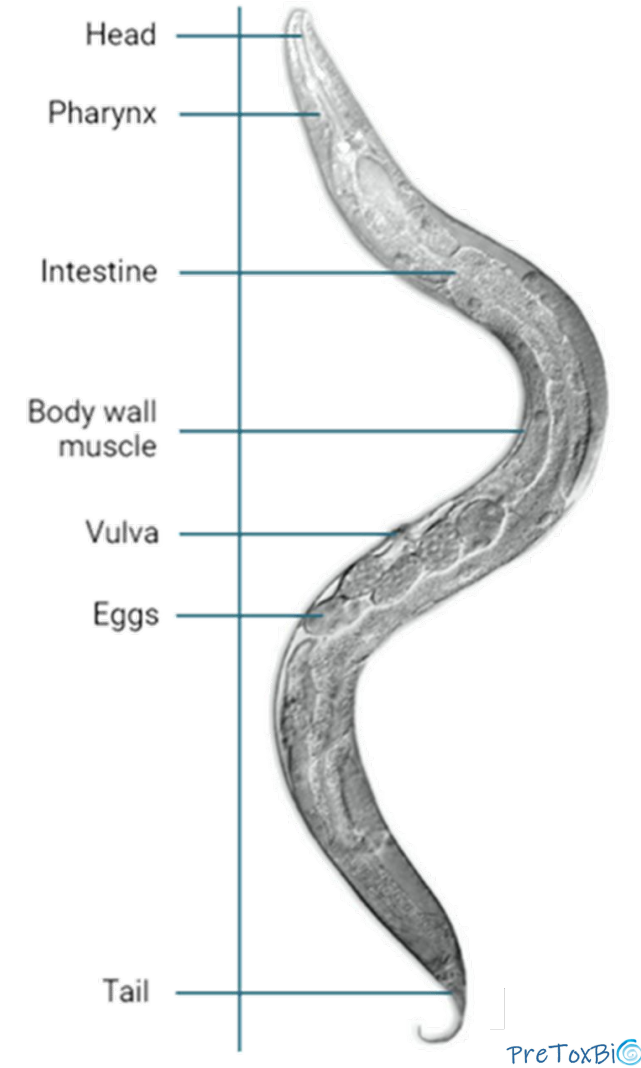
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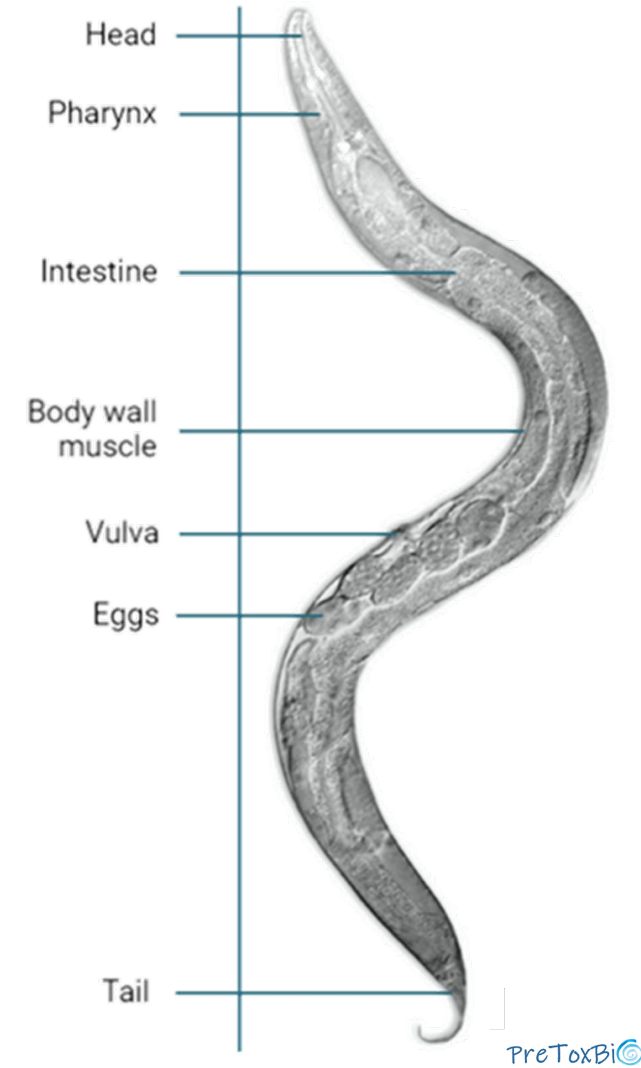
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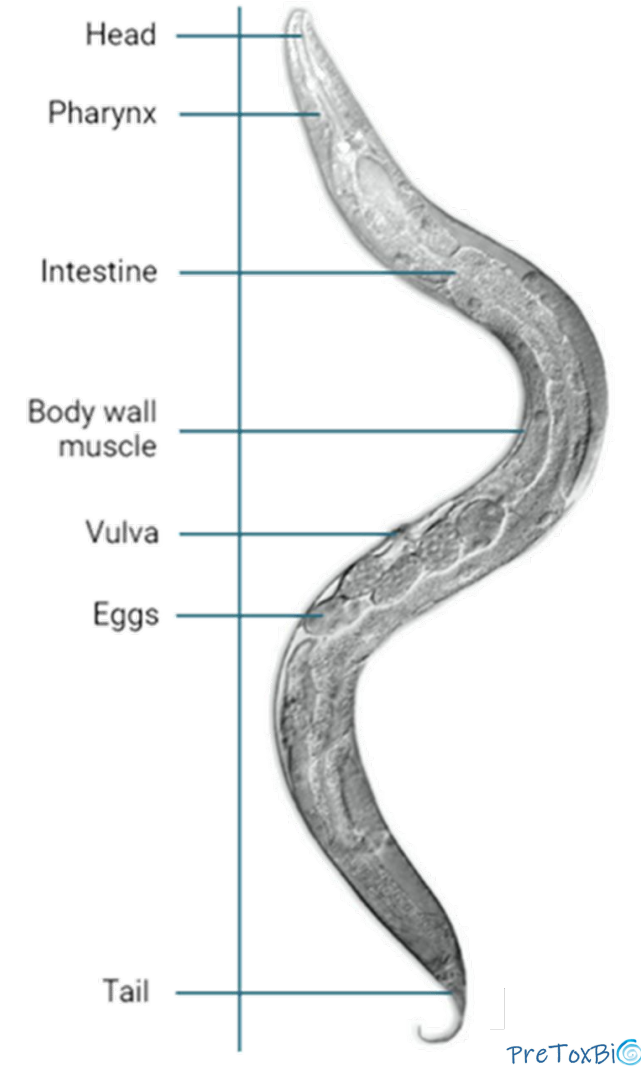
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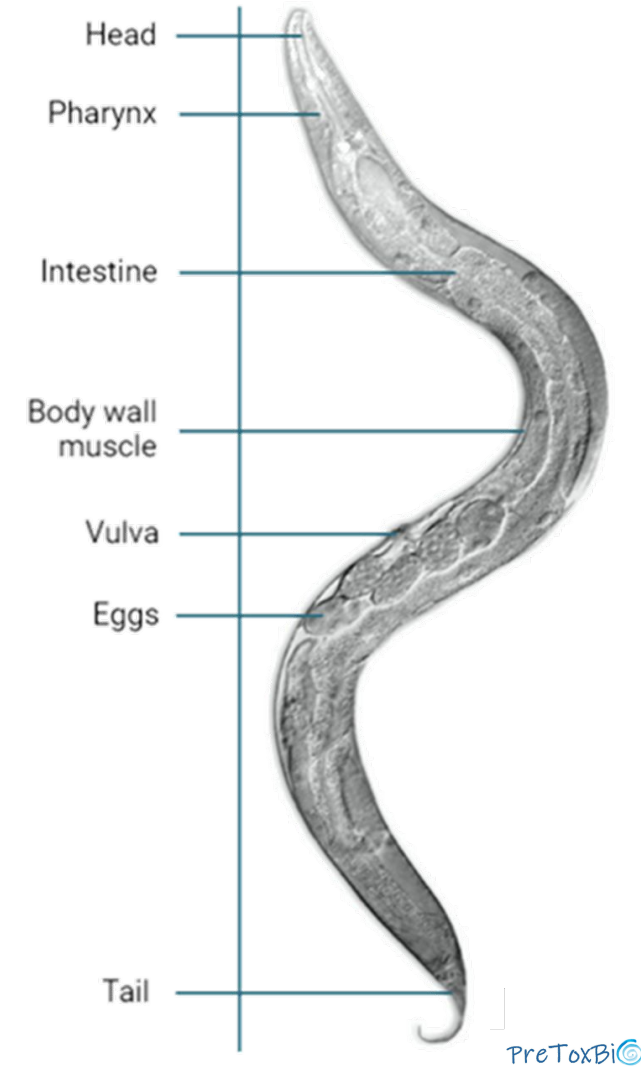
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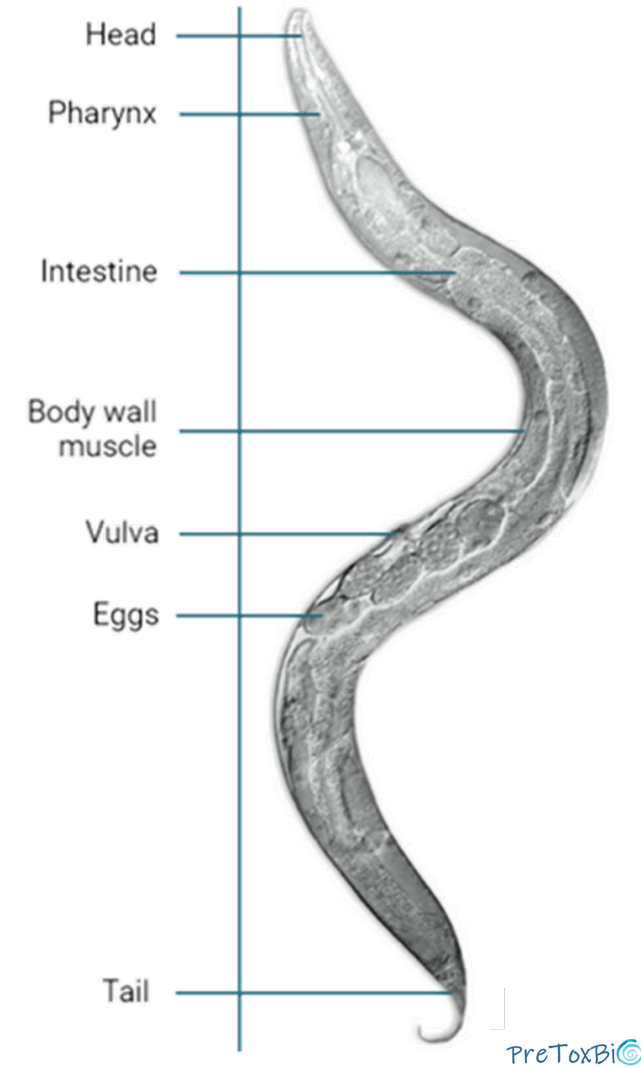
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C. elegans for vertebrate toxicity

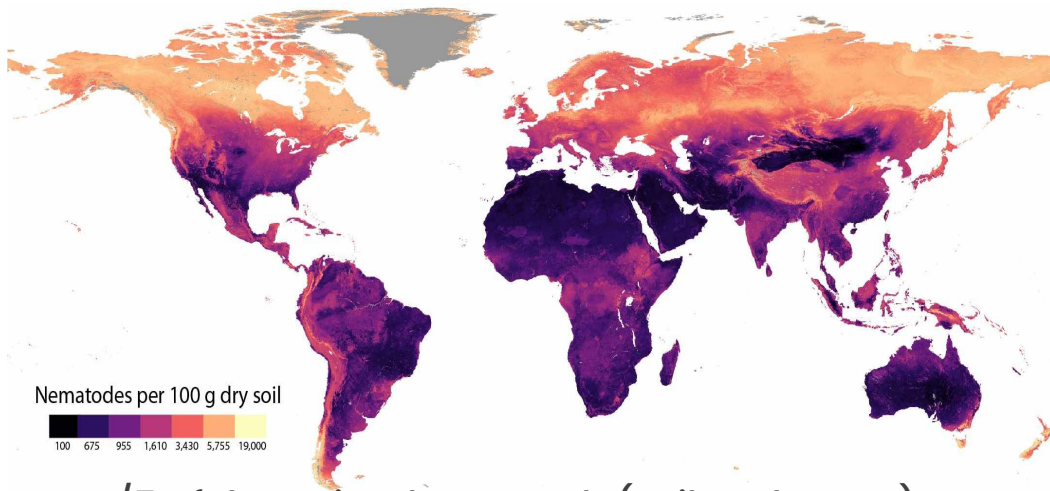
C. elegans toxicity assays provide data from a whole animal with intact and metabolically active digestive, reproductive, endocrine, sensory and neuromuscular systems.

There are many instances of conservation of the mode of toxic action between *C. elegans* and mammals.

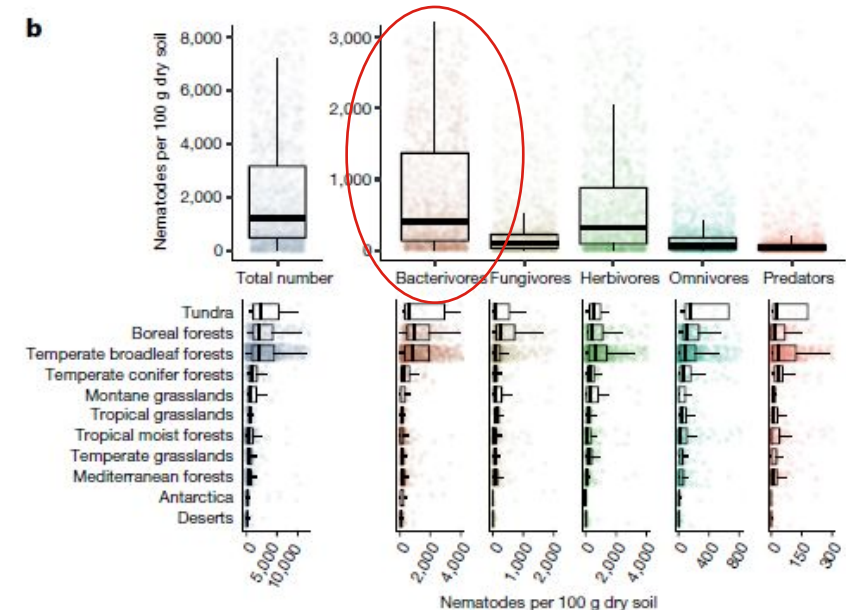
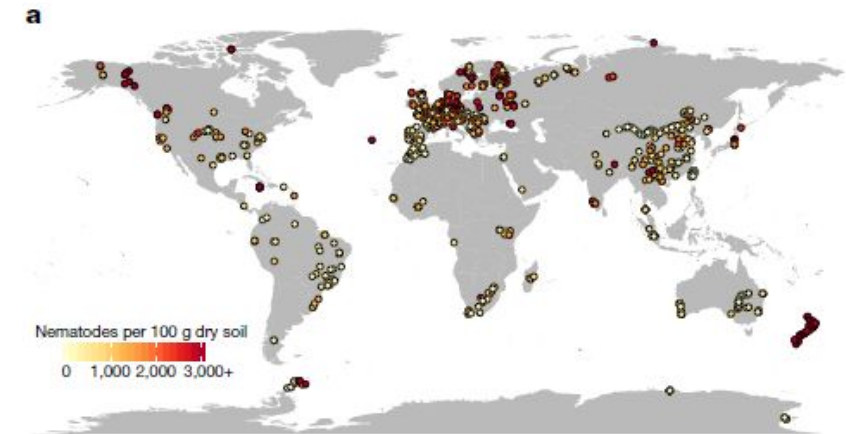
Toxicity ranking screens in *C. elegans* have repeatedly been shown to be as predictive of rodents LD₅₀ ranking.

C. elegans is included in early safety testing and as one component in integrated toxicity testing strategies.

Nematodes are the most abundant animals on Earth



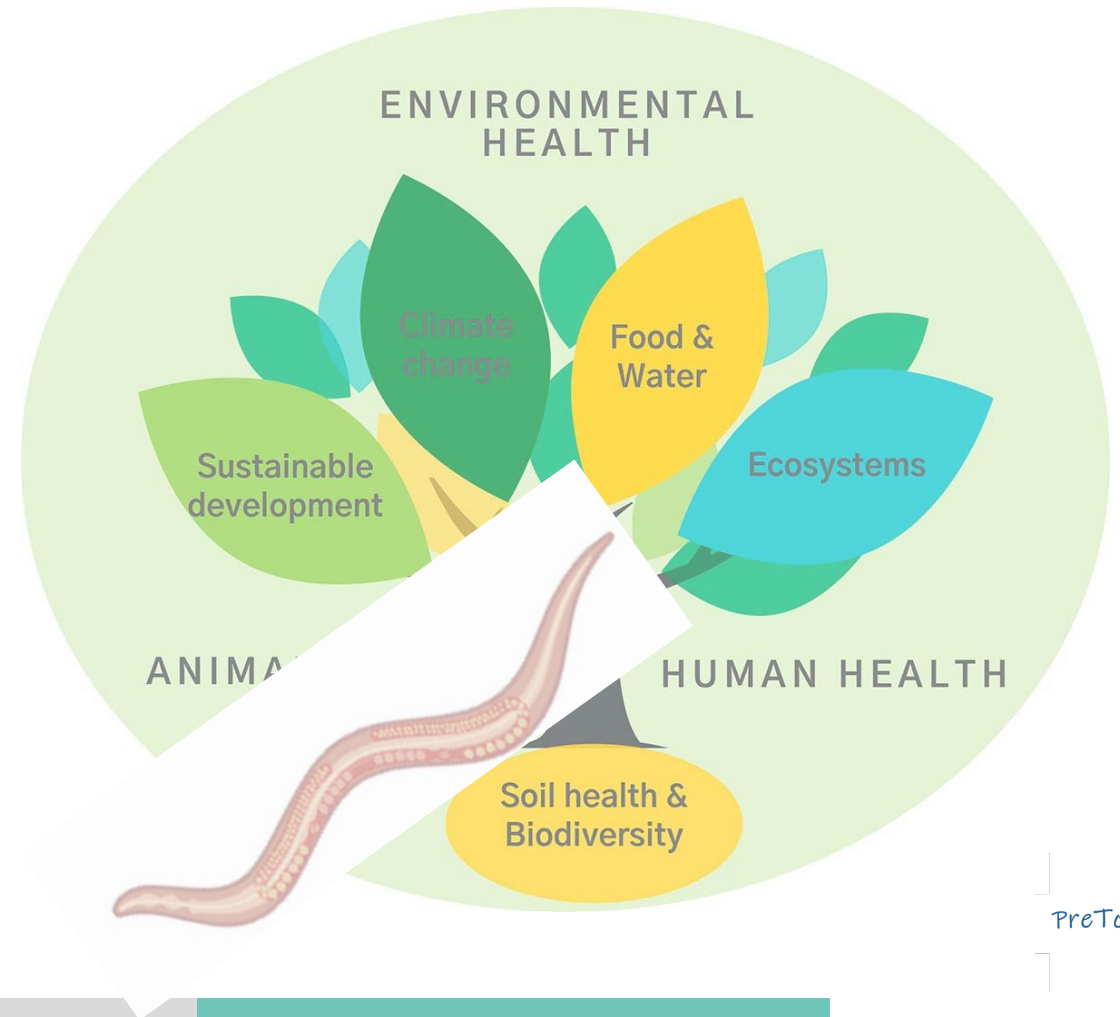
- 4/5 of the animals on earth (soil and water) are nematodes.
- 38% are concentrated in the sub-Arctic regions, 24% in temperate zones, and 21% in tropical zones.
- They play a fundamental role in the process of transformation of organic micronutrients and in the control of the population of soil and aquatic microorganisms.
- **They are good indicators of biological activity in the soil and water.**



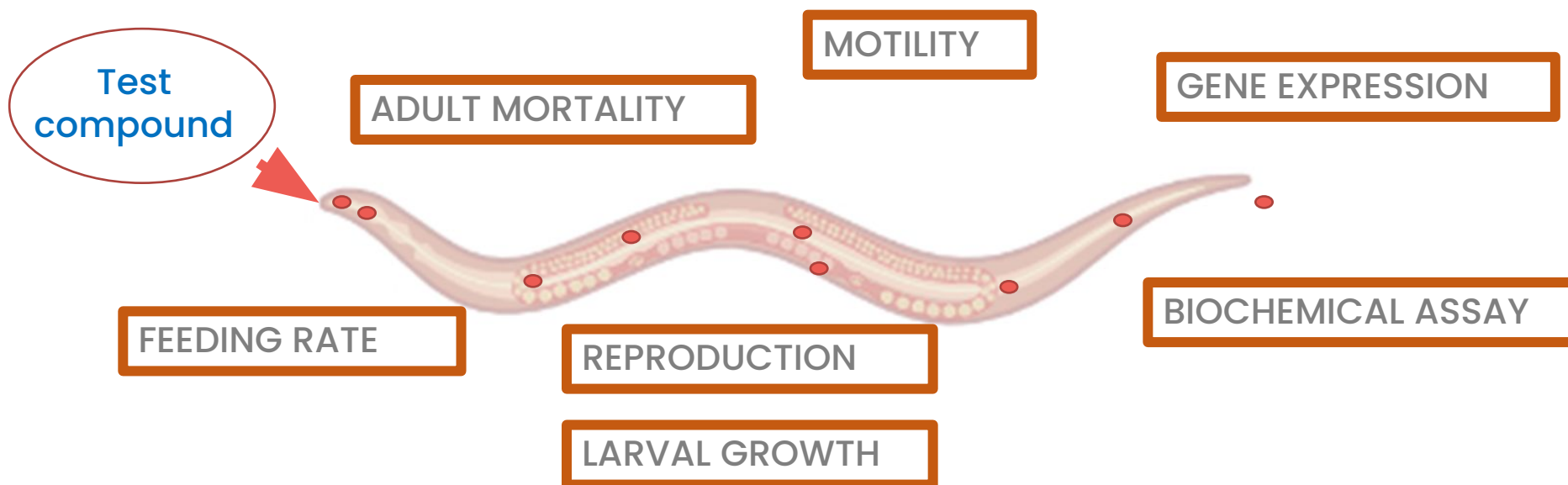
C. elegans for enviromental toxicity

With respect to its abundances, its role as an intermediary between microorganisms and higher trophic levels, and its ubiquitous occurrence in all habitats, *C. elegans* is of strong potential interest as an environmental indicator and for ecotoxicological studies.

Standardized test systems developed for evaluating water and soil quality using *C. elegans* include: ASTM E2172-01: ASTM, 2001; ISO 10872: ISO, 2010.



Toxicity tests



Brief overview on the topic.....

.....available for details until Thursday, 12.00

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luisa.diomedede@marionegri.it

Acknowledgements



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FARMACOLOGICHE
MARIO NEGRI · IRCCS



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