



UNIMORE

UNIVERSITÀ DEGLI STUDI DI
MODENA E REGGIO EMILIA

Dipartimento di Scienze della Vita

***The apple snail *Pomacea canaliculata*: a new and
alternative animal model for testing innovative
nanomedicines***

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 **DIRECTIVE 2010/63/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL**
of 22 September 2010

on the protection of animals used for scientific purposes

(Text with EEA relevance)

THE EUROPEAN PARLIAMENT AND THE COUNCIL OF THE EUROPEAN UNION,

Having regard to the Treaty on the Functioning of the European Union, and in particular Article 114 thereof,

Having regard to the proposal from the European Commission,

Having regard to the opinion of the European Economic and Social Committee ⁽¹⁾,

After consulting the Committee of the Regions,

Acting in accordance with the ordinary legislative procedure ⁽²⁾,

There is a strong trend to apply the “3 Rs” rule in experiments that involve animal use, which are:

- reduce the number of animals used in the laboratory;*
- refine the protocols to increase animal comfort and reduce pain;*
- replace animals for other models that do not have bioethical problems associated*

Trevijano-Contador and Zaragoza, Virulence 5:4, 454–456; May 15, 2014

Subject matter and scope

1. This Directive establishes measures for the protection of animals used for scientific or educational purposes.

To that end, it lays down rules on the following:

- (a) the replacement and reduction of the use of animals in procedures and the refinement of the breeding, accommodation, care and use of animals in procedures;
- (b) the origin, breeding, marking, care and accommodation and killing of animals;
- (c) the operations of breeders, suppliers and users;
- (d) the evaluation and authorisation of projects involving the use of animals in procedures.

2. This Directive shall apply where animals are used or intended to be used in procedures, or bred specifically so that their organs or tissues may be used for scientific purposes.

This Directive shall apply until the animals referred to in the first subparagraph have been killed, rehomed or returned to a suitable habitat or husbandry system.

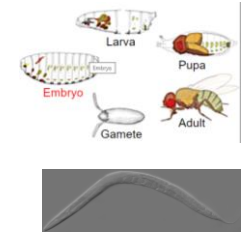
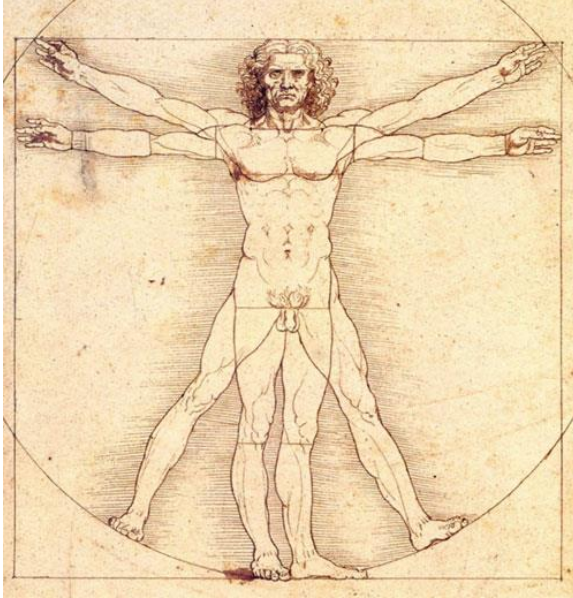
The elimination of pain, suffering, distress or lasting harm by the successful use of anaesthesia, analgesia or other methods shall not exclude the use of an animal in procedures from the scope of this Directive.

3. This Directive shall apply to the following animals:

- (a) live non-human vertebrate animals, including:
 - (i) independently feeding larval forms; and
 - (ii) foetal forms of mammals as from the last third of their normal development;
- (b) live cephalopods.

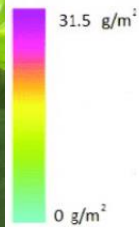
4. This Directive shall apply to animals used in procedures, which are at an earlier stage of development than that referred to in point (a) of paragraph 3, if the animal is to be allowed to live beyond that stage of development and, as a result of the procedures performed, is likely to experience pain, suffering, distress or lasting harm after it has reached that stage of development.

How to reliably reproduce the complexity of human beings, and assess the efficacy and safety of new nanoparticle-based drugs and nano-technological products?

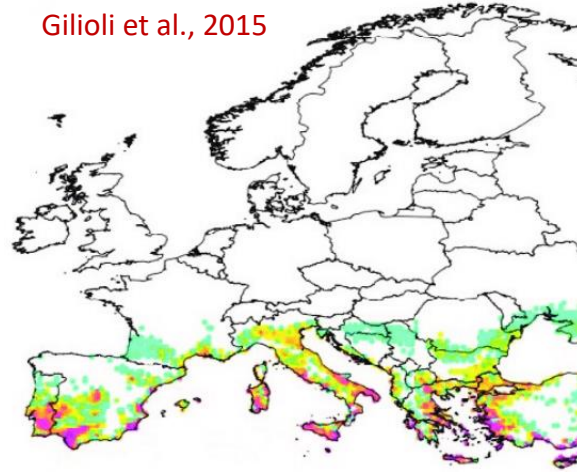


The invertebrate models most frequently adopted in nanomedicine-based experimental protocols are the roundworm *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster*, especially because of the numerous forward and reverse genetic approaches that can be employed in these animals

Pomacea canaliculata (Gastropoda, Caenogastropoda)



Gilioli et al., 2015



- *P. canaliculata* is original from South America and spread in South East Asia, North America and has been reported in Spain (EU)
- *P. canaliculata* is indexed among the 100 world's worst invasive alien species
- *P. canaliculata* has been marked as pest and invading species by the EU Parliament

An invasive pest but also a potential new model



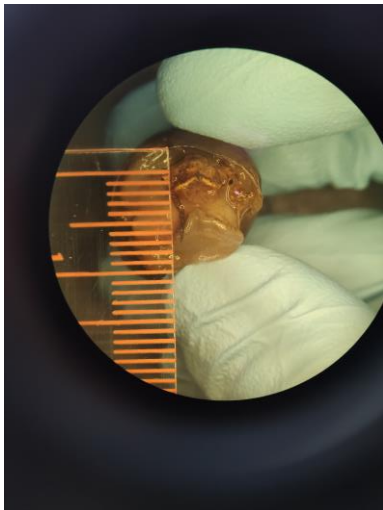
Separated sex (gonochoric)



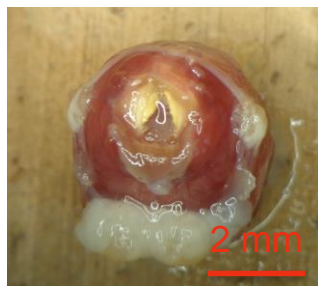
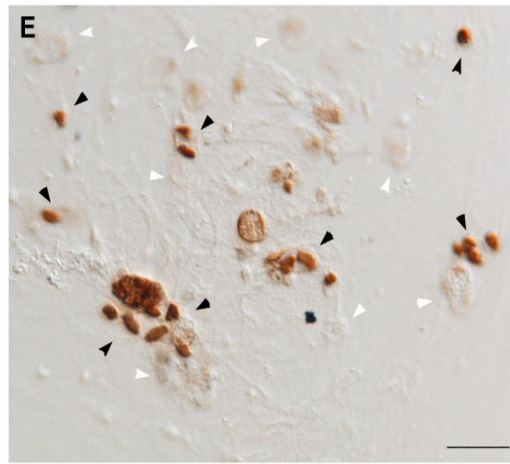
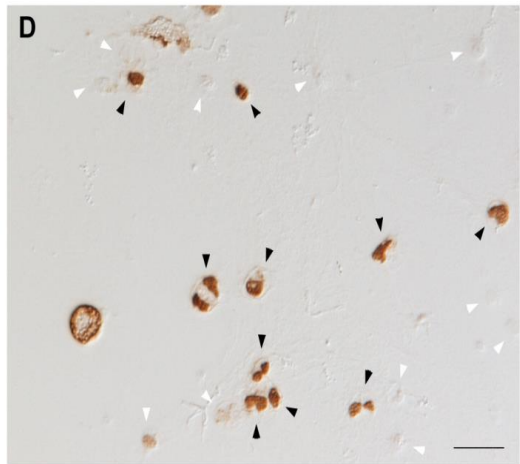
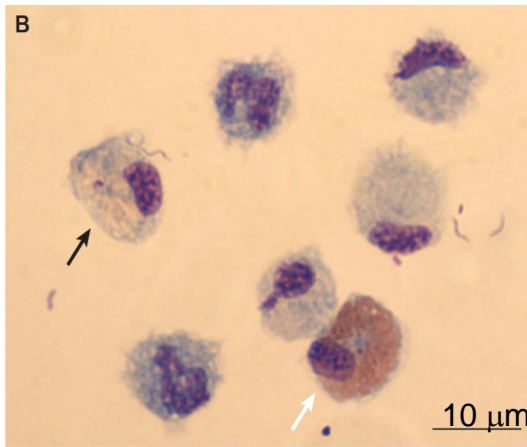
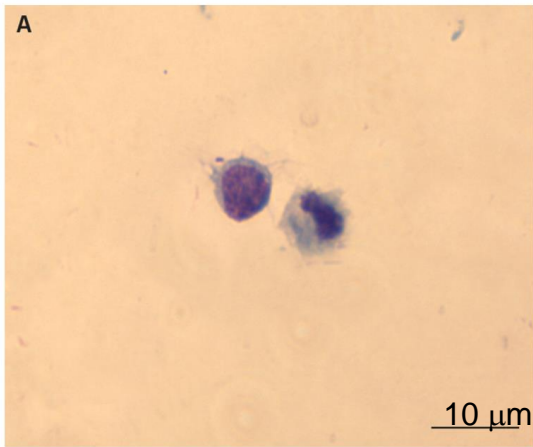
Neurotoxic eggs are laid outside of the water



P. canaliculata is highly prolific. Eggs directly develop into small snails within 16-18 days



In 4-6 months the cycle is closed

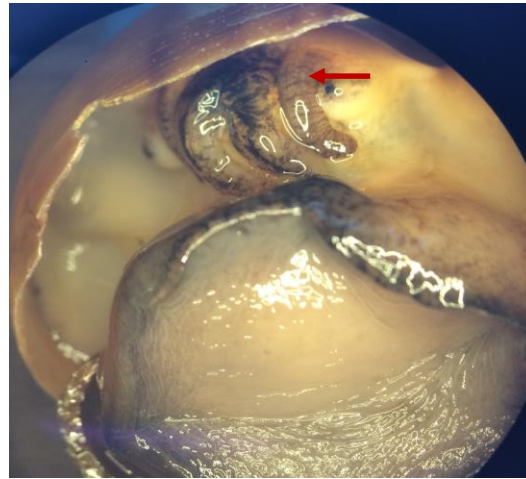
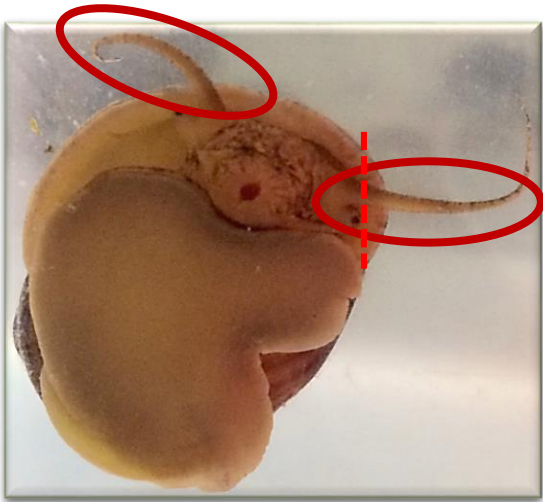


Gangliar ring
around buccal
apparatus

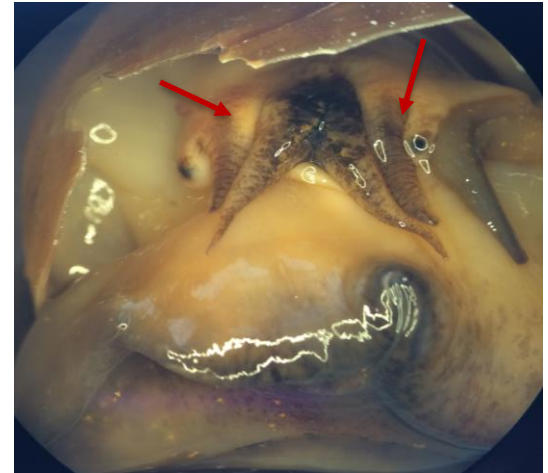
Adult *P. canaliculata* possesses a complex immune system and identifiable central nervous components



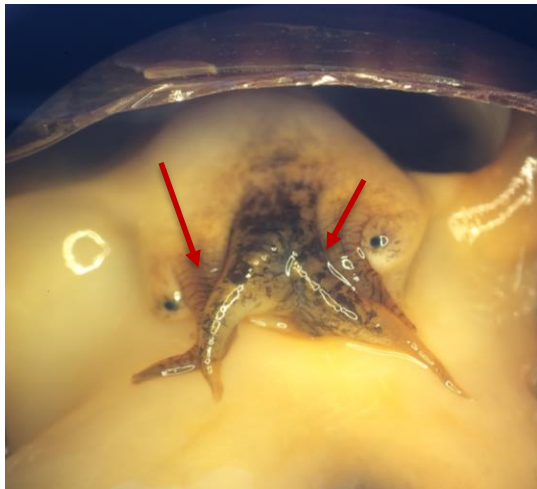
Adult *P. canaliculata* can regenerate sensory organs



24 hpa



48 hpa



72 hpa



3 mpa

hpa = hours post-amputation
mpa = months post-amputation

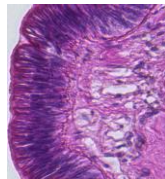
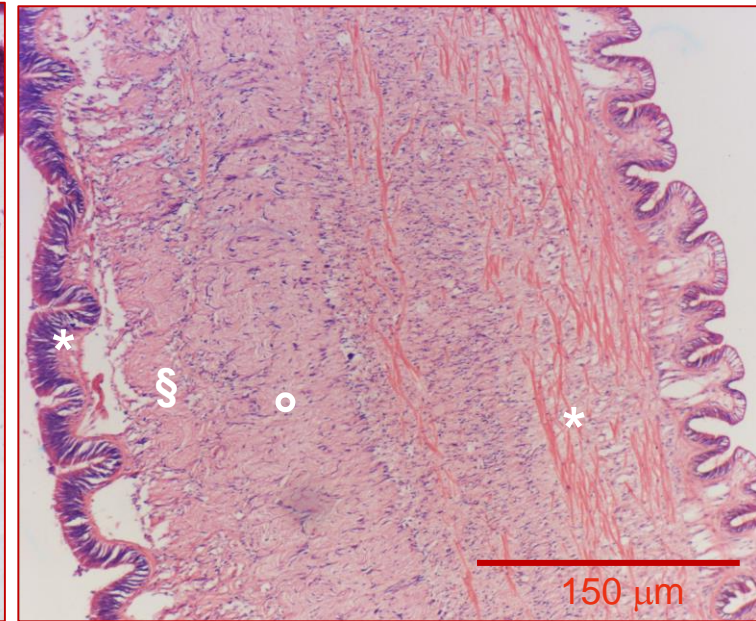
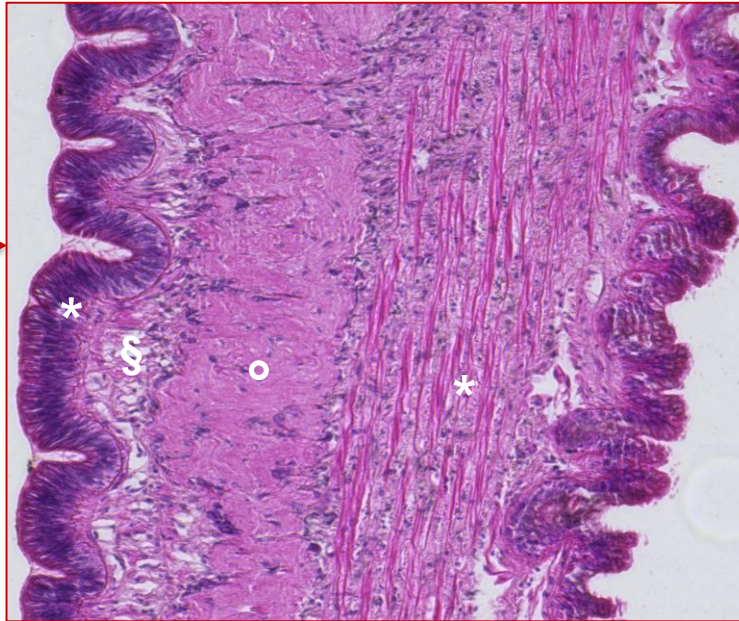
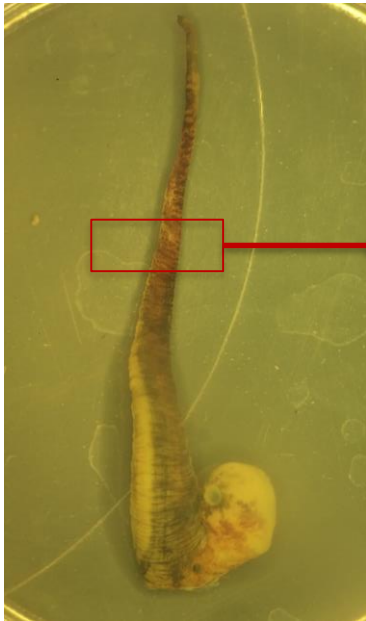
***Adult *P. canaliculata*
regenerates cephalic
tentacles within 3
months***

Adult *P. canaliculata* can regenerate sensory organs

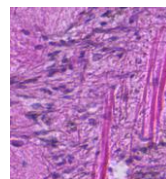


Control

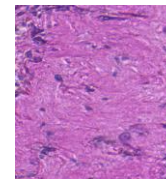
3 mpa



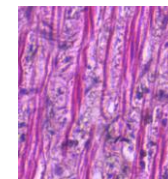
* Epithelium



§ Connective tissue



° Nerve

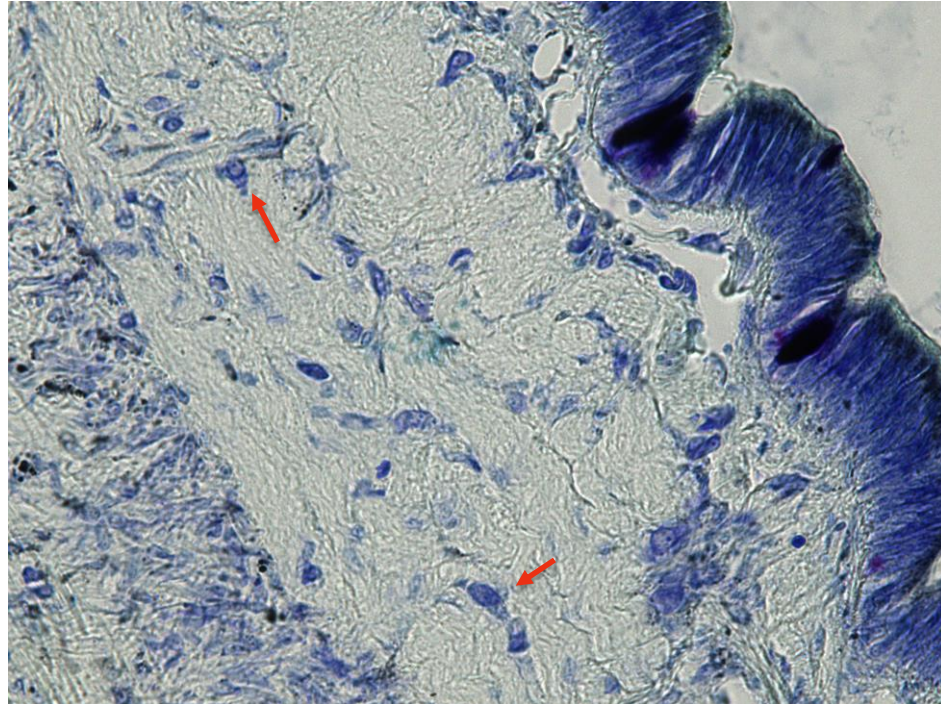


^ Muscles

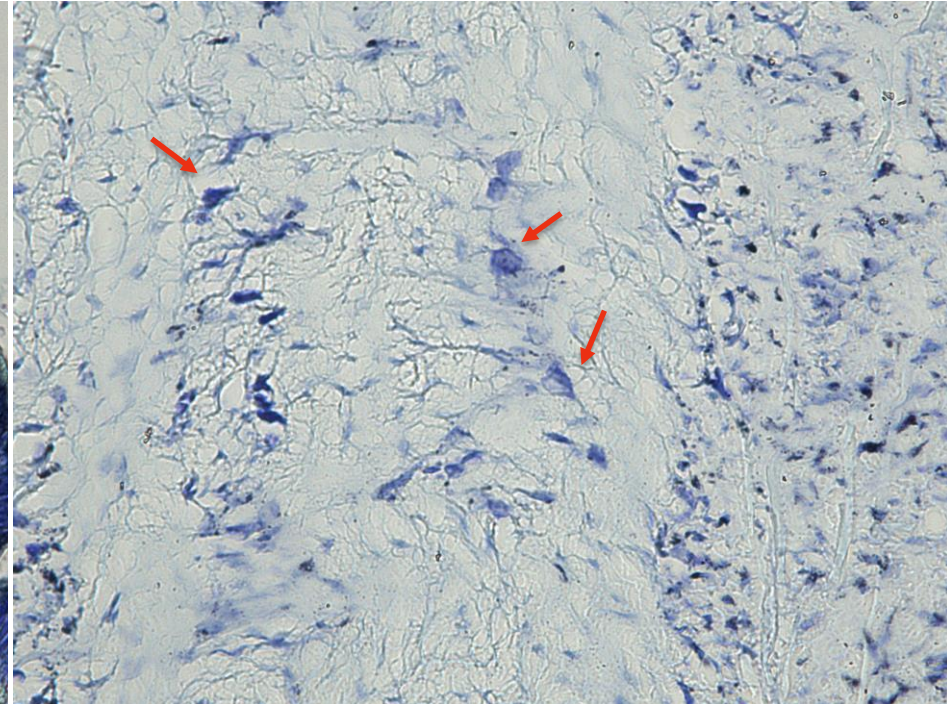
Adult *P. canaliculata* can regenerate sensory organs



Control



3 mpa



Can *P. canaliculata* replace rodents in some experimental protocols?

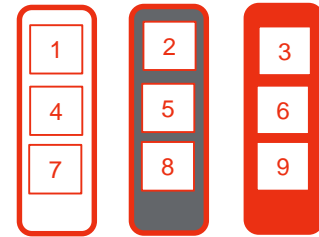
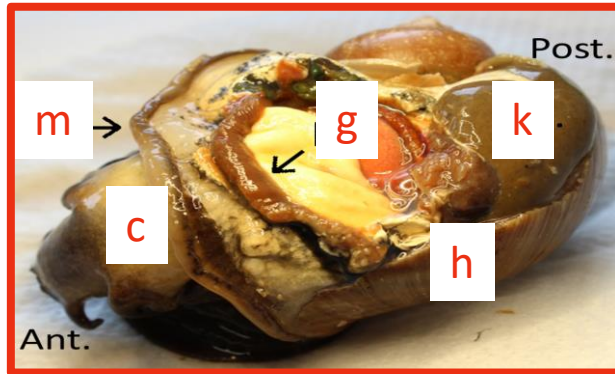


- Size
- Weight
- Separated sex
 - Longevity
- Recognizable organs
- Direct development
 - Genomes, transcriptomes and tissue-specific proteomes available



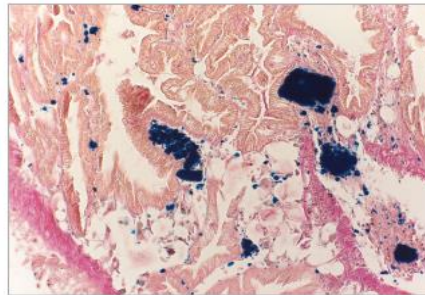


Injection with superparamagnetic iron oxide nanoparticles (SPIONs)

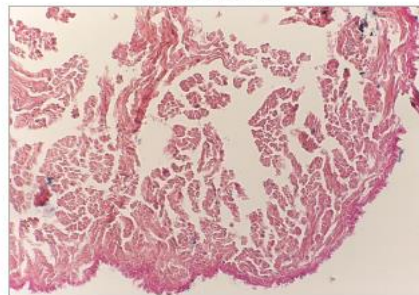


Serial slices for different staining

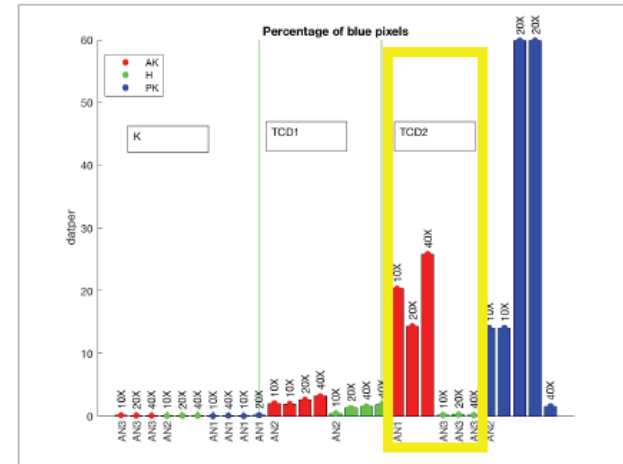
Kidney



Heart



Quantitative automatic evaluation of staining, through MATLAB® script



More in Poster PS 41-
Fiorino *et al.*
Ref. D. Malagoli

Can *P. canaliculata* replace rodents in some experimental protocols?



- Direct development
 - Size
 - Weight
- Separated sex
 - Longevity
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Our preliminary observations suggest that *Pc* is a possible substitute of small rodents, in preliminary studies for testing bioaccumulation and bio-safety of nanoparticle-based drugs and nano-technological products



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Thank you for your attention

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