(Eco)toxicologia e análise de risco – modelos biológicos e ferramentas

- Teresa Dias –
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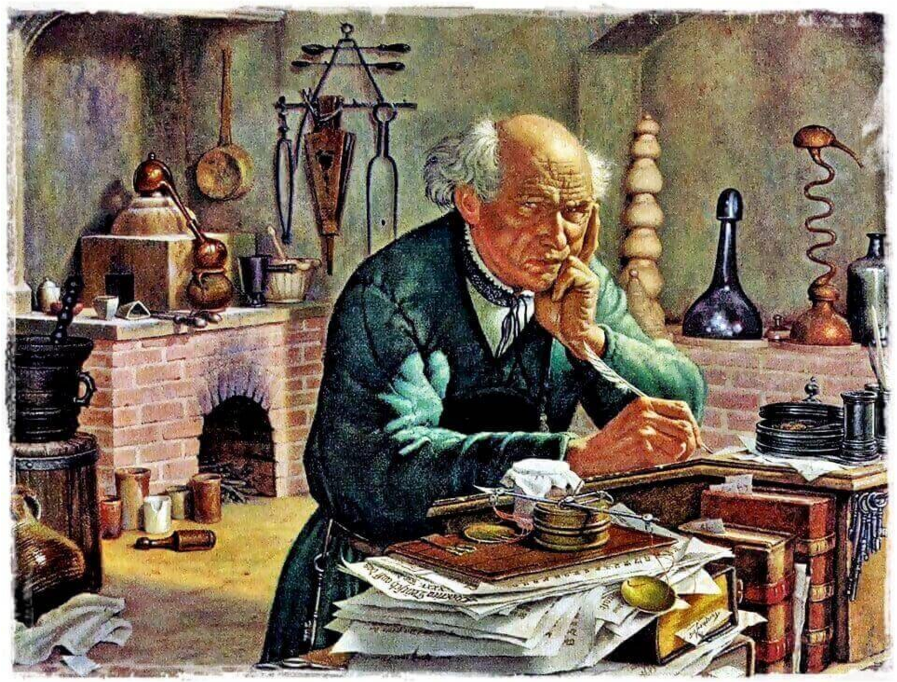
O que tem em comum estas imagens?



“All substances are poisons. There is none which is not a poison. The right dose differentiates the poison from a remedy”

Philippus Aureolus Theophrastus Bombastus von Hohenheim, mais conhecido por Paracelcius (1493-1541) foi um médico, alquimista, físico, astrólogo e ocultista Suiço-alemão.
Seu pseudónimo significa "superior a Celso (médico romano)".
Também é aclamado pelo seu trabalho na Química e como fundador da Bioquímica e da Toxicologia.

E assim nasce a… toxicologia

Toxicologia

A **toxicologia** é uma ciência multidisciplinar que tem como objeto de estudo os efeitos adversos das substâncias químicas sobre os organismos.

Ramos da toxicologia:

- Clínica: trata pacientes intoxicados, fazendo diagnóstico e definindo tratamento
- Experimental: utiliza animais para elucidar o mecanismo de ação, espectro de efeitos tóxicos e órgão alvos para cada agente tóxico
- Analítica: identifica/quantifica tóxicos em diversas matrizes (biológicas ou não)
- Ambiental
- Forense
- Ecotoxicologia: descreve os efeitos tóxicos em organismos vivos, especialmente em populações e em comunidades (Thuhaut, 1969)

PERIGO *versus* RISCO

Risco = perigo x exposição

- **Análise de risco prospetiva** (no futuro) – identificar se uma determinada substância vai ocorrer no ambiente, onde e quais os organismos que irão ser afetados.
- **Análise de risco retrospetiva** (passado ou em curso) – avaliar o risco de locais contaminados, análise toxicológica direta, etc.

Caracterização do risco

Exposição

PEC (Predicted Environmental Concentration)

ERC (Ecotoxicologically relevant concentration)

+

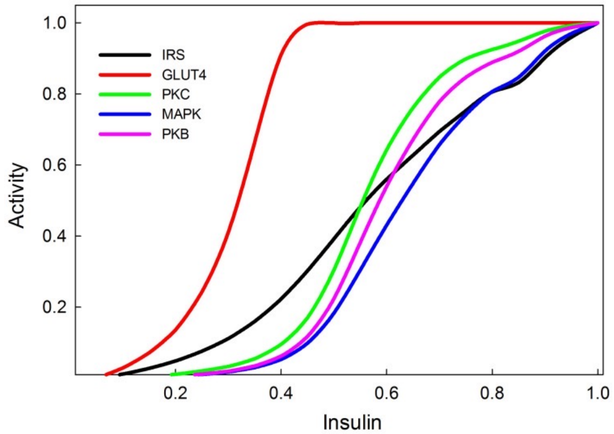
Efeitos

Testes ecotoxicológicos em diferentes “tiers” (~níveis)

Determinação da TC (toxic concentration) ou RAC (Regulatory accepted concentration)

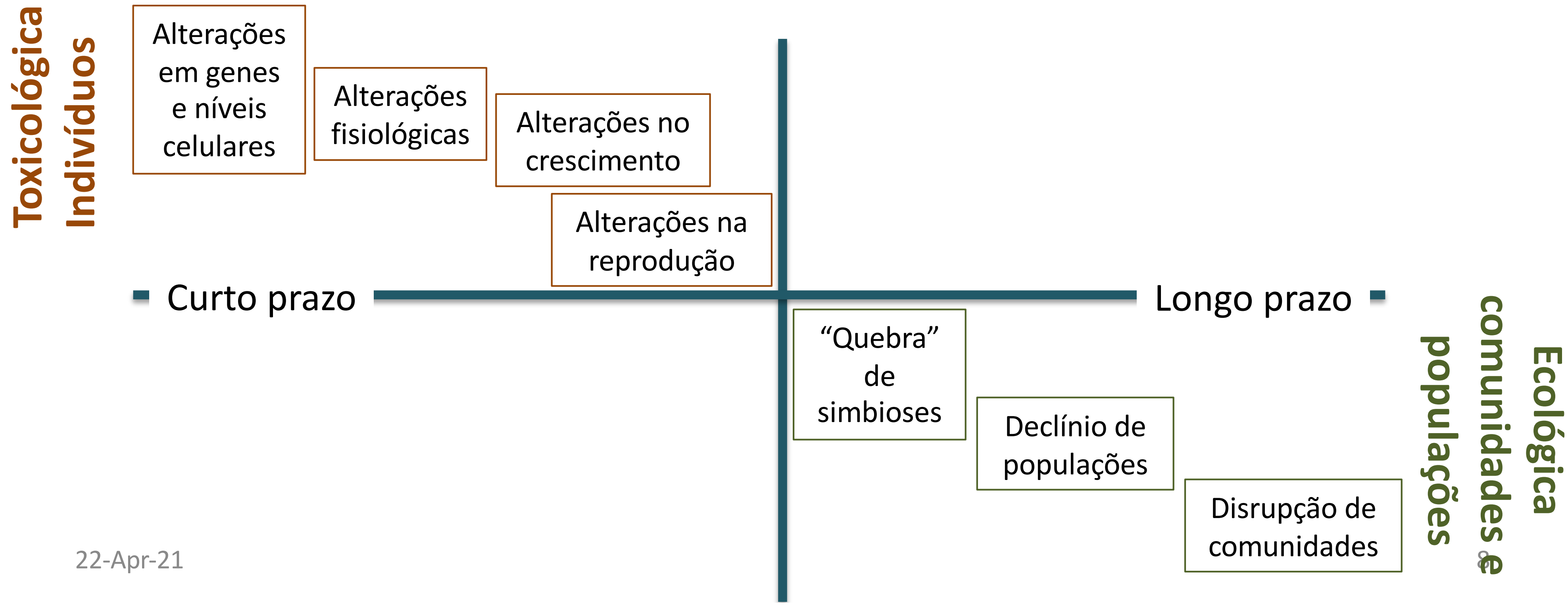
Questões fundamentais

1. Doses da(s) substância(s) a ser testada



Questões fundamentais

2. O “QUE” testar e em “QUEM” (cadeia de evidência ecotoxicológica)



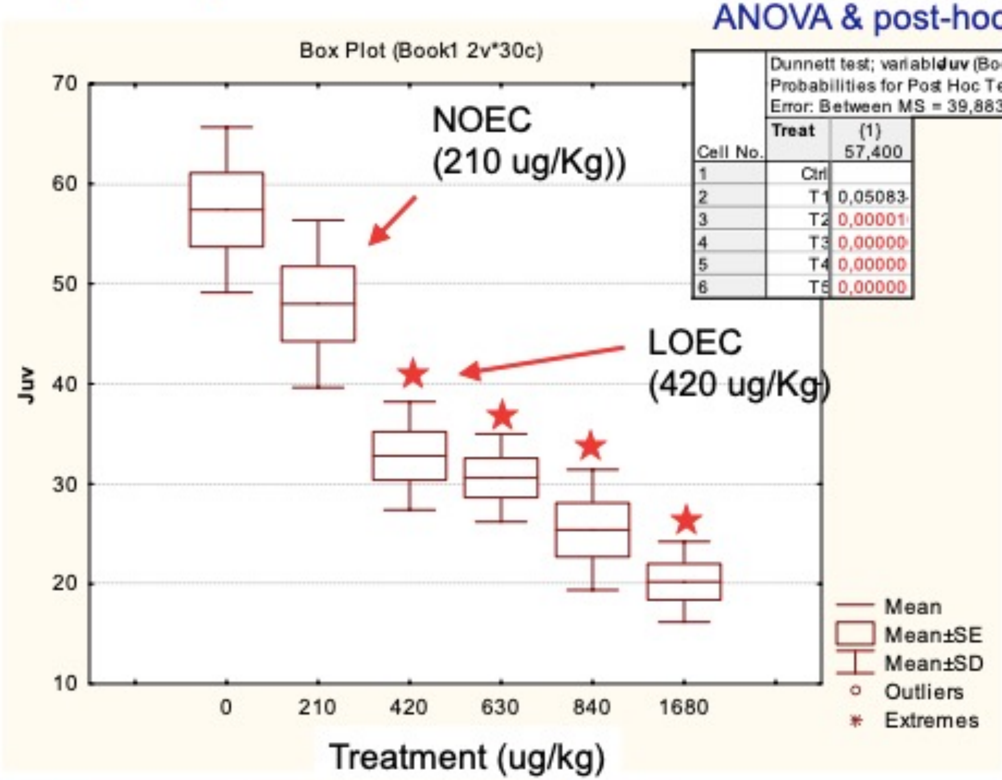
Alguns exemplos de testes com organismos do solo

Testes no laboratório: testes com uma única espécie; testes com invertebrados do solo; testes com plantas, testes com microrganismos, testes com várias espécies

Testes intermédios: testes em micro- ou mesocosmos

Testes no campo: testes com minhocas, com artrópodes predadores, etc.

Alguns exemplos de testes com organismos do solo



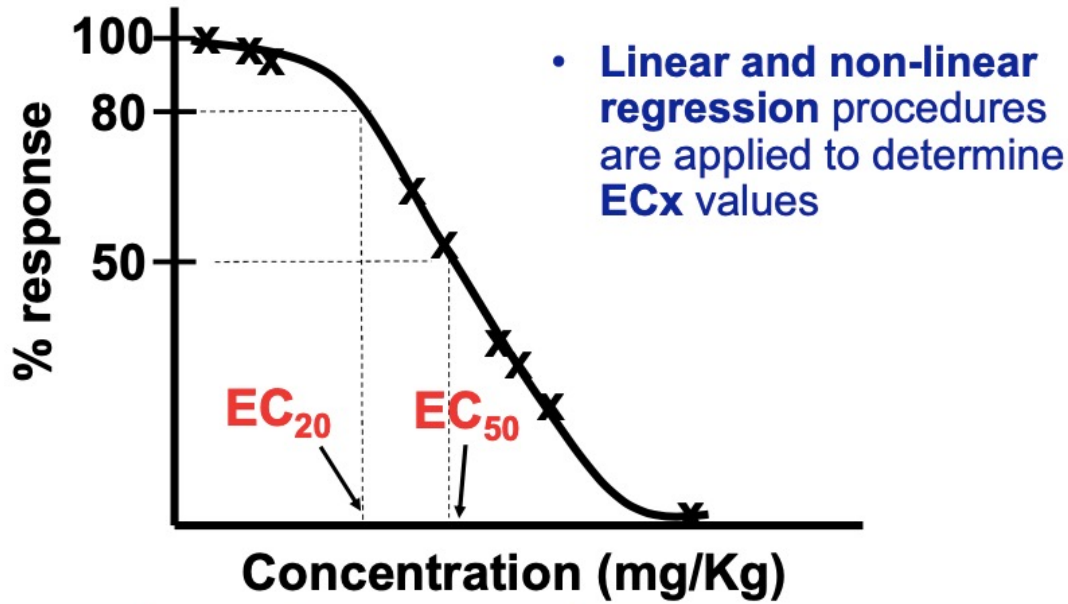
Dunnett test; variablJuv (Book1)
Probabilities for Post Hoc Tests (M<Contr
Error: Between MS = 39,883, df = 24,000

Cell No.	Treat	(1) 57,400
1	Ctrl	
2	T1	0,05083
3	T2	0,00001
4	T3	0,00000
5	T4	0,00000
6	T5	0,00000

NOEC – No observed effect concentration.
LOEC – Lowest observed effect concentration.
EC50 – median effective concentration. It is the concentration of the substance that causes a specific toxic effect to 50% of the test organism

Fonte: Prof José Paulo Sousa

Alguns exemplos de testes com organismos do solo



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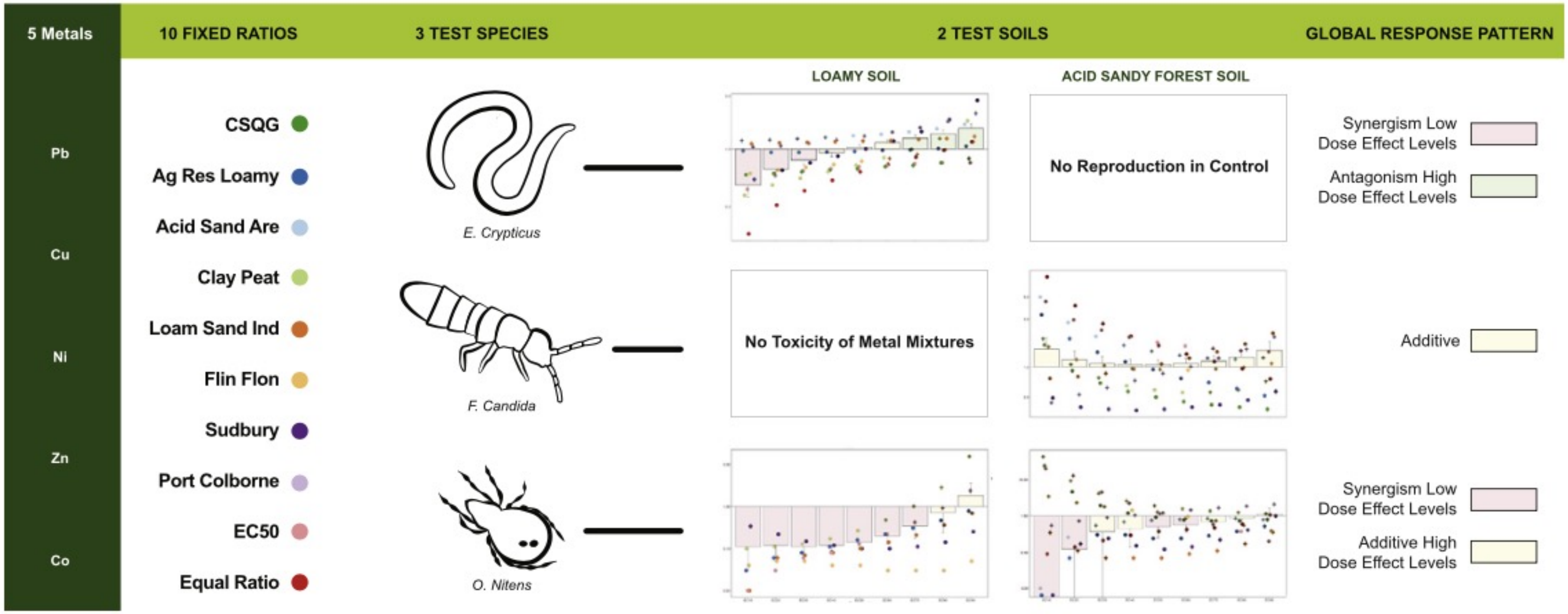

Renaud et al 2020

Alguns exemplos de testes com vários "tiers"

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ELSEVIER

Toxicity of ionic liquids prepared from biomaterials

W. Gouveia [a], T.F. Jorge [a], S. Martins [a], M. Meireles [a], M. Carolino [b], C. Cruz [b], T.V. Almeida [c], M.E.M. Araújo [a,*]

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HIGHLIGHTS

- Eight ionic liquids from biosources, were prepared with moderate to good yields.
- The toxicity of ILs was checked against organisms of various levels of organization.
- The toxicity was observed to depend on both the cation and anion.
- Choline-amino acid ILs showed a remarkable low toxicity to *A. salina* and HeLa cell.
- None of ionic liquids exhibited marked toxicity to bacteria.

ARTICLE INFO

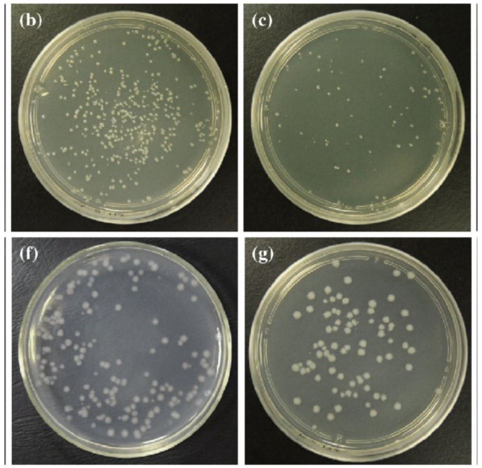
Article history:

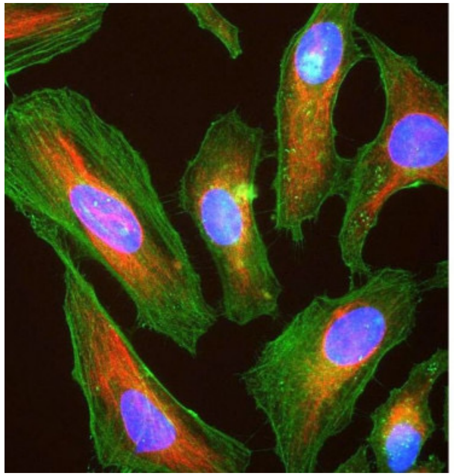
ABSTRACT

In search of environmentally-friendly ionic liquids (ILs), 14 were prepared based on the imidazolium,

Gouveia et al 2014

Alguns exemplos de testes com vários “tiers”





Henrietta Lacks was diagnosed with cervical cancer at the age of 30 and died within a year of her diagnosis. During her treatment at Johns Hopkins Hospital, doctors took two cervical samples without her consent. To protect her identity, the samples were logged under the pseudonym Helen Lane and abbreviated as “HeLa” cells. HeLa cells are remarkable because they continuously divide, allowing them to be used indefinitely in laboratory settings.

Prior to the discovery of these cells, regular cells typically lasted just a few days in a lab. The HeLa cells' ability to multiply offered scientists more time for their research. HeLa cells also allowed for experiments to have more reliability because of the novel ability to run tests on the same cells at different times and in different places.

Alguns exemplos de testes com vários "tiers"

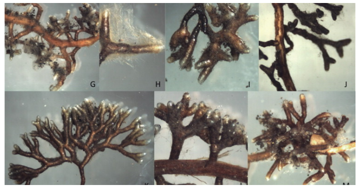
Bactérias: *Bacillus subtilis* subsp. *subtilis* (Gram +) and *Escherichia coli* (Gram -)
Após 48h de exposição, avaliou-se o efeito inibitório no crescimento bacteriano em comparação com o antibiótico cloranfenicol.

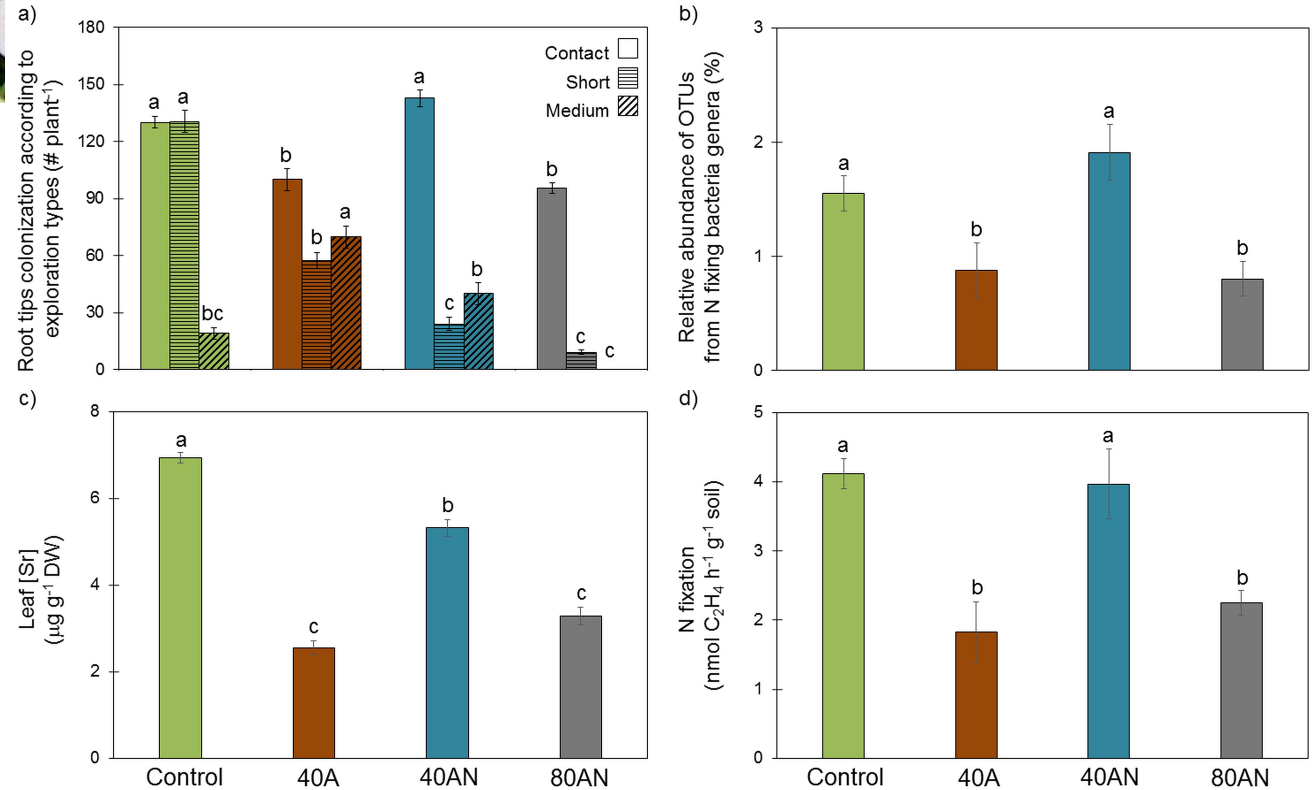
Crustáceo *Artemia salina* L. (Artemiidae)
é um organismo invertebrado que faz parte da fauna de ecossistemas aquáticos salinos e marinhos. Após 24h de exposição, avaliou-se a sobrevivência das larvas.

Células humanas HeLa (carcinoma cervical)
constituem um model para avaliar o dano que células humanas podem sofrer após exposição direta. Após 48h de exposição, a viabilidade cellular através da função mitochondrial (teste colorimétrico MTT)

Gouveia et al 2014

Alguns exemplos de testes com quebra de simbioses





Dias et al
2017

Outros modelos

Testicular damage and farming environments: An integrative ecotoxicological link

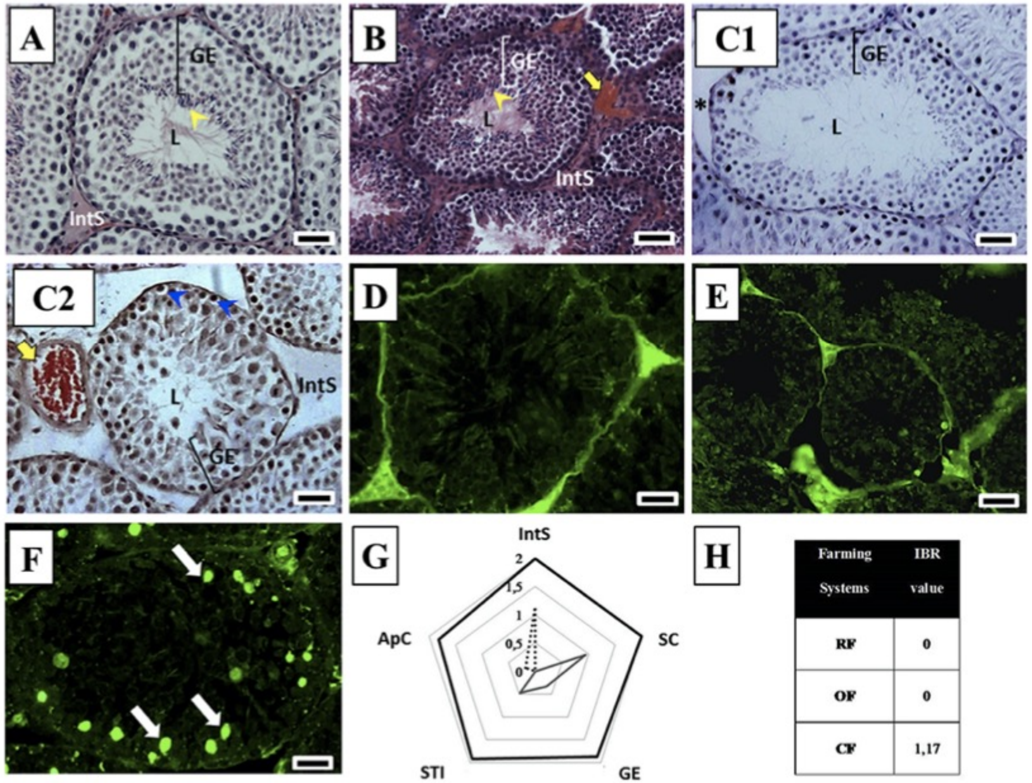
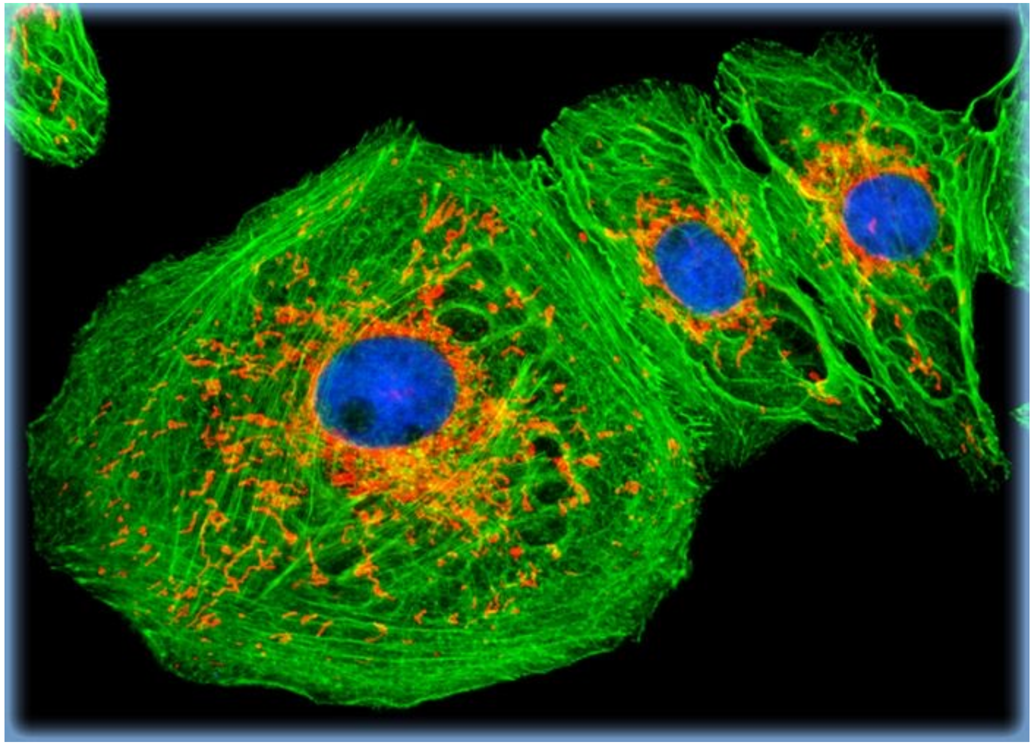


Fig. 1. Histology of the seminiferous tubules, TUNEL assay for apoptotic DNA fragmentation and Integrative biomarker response (IBR) for testicular damage biomarker toxylin and eosin staining (A, B and C) and TUNEL assay (D, E and F) of the seminiferous tubules of mice from the reference site (A and D), organic (B and E) and con farming sites (C and F); germinal epithelium (GE), interstitial space (IntS), lumen (L). IBR score star plot (G) and IBR value (H) for testicular damage biomarkers [apopt (ApC); germinal epithelium (GE); interstitial space (IntS); sperm cells (SC); seminiferous tubule injury index (STI)] of mice from conventional (black line, CF), and organi

Parelho et al 2016

Outros modelos

African Green Monkey Kidney (Vero) Cells



Journal of Virology, Aug. 1996, p. 5519–5524 Vol. 70, No. 8
0022-538X/96/$04.00+0

African Green Monkey Kidney (Vero) Cells Provide an Alternative Host Cell System for Influenza A and B Viruses

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The preparation of live, attenuated human influenza virus vaccines and of large quantities of inactivated vaccines after the emergence or reemergence of a pandemic influenza virus will require an alternative host cell system, because embryonated chicken eggs will likely be insufficient and suboptimal. Preliminary studies indicated that an African green monkey kidney cell line (Vero) is a suitable system for the primary isolation and cultivation of influenza A viruses (E. A. Govorkova, N. V. Kaverin, L. V. Gubareva, B. Meignier, and R. G. Webster, J. Infect. Dis. 172:250–253, 1995). We now demonstrate for the first time that Vero cells are suitable for isolation and productive replication of influenza B viruses and determine the biological and genetic properties of both influenza A and B viruses in Vero cells; additionally, we characterize the receptors on Vero cells compared with those on Madin-Darby canine kidney (MDCK) cells. Sequence analysis indicated that the hemagglutinin of Vero cell-derived influenza B viruses was identical to that of MDCK-grown counterparts but differed from that of egg-grown viruses at amino acid positions 196 to 198. Fluorescence-activated cell sorting analysis showed that although Vero cells possess predominantly α2,3 galactose-linked sialic acid, they are fully susceptible to infection with either human influenza A or B viruses. Moreover, all virus-specific polypeptides were synthesized in the same proportions in Vero cells as in MDCK cells. Electron microscopic and immunofluorescence studies confirmed that infected Vero cells undergo the same morphological changes as do other polarized epithelial cells. Taken together, these results indicate that Vero cell lines could serve as an alternative host system for the cultivation of influenza A and B viruses, providing adequate quantities of either virus to meet the vaccine requirements imposed by an emerging pandemic.