BIOENSAIOS PARA AVALIAÇÃO DE RISCO ECOTOXICOLÓGICO DE AGROTÓXICOS

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BIOENSAIOS

- Preveem o comportamento da substância sobre os organismos vivos
- Efeito interativo entre contaminantes
- Toxicidade de subprodutos gerados





BIOENSAIOS

- **Procariontes:**
  - *Salmonella*
  - *Vibrio fischeri*
- **Eucariontes:**
  - Vegetais (*Allium cepa, Tradescantia, Vicia faba)*
  - Animais
    - Invertebrados (planária, D*aphnia*, hidras)
    - Vertebrados (peixes, anfíbios, répteis, mamíferos)
  - Cultura de células

PARÂMETROS AVALIADOS

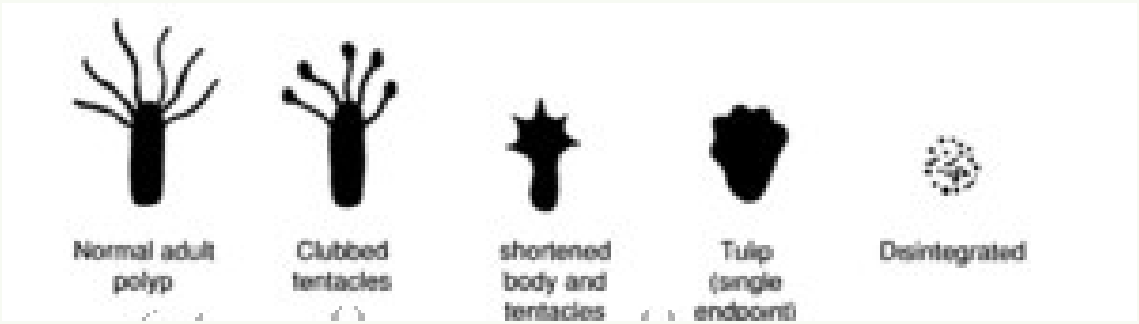
- Morte de indivíduos



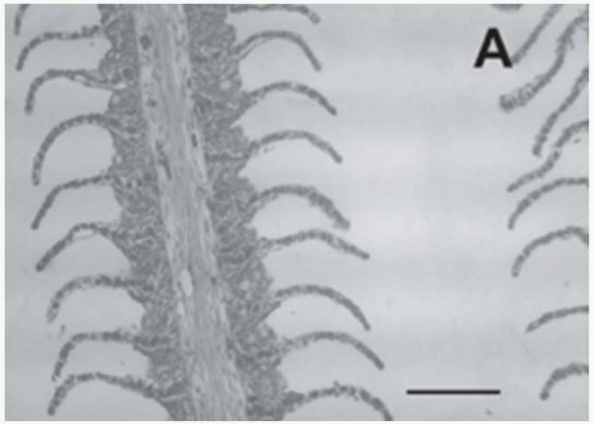

- Perda da capacidade reprodutiva

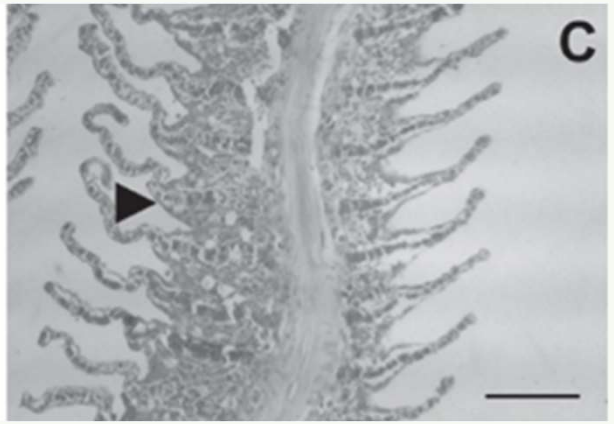

PARÂMETROS AVALIADOS

- Alterações morfológicas



- Alterações histológicas



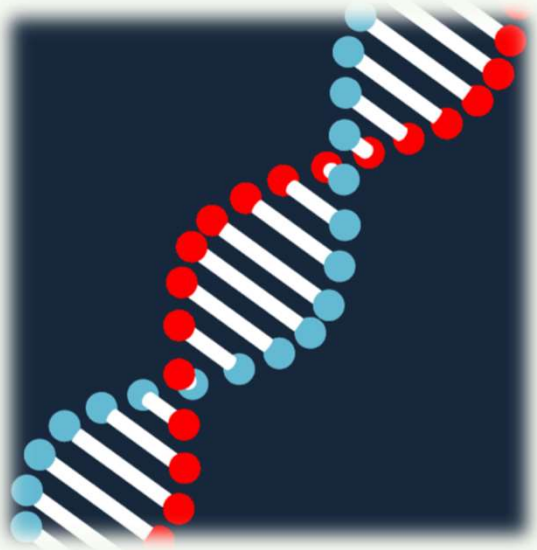


PARÂMETROS AVALIADOS

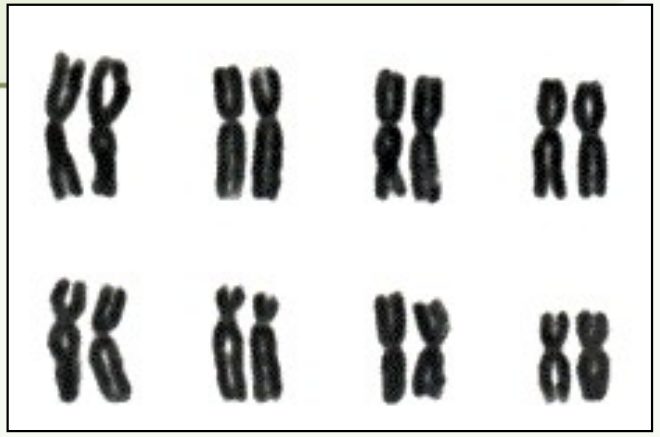
- Alterações no DNA

  - **Genotoxicidade:** danos ao material genético da célula ou organismo exposto;

  - **Mutagenicidade:** surgimento de mutações devido à danos no material genético.



ORGANISMO-TESTE



Allium cepa

- Baixo custo e fácil manuseio
- Alta sensibilidade
  - sensibilidade de 76% para a avaliação de 148 químicos pelo teste de *Allium* (GRANT, 1982)
  - praticamente a mesma de sistemas-teste de algas e de linfócitos humanos (FISKESJÖ, 1985)

- Boa correlação com outros sistemas testes
  - 82% com o teste de carcinogenicidade em roedores e mais sensível que os testes de Ames (RANK e NIELSEN, 1994)

ORGANISMO-TESTE

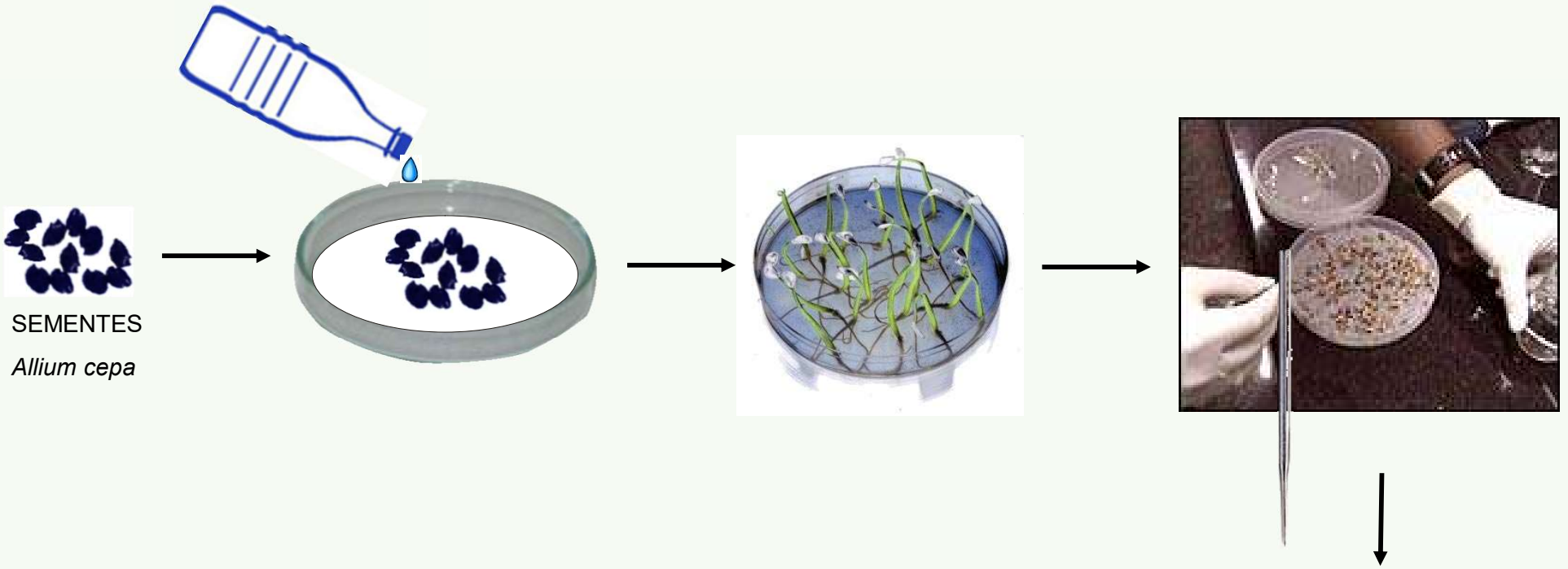
Allium cepa

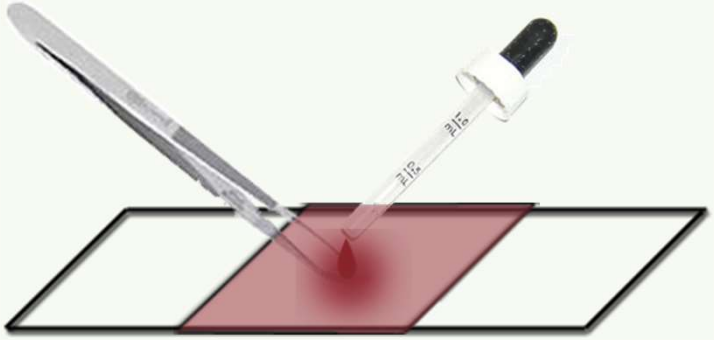
- Avalia os efeitos de diferentes contaminantes (substâncias puras, misturas, amostras ambientais, etc);

- Exposição direta à amostra ou ao químico
  - Sem necessidade de métodos de extração e concentração

- Presença de sistema de ativação metabólica
- Avalia diferentes parâmetros
  - mecanismo de ação

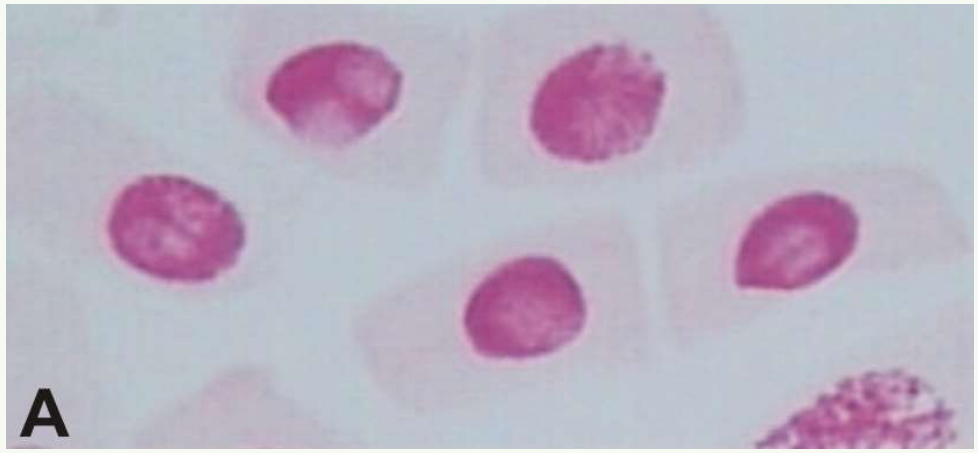
REALIZAÇÃO DO ENSAIO DE ABERRAÇÕES CROMOSSÔMICAS E DO TESTE DO MICRONÚCLEO EM *Allium cepa*



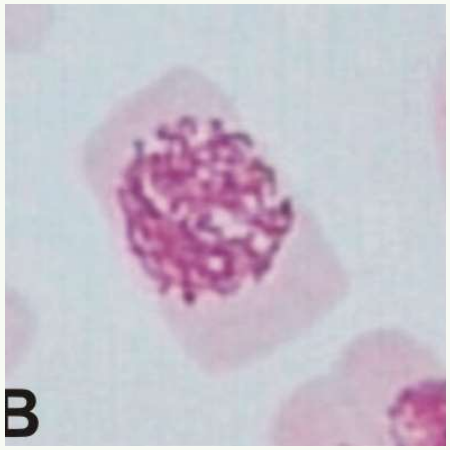


Animação: Roberto, M.M. ; LEME, D.M. (2007)

ORGANISMO-TESTE



Intérfase



Prófase

Metáfase

Anáfase

Telófase

EXEMPLO

Ecotoxicology and Environmental Safety 72 (2009) 1680–1686

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Origin of nuclear and chromosomal alterations derived from the action of an aneugenic agent—Trifluralin herbicide

Thaís Cristina Casimiro Fernandes, Dânia Elisa Christofoletti Mazzeo, Maria Aparecida Marin-Morales *

São Paulo State University, Av. 24 A, 1515, Bela Vista, CEP 13506-900 Rio Claro, SP, Brazil

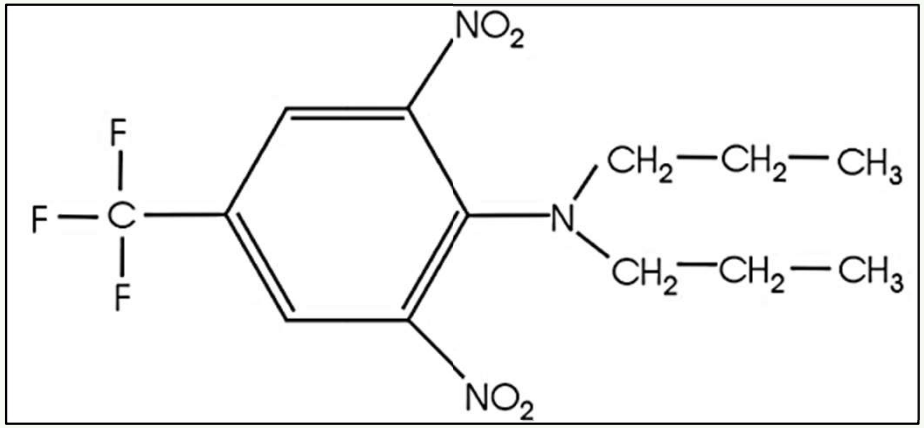
EXEMPLO

Agrotóxico testado:

- 3,34 mg/L (campo)
- 1,67 mg/L
- 0,84 mg/L
- 0,42 mg/L

Trifluralina
(445 g/L)

Grupo químico:
Dinitroanilina



EXEMPLO

Tratamento:

Germinação:
Água ultrapura
(1 cm)

Exposição:
24 h
(trifluralina)

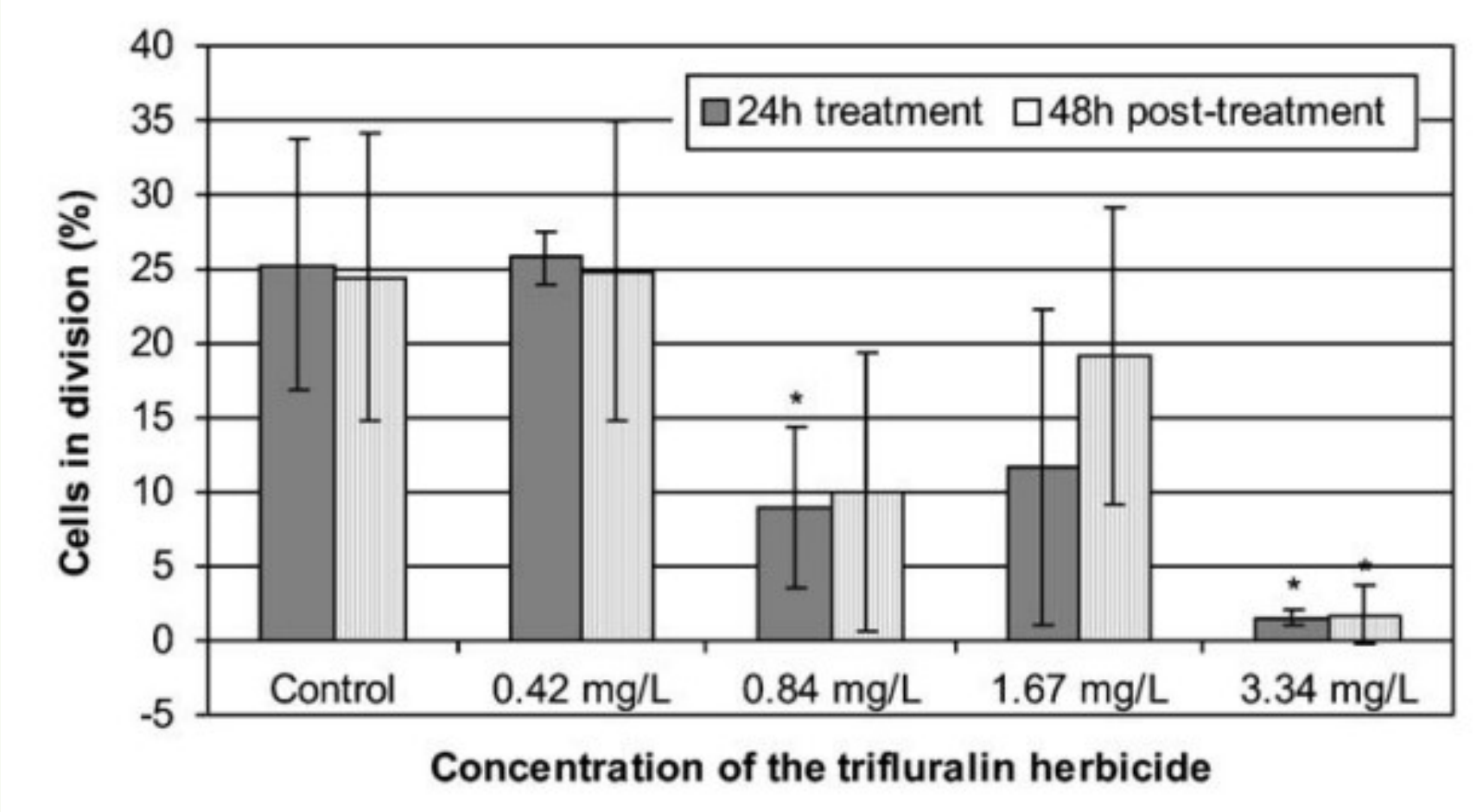
Coleta e fixação
(metade)

Recuperação:
48 h
(água ultrapura)

Coleta e fixação
(restante)

EXEMPLO

Resultados:



EXEMPLO

Resultados:

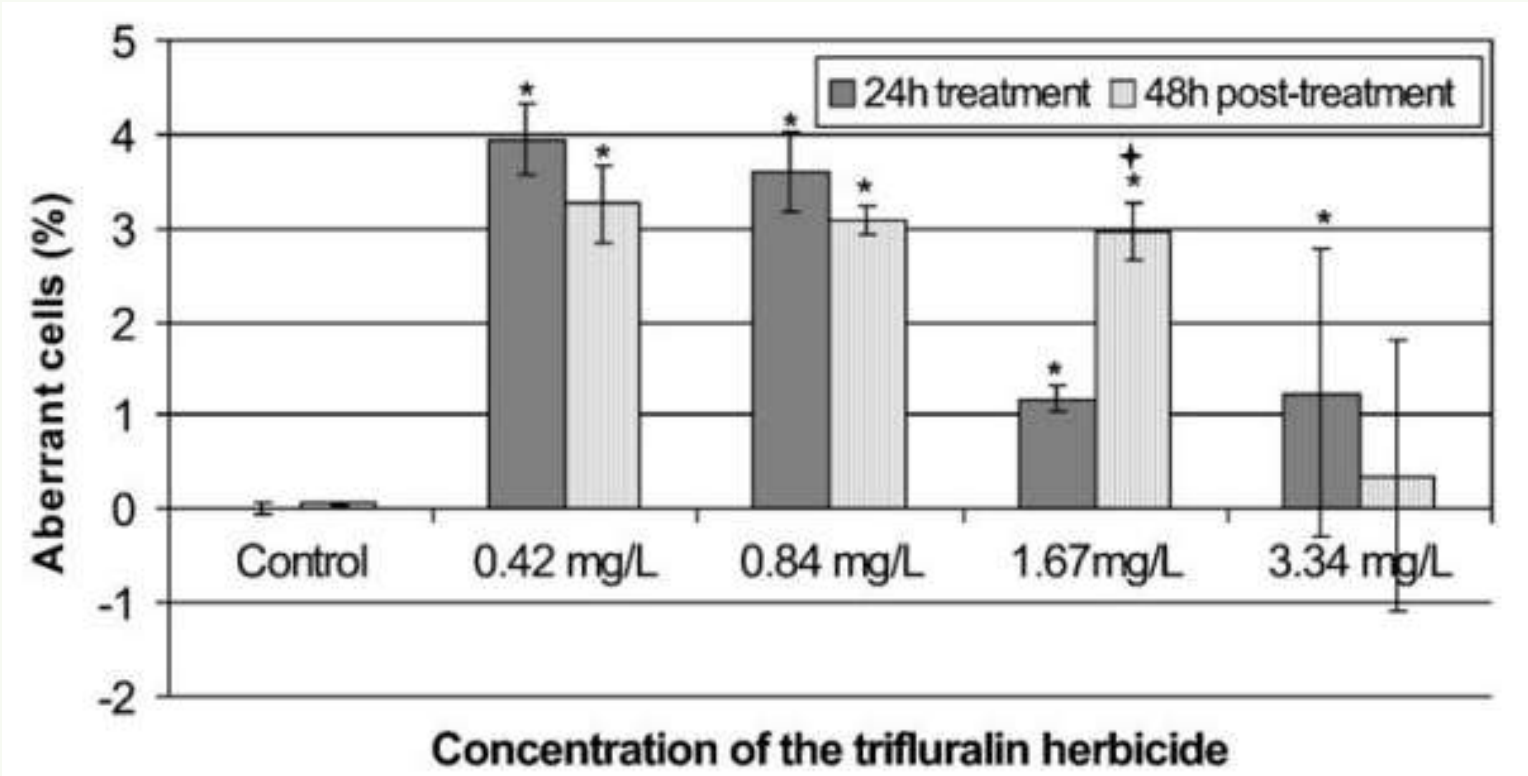
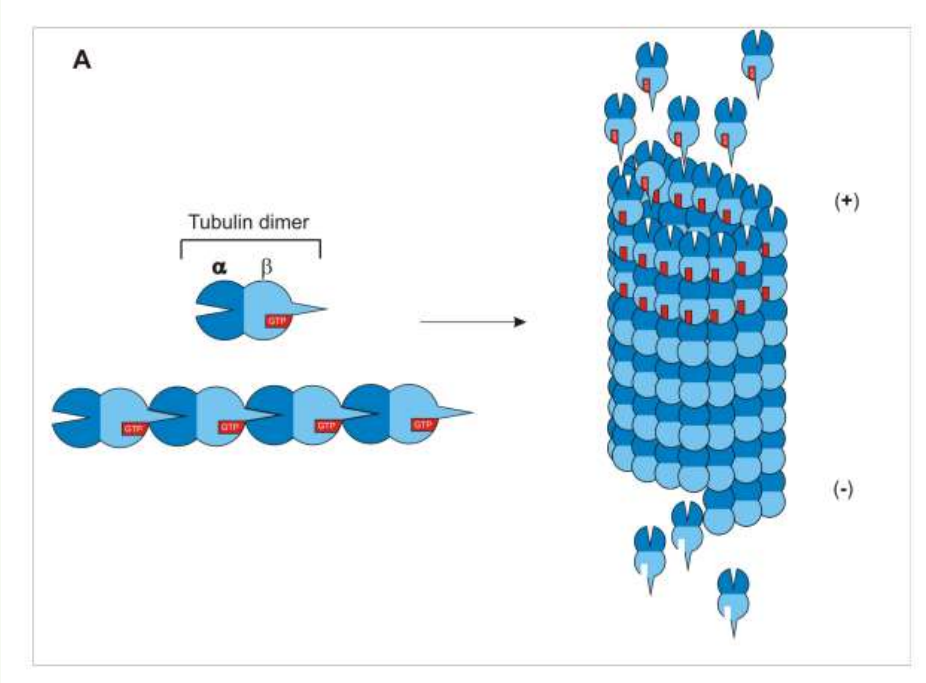
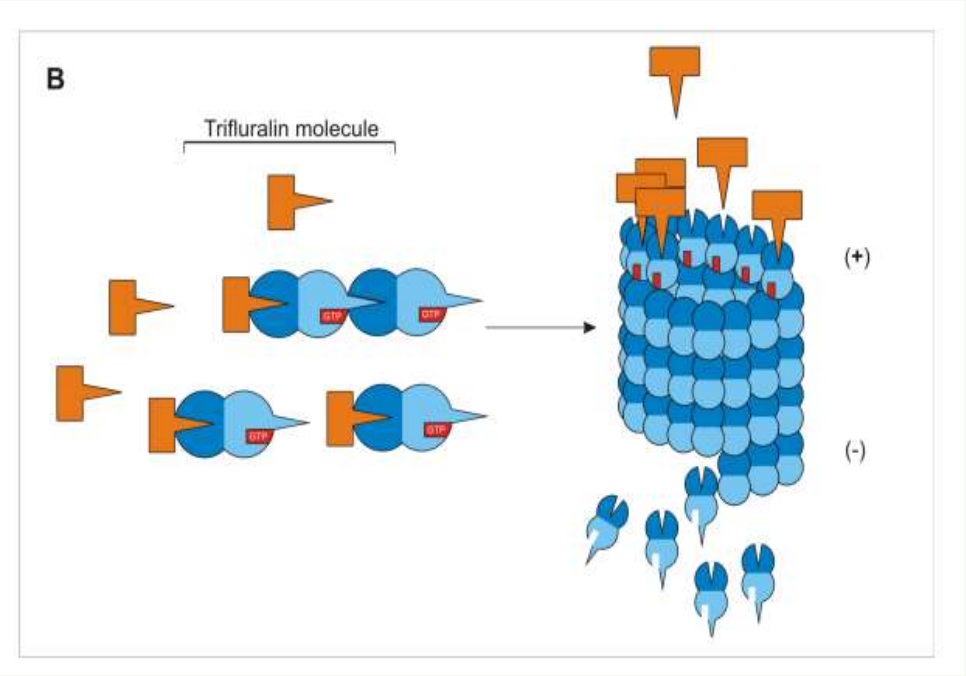


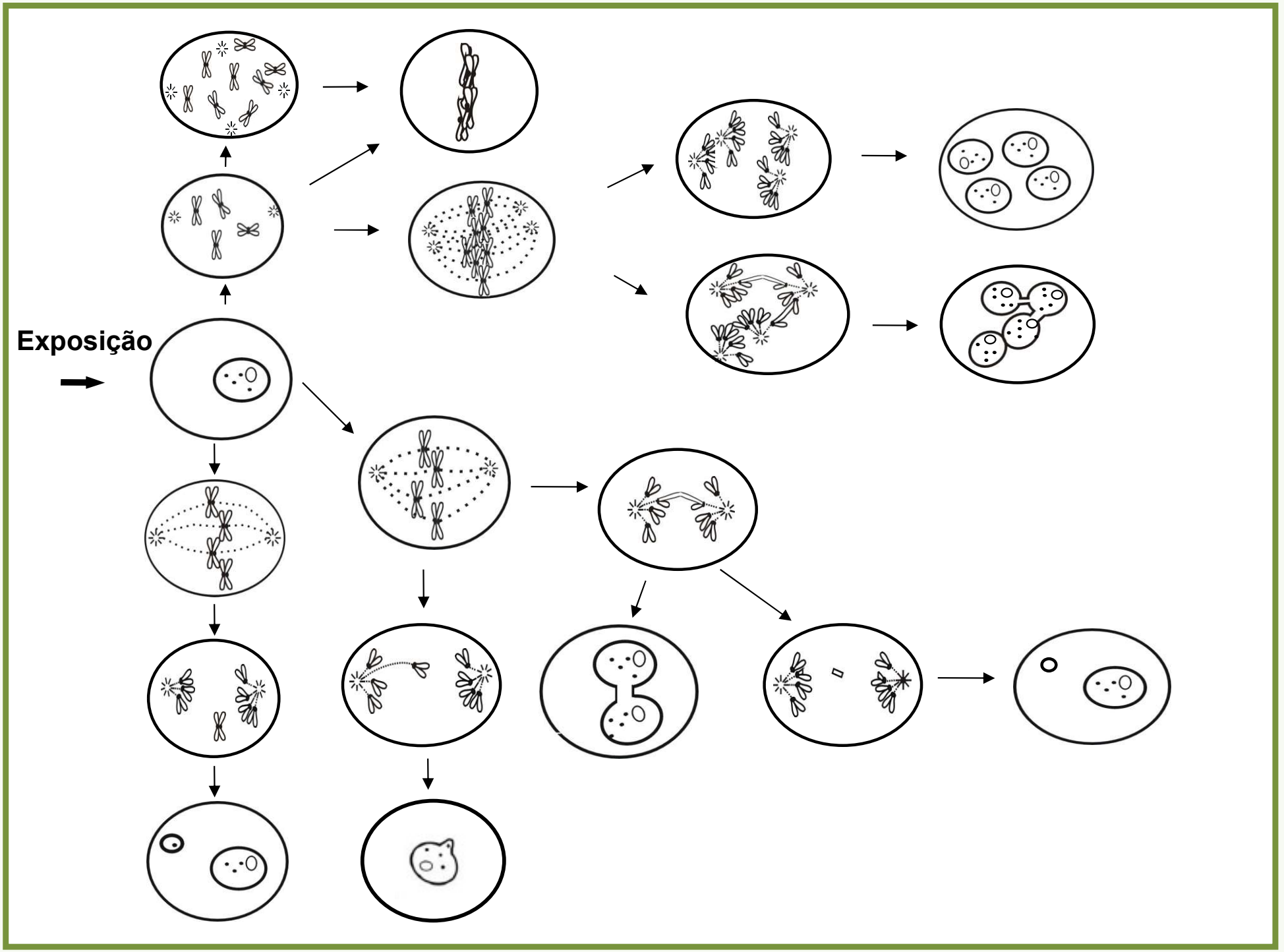
Fig. 3. Total frequency of aberrant cells for all tested concentrations of the trifluralin herbicide after 24 h trifluralin treatment and 48 h recovery post-treatment. *Significant $p<0.05$ (compared to the control). +Significant $p<0.05$ (24 h treatment versus 48 h recovery post-treatment).

EXEMPLO

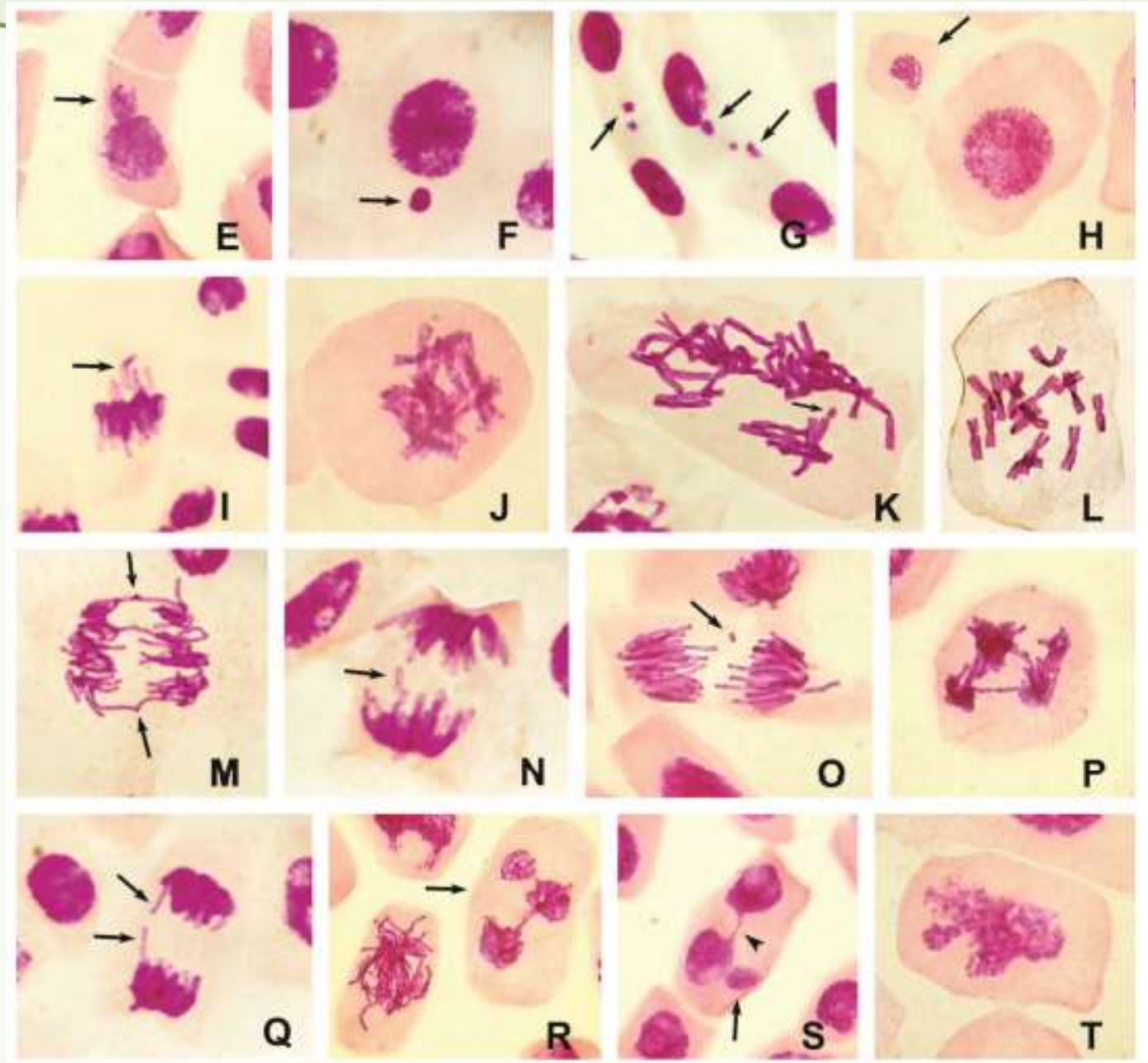
Resultados:






Exposição

EXEMPLO



EXEMPLO

Environ Sci Pollut Res (2015) 22:9796–9806
DOI 10.1007/s11356-015-4134-2

RESEARCH ARTICLE

Genotoxicity evaluation of environmental pollutants using analysis of nucleolar alterations

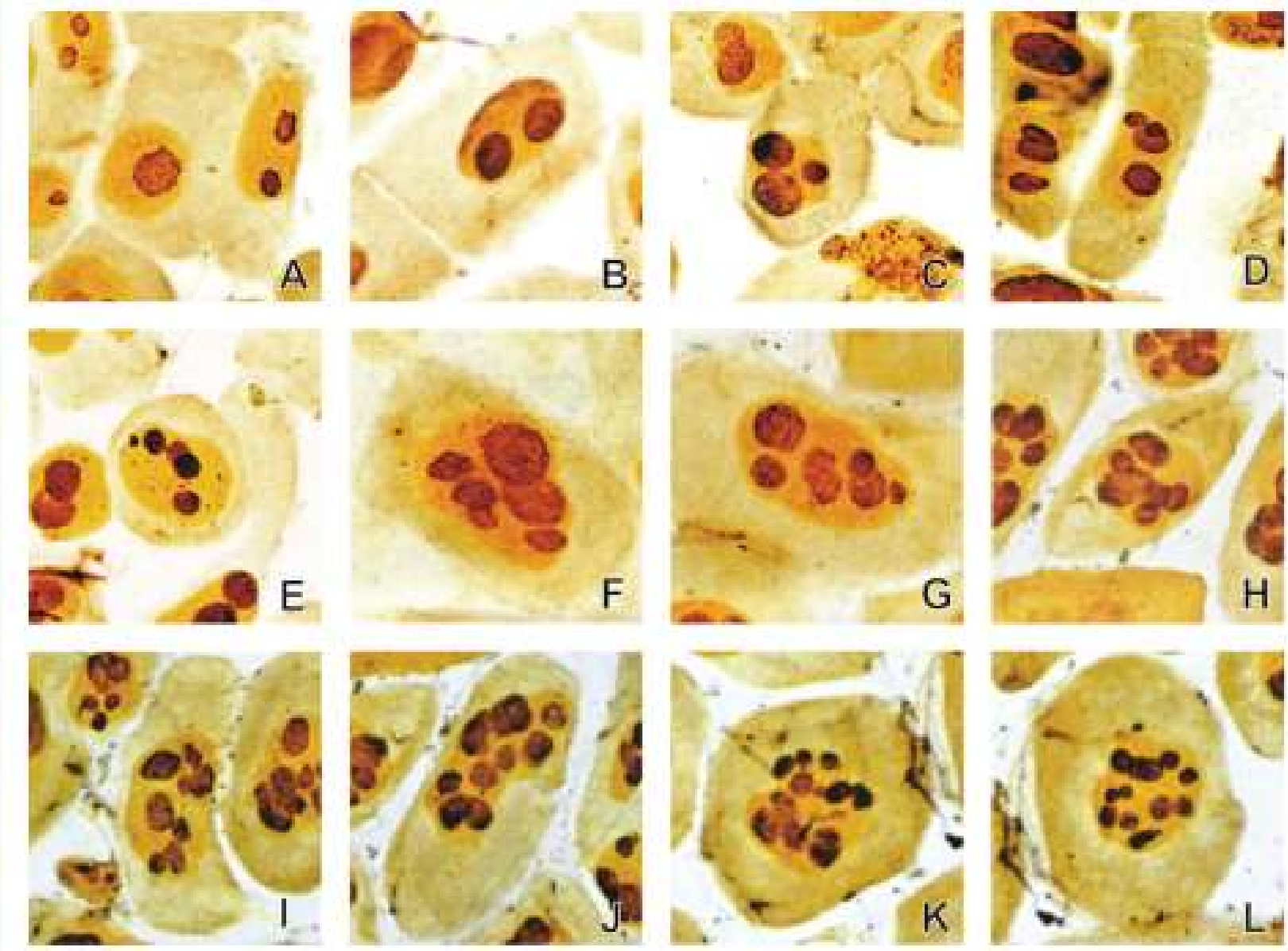
Dânia Elisa Christofoletti Mazzeo · Maria Aparecida Marin-Morales

- Bandamentos cromossômicos

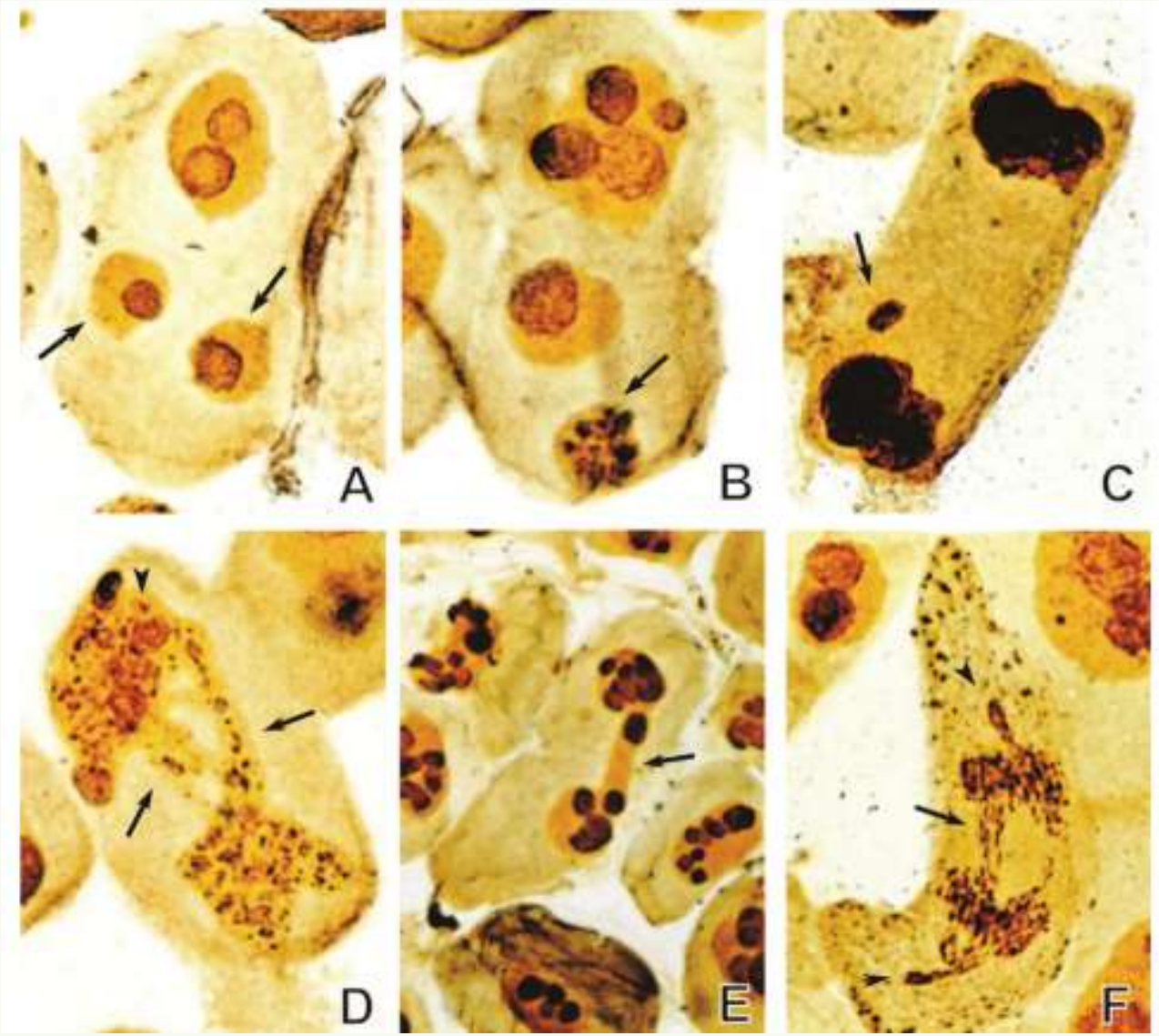
Fornecem informações qualitativas sobre a composição do DNA.

Ex. RON ($AgNO_3$)

EXEMPLO



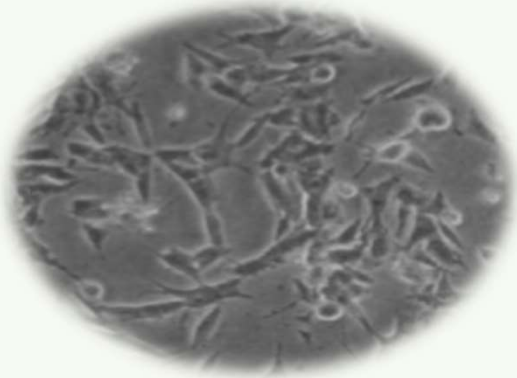
EXEMPLO



SISTEMA-TESTE

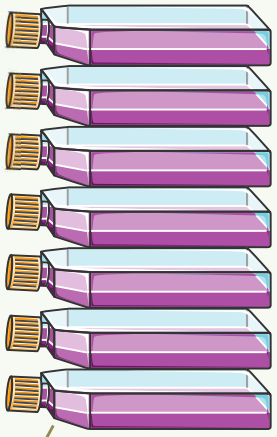
Cultura de células

- Linhagem homogênea
- Resultados semelhantes com o de animais
- Não há necessidade de sacrifício de animais
- Tempo e custo reduzidos, mais réplicas
- Estocagem em nitrogênio líquido

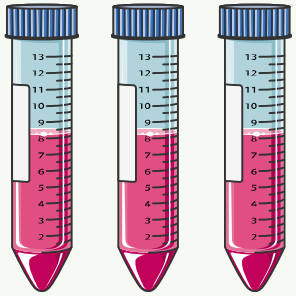


ENSAIO DO COMETA

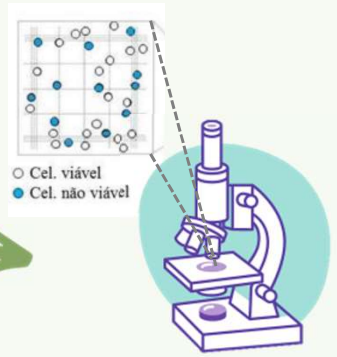
Amostra



Exposição
(3 a 24h)



Colheita e fixação das células

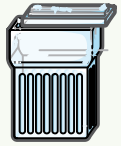


Viabilidade celular

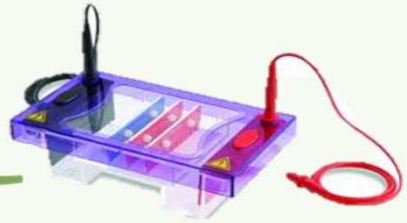
Agarose LMP

Células tratadas

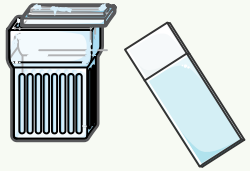
Lâmina com agarose (triplicata)



Sol. Lise (1h)



Tampão alcalino pH>12



Neutralização (pH 7) **e fixação**

Software *Comet Assay IV*
(momento da cauda de Olive e intensidade da cauda)

Coloração: Gel Red
Microscópio de Fluorescência
300 nucleiodes/tratamento

ENSAIO DO COMETA

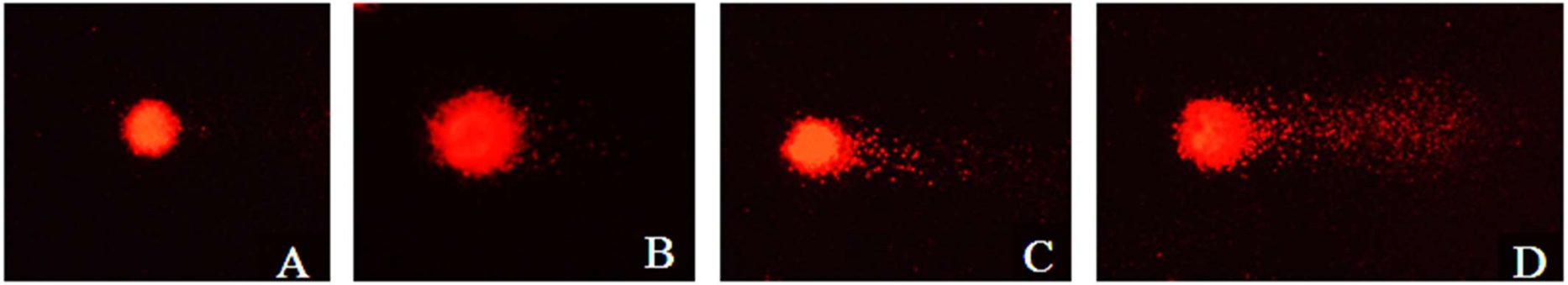
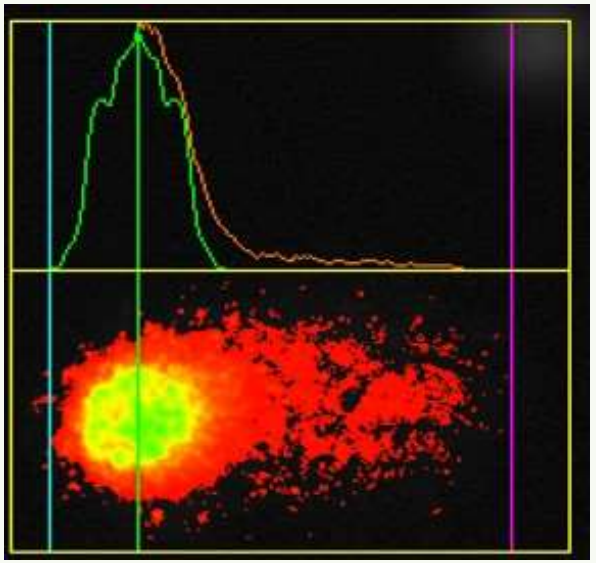
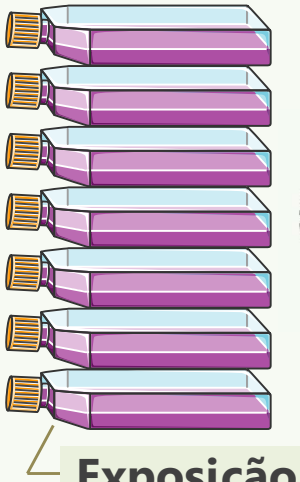


Figura 1. A: classe 0; B: classe 1; C: classe 2; D: classe 3.



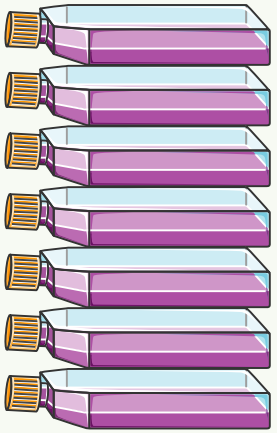
TESTE DO MICRONÚCLEO

Amostra

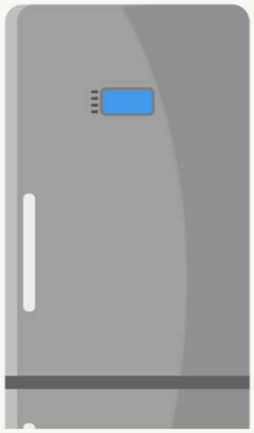


Exposição
(3 a 24h)

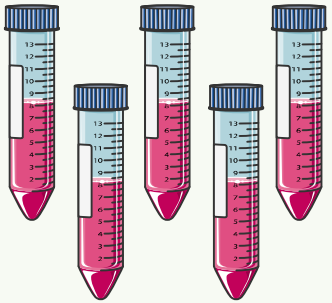
CITOCALASINA B



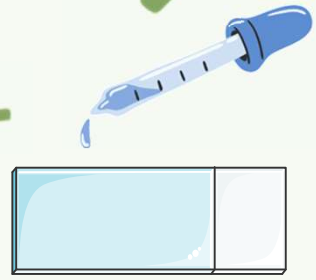
Incubação
(30 h)

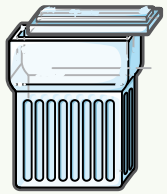


37 °C
5% CO_2

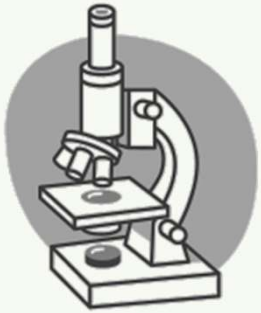


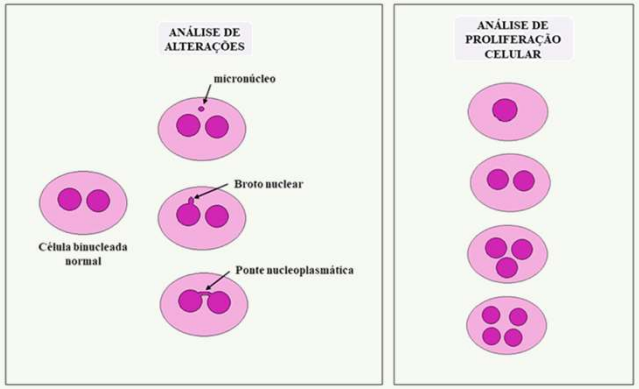
Colheita e fixação das células





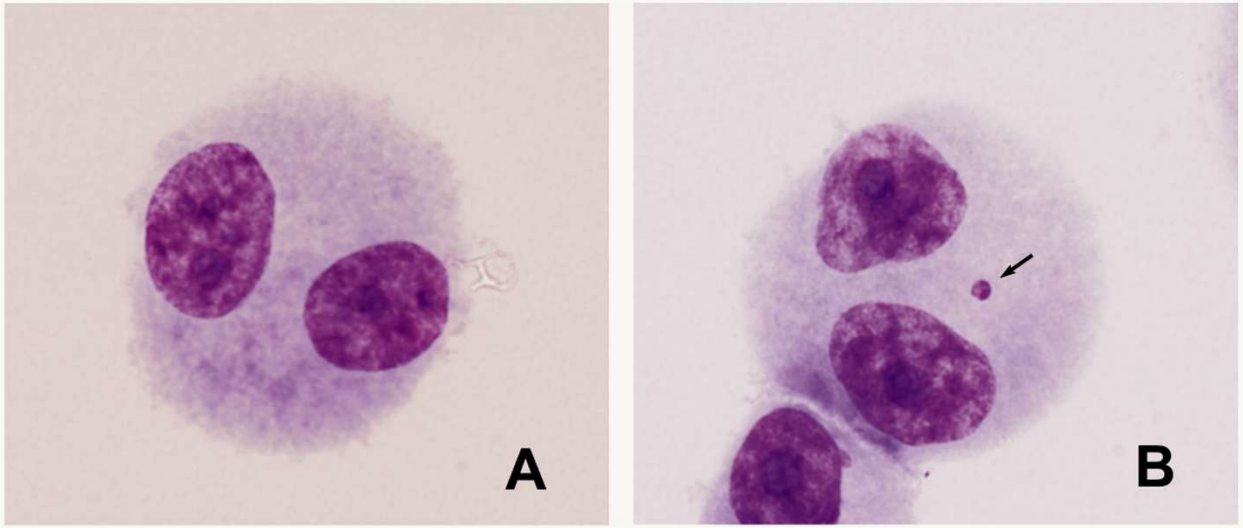
Coloração com Giemsa



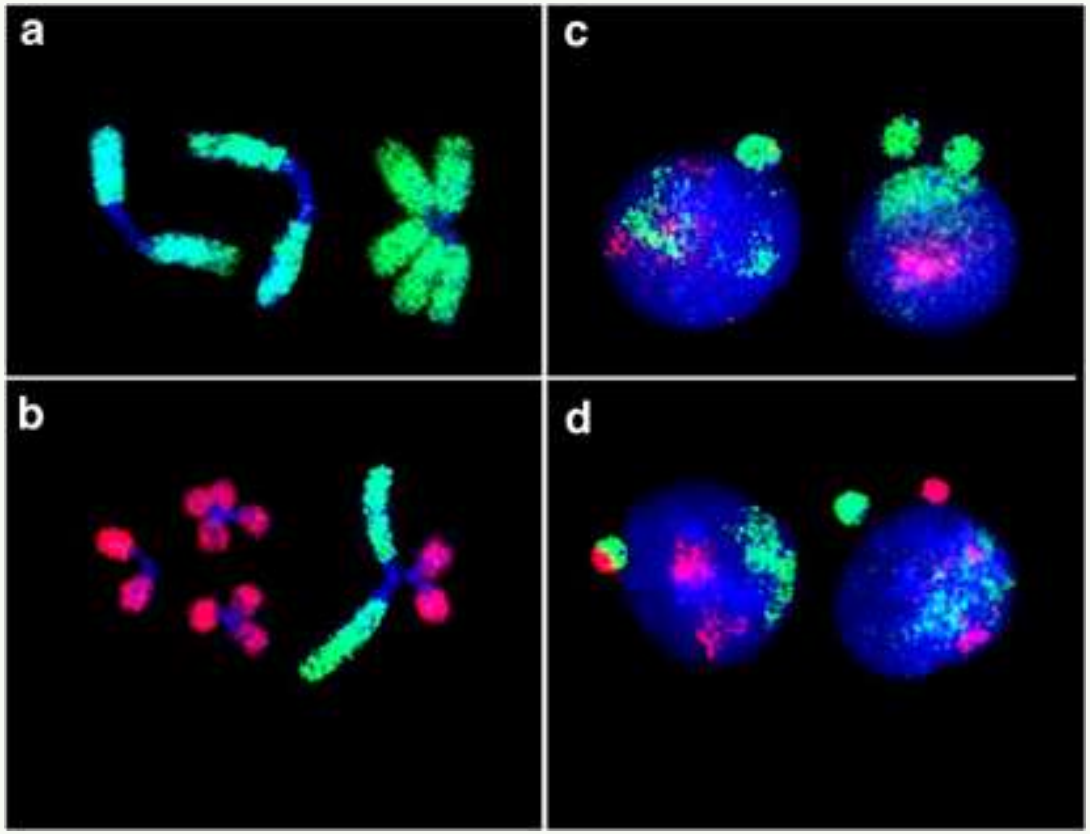


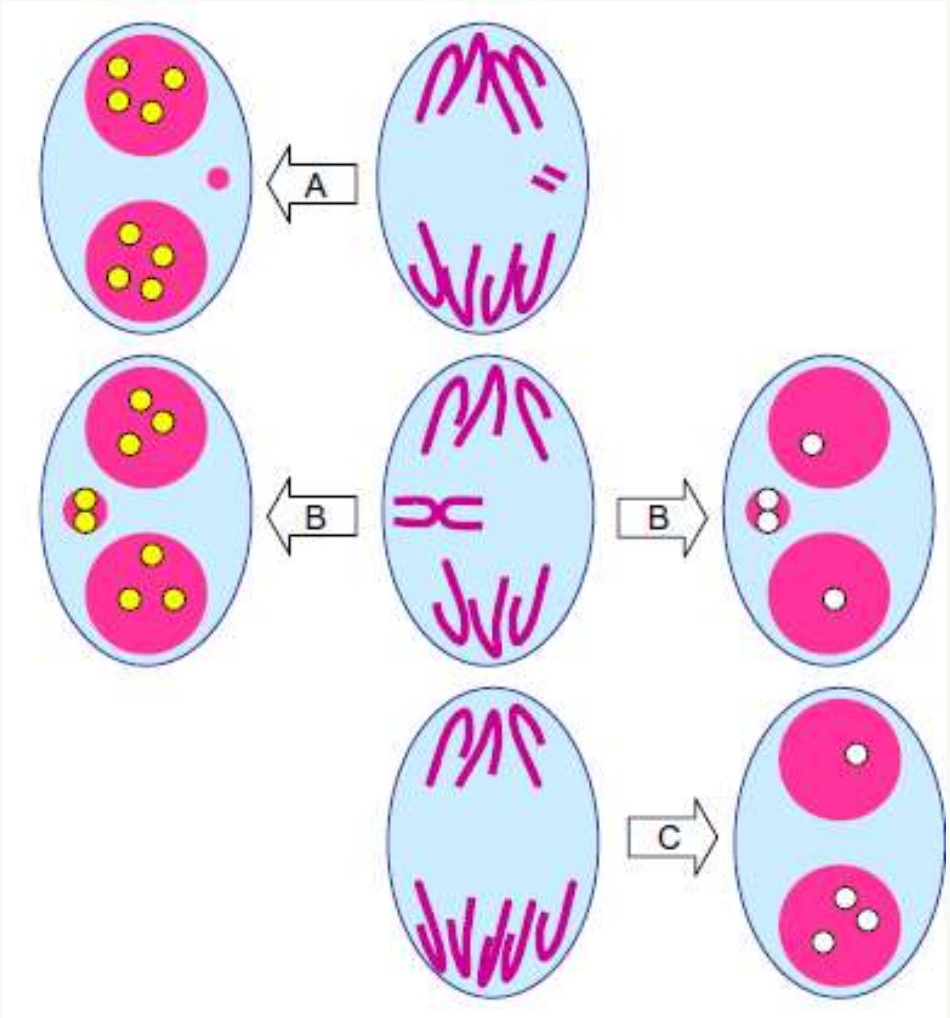
Genotoxicidade → 6000 células/tratamento ;
IPBC → 3000 células/tratamento

TESTE DO MICRONÚCLEO



HIBRIDIZAÇÃO *IN SITU*





EXEMPLO

Ecotoxicology and Environmental Safety 120 (2015) 174–183

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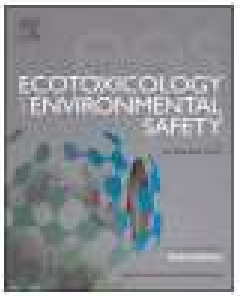


Toxicogenetic effects of low concentrations of the pesticides imidacloprid and sulfentrazone individually and in combination in in vitro tests with HepG2 cells and *Salmonella typhimurium*

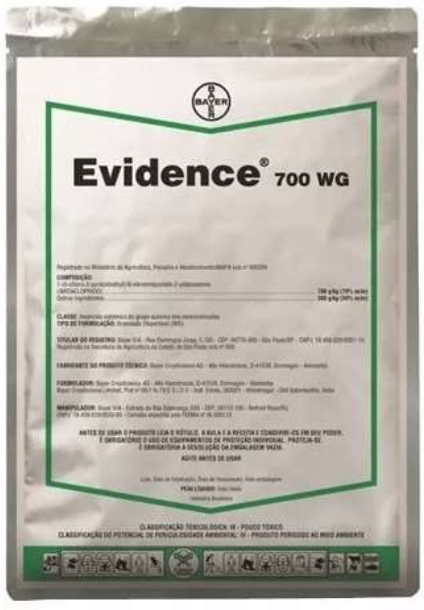
Jaqueline Bianchi, Diogo Cavalcanti Cabral-de-Mello, Maria Aparecida Marin-Morales *

Department of Biology, Institute of Biosciences, São Paulo State University (UNESP), Av. 24A, 1515, Bela Vista, Rio Claro, São Paulo CEP 13506-900, Brazil

EXEMPLO

Agrotóxicos testados:

- 0,06 g/L
- 0,006 g/L
- 0,0006 g/L



- 0,036 g/L
- 0,0036 g/L
- 0,00036 g/L

Sulfentrazona
(500 g/L)

Herbicida
Triazolinona

Imidacloprida
(480 g/L)

Inseticida
Neonicotinoide

Misturas

RESULTADOS

Results of the comet assay with HepG2 cells, after exposure for 24 h to the different concentrations of the insecticide imidacloprid, herbicide sulfentrazone and to the mixture of these two pesticides.

	Number of cells analyzed	Score[a] (mean ± sd)
Imidacloprid		
NC	670	2.4 ± 4.7
0.036 g/L	631	3.3 ± 3.5
0.0036 g/L	672	9.1 ± 5.4*
0.00036 g/L	662	11.0 ± 12.3*
PC-MMS	637	174.3 ± 29.6*
Sulfentrazone		
NC	604	11.5 ± 5.7
0.06 g/L	605	16.0 ± 6.2
0.006 g/L	614	16.2 ± 7.3
0.0006 g/L	603	13.6 ± 3.9
PC-MMS	600	211.8 ± 8.4*
Mixture		
NC	605	11.7 ± 1.8
M1	607	14.6 ± 4.7
M2	615	19.8 ± 8.1*
M3	614	25.4 ± 3.4*
PC-MMS	601	252.8 ± 18.9*

M1: mixture of the herbicide sulfentrazone (0.06 g/L) with the insecticide imidacloprid (0.036 g/L); M2: mixture of the herbicide sulfentrazone (0.006 g/L) with the insecticide imidacloprid (0.0036 g/L); M3: mixture of the herbicide sulfentrazone (0.0006 g/L) with the insecticide imidacloprid (0.00036 g/L).

[a] Score: (percentage of cells in class 0 × 0)+(percentage of cells in class 1 × 1)+(percentage of cells in class 2 × 2)+(percentage of cells in class 3 × 3).

* Statistically significant ($p < 0.05$).

RESULTADOS

Results of the comet assay with HepG2 cells, after exposure for 24 h to the different concentrations of the insecticide imidacloprid, herbicide sulfentrazone and to the mixture of these two pesticides.

	Number of cells analyzed	Score[a] (mean ± sd)
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NC	670	2.4 ± 4.7
0.036 g/L	631	3.3 ± 3.5
0.0036 g/L	672	9.1 ± 5.4*
0.00036 g/L	662	11.0 ± 12.3*
PC-MMS	637	174.3 ± 29.6*
Sulfentrazone		
NC	604	11.5 ± 5.7
0.06 g/L	605	16.0 ± 6.2
0.006 g/L	614	16.2 ± 7.3
0.0006 g/L	603	13.6 ± 3.9
PC-MMS	600	211.8 ± 8.4*
Mixture		
NC	605	11.7 ± 1.8
M1	607	14.6 ± 4.7
M2	615	19.8 ± 8.1*
M3	614	25.4 ± 3.4*
PC-MMS	601	252.8 ± 18.9*

M1: mixture of the herbicide sulfentrazone (0.06 g/L) with the insecticide imidacloprid (0.036 g/L); M2: mixture of the herbicide sulfentrazone (0.006 g/L) with the insecticide imidacloprid (0.0036 g/L); M3: mixture of the herbicide sulfentrazone (0.0006 g/L) with the insecticide imidacloprid (0.00036 g/L).

[a] Score: (percentage of cells in class 0 × 0)+(percentage of cells in class 1 × 1)+(percentage of cells in class 2 × 2)+(percentage of cells in class 3 × 3).

* Statistically significant ($p < 0.05$).

RESULTADOS

Results of the MN test with the HepG2 cells exposed for 24 h to the three different concentrations of the insecticide imidacloprid, herbicide sulfentrazone and to the mixture of these two pesticides.

Treatments	Concentrations	Total of analyzed cells	Mean ± sd
NC	–	6032	09.00 ± 1.26
Imidacloprid	0.036 g/L	6029	33.16 ± 5.71**
	0.0036 g/L	6036	30.33 ± 8.91**
	0.00036 g/L	6014	44.83 ± 15.11**
PC (MMS)	4×10^{-2} M	6049	53.16 ± 14.27**
NC	–	6010	19.00 ± 3.16
Sulfentrazone	0.06 g/L	6032	26.16 ± 4.57*
	0.006 g/L	6020	37.67 ± 7.55**
	0.0006 g/L	6025	40.50 ± 9.41**
PC (MMS)	4×10^{-2} M	6024	58.66 ± 5.75**
NC	–	6013	16.50 ± 3.27
Mixture	M1	6029	23.00 ± 6.98
	M2	6031	19.17 ± 3.30
	M3	6022	28.50 ± 15.37
PC (MMS)	4×10^{-2} M	6036	78.33 ± 15.15**

M1: mixture of the herbicide sulfentrazone (0.06 g/L) with the insecticide imidacloprid (0.036 g/L); M2: mixture of the herbicide sulfentrazone (0.006 g/L) with the insecticide imidacloprid (0.0036 g/L); M3: mixture of the herbicide sulfentrazone (0.0006 g/L) with the insecticide imidacloprid (0.00036 g/L).

* Statistically significant ($p < 0.05$).

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** Statistically significant ($p < 0.001$).

RESULTADOS

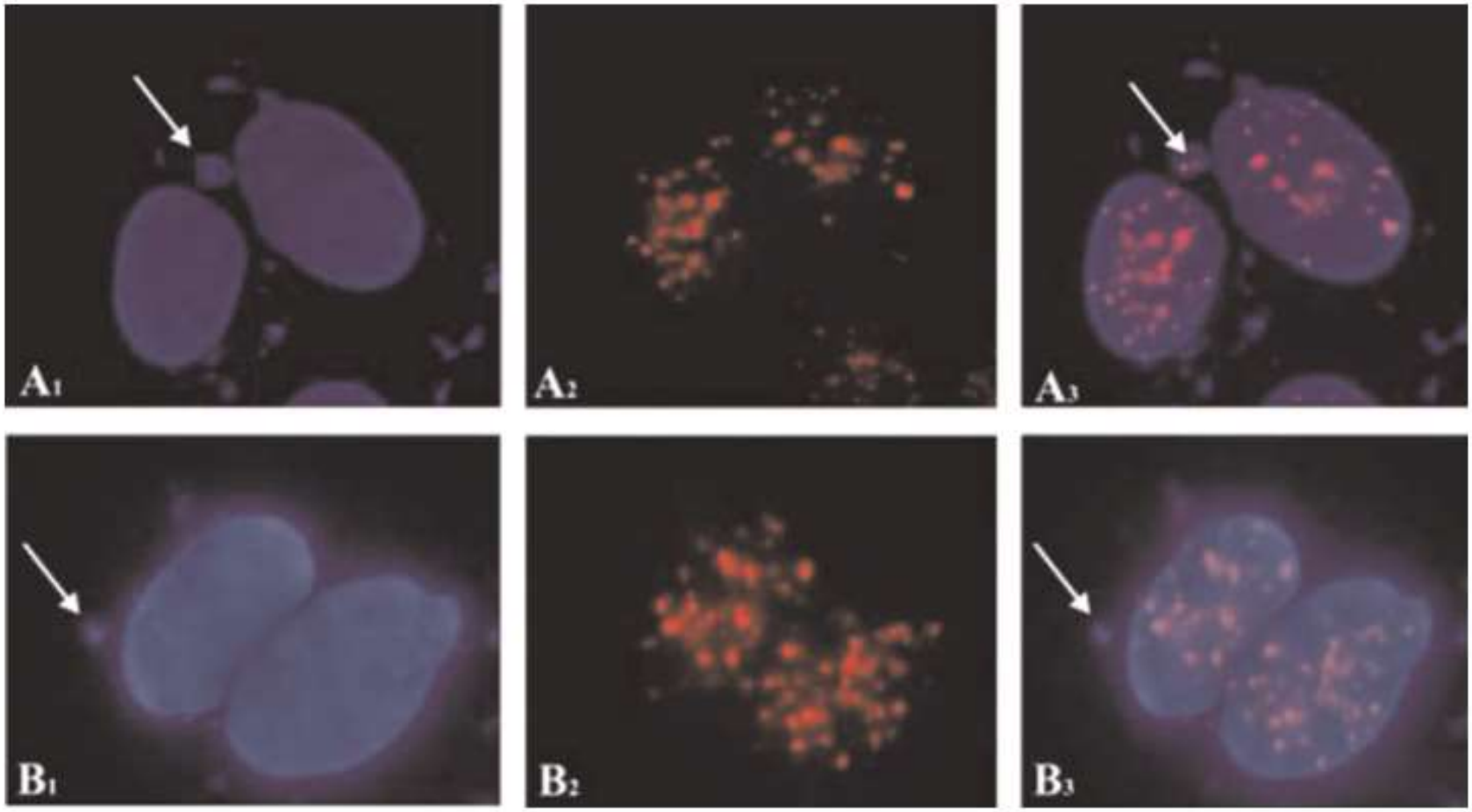


Fig. 3. HepG2 cell treated with the herbicide sulfentrazone, submitted to the FISH technique: (A_1 and B_1) binucleated cells with one micronucleus (arrow) stained with DAPI; (A_2 and B_2) location of the centromeres of the chromosomes labelled with rhodamine (red points); (A_3) overlay of the images A_1 and A_2 showing the location of the centromere within the MN (arrow: indicative of aneugenicity); (B_3) overlapping of the images B_1 and B_2 showing absence of centromere within the MN (indicative of clastogenicity). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

RESULTADOS

Results of the fluorescent in situ hybridization with centromeric probe in HepG2 cells exposed to insecticide imidacloprid, herbicide sulfenrazone and the mixture of these two pesticides.

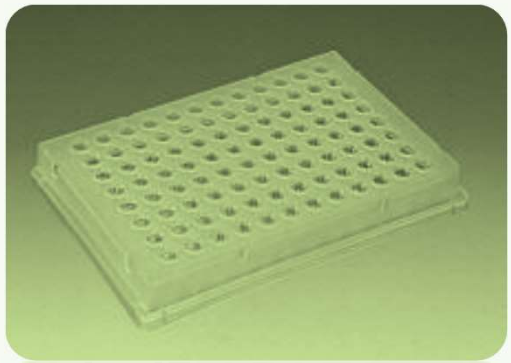
	Number of micronucleated cells analyzed	Number of MNC+	Number of MNC−	% MNC+	% MNC−
Imidacloprid	50	12	38	24	76
Sulfentrazone	50	24	26	48	52
Mixture	50	26	24	52	48

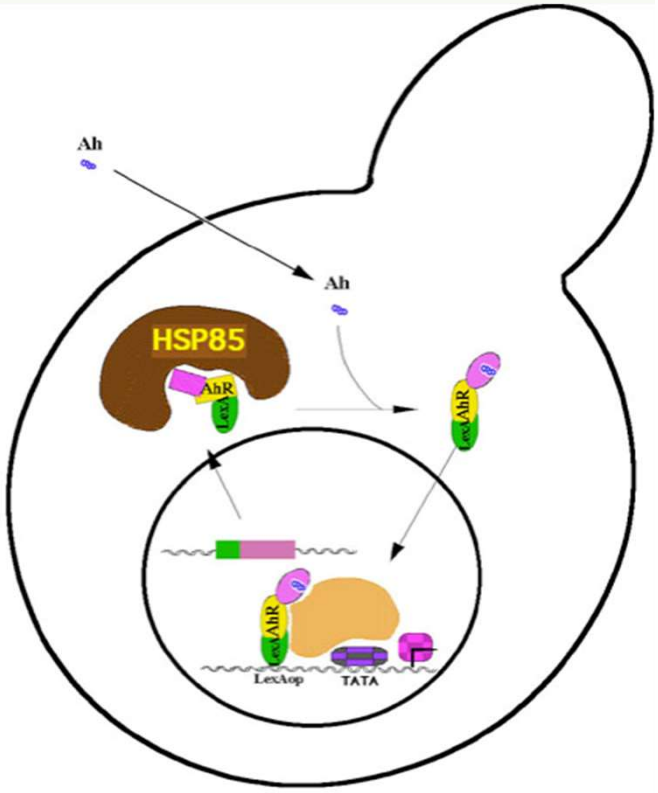
MNC+: micronucleus showing centromere signal. MNC−: micronucleus without centromere signal.

SISTEMA-TESTE

Ensaio com leveduras recombinantes

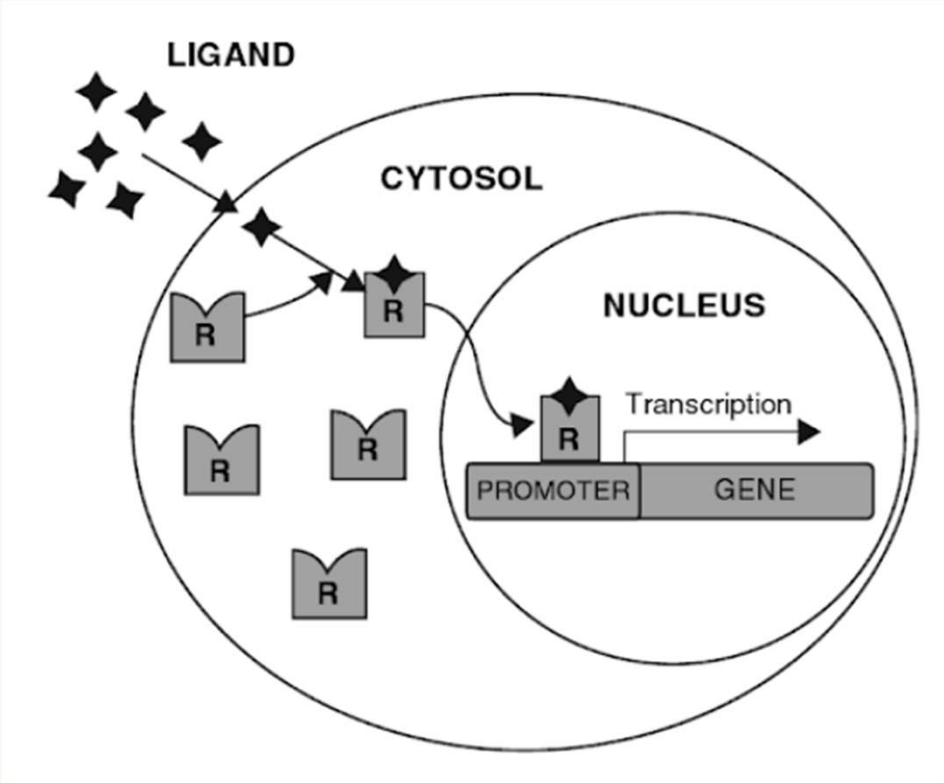
- Sítios de ligação para poluentes específicos
- Alteradores endócrinos
- Gene repórter = *lacZ*
- Quantificação = β-galactosidase + substrato fluorescente





SISTEMA-TESTE

Ensaio com leveduras recombinantes



RECEPTORES NUCLEARES

HORMONAIS

Estrogenic Androgenic

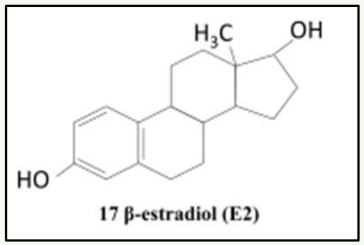
Retinoic-like Thyroid

Glucocorticoid

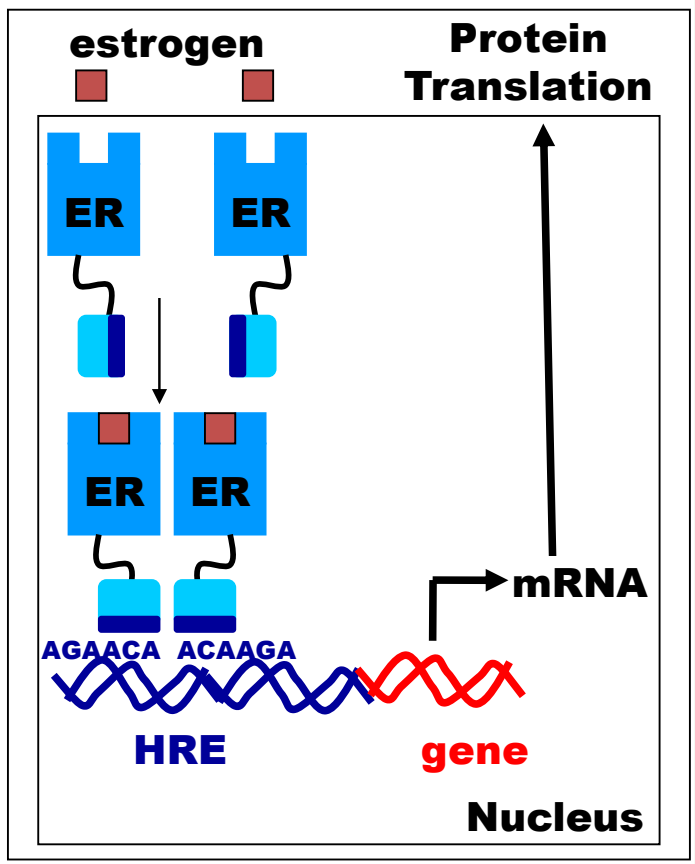
Mineralocorticoid

Vitamin D Progestins

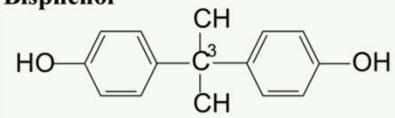
SISTEMA-TESTE



17 β-estradiol (E2)

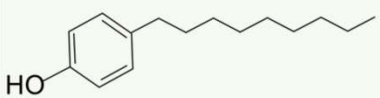


Bisphenol

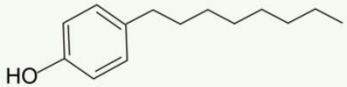


Bisphenol A (BPA)

Alkylphenols

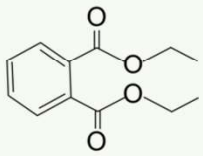


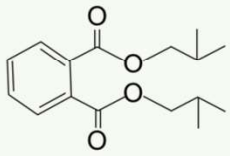
4-n-Nonyphenol (4NP)

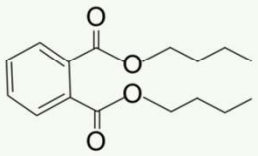


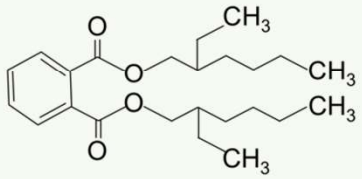
4-n-Octylphenol (OP)

Phthalates


Diethyl phthalate (DEP)

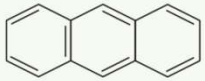

Diisobutyl phthalate (DiBP)

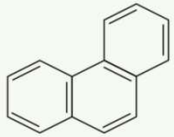

Dibutyl phthalate (DnBP)

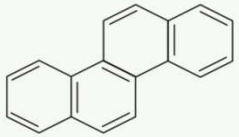


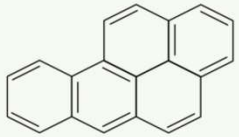
Di (2-ethylhexyl) phthalate (DEHP)

Polycyclic Aromatic Hydrocarbons

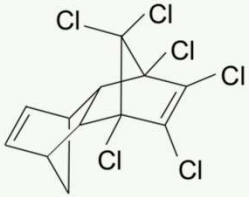

Anthracene (ANT)


Phenanthrene (PHE)


Chrysene (CHR)

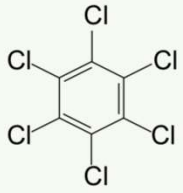

Benzo[a]pyrene (B[a]P)

Agrochemicals

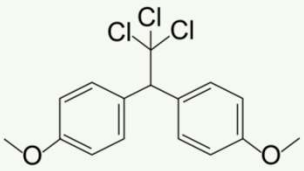


Aldrin

Dichlorodiphenyltrichloroethane (o, p' - DDT)



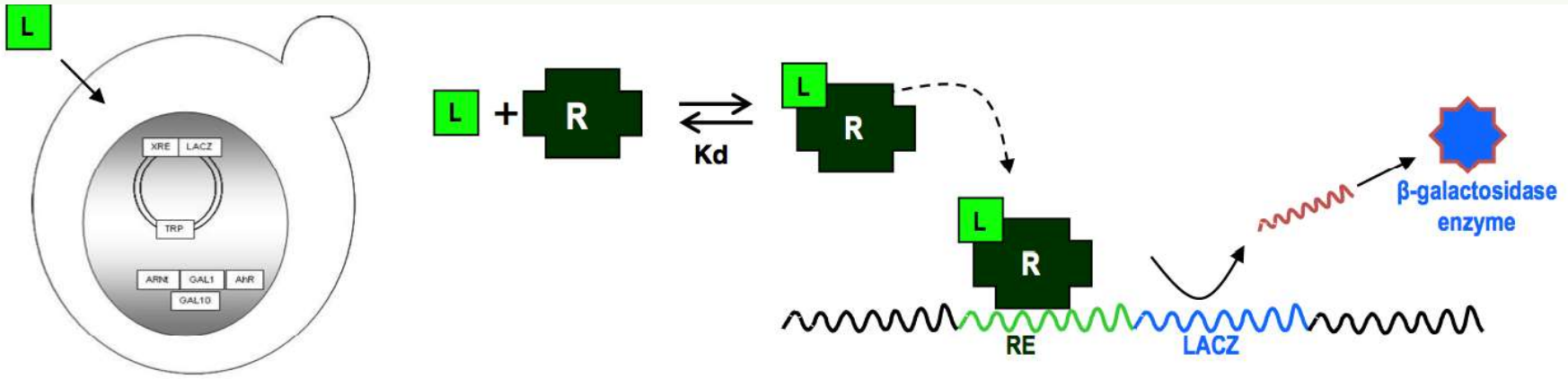
Hexachlorobenzene (BHC)



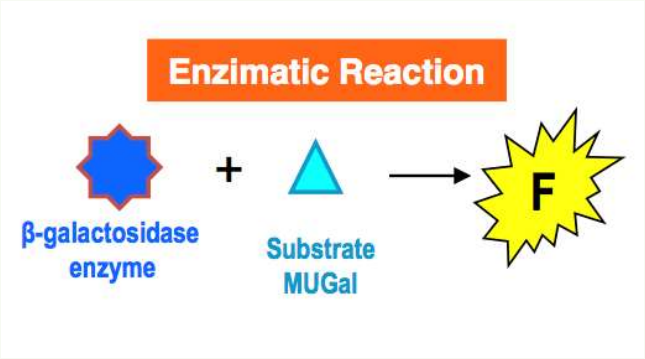
Methoxychlor

SISTEMA-TESTE

Ensaio com leveduras recombinantes

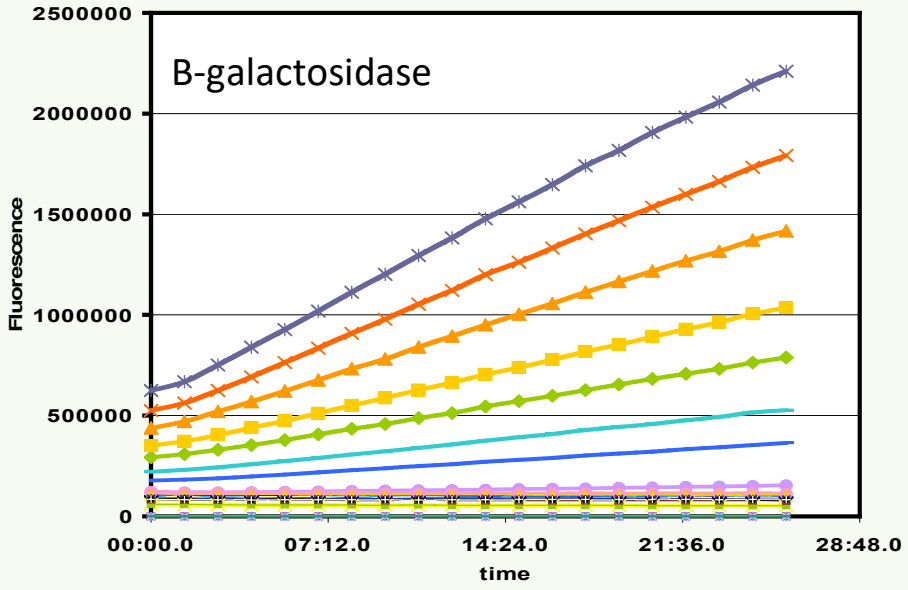


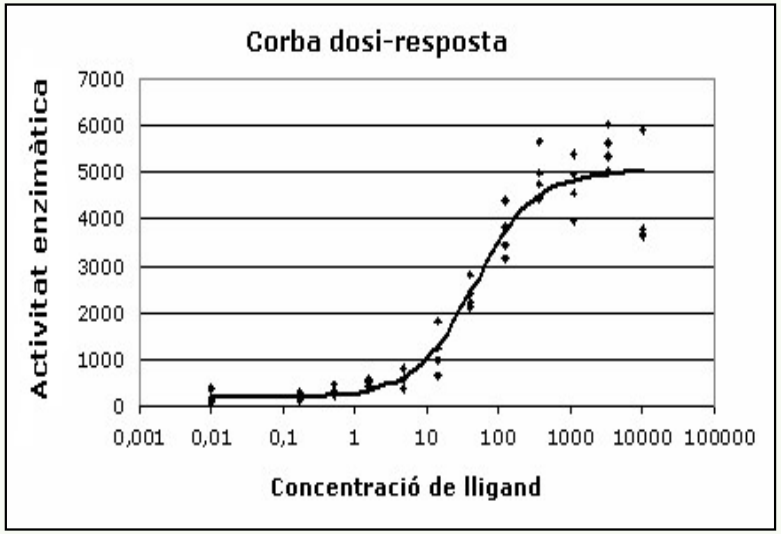
R vertebrate nuclear hormone receptors: Estrogen receptor (estrogenic activity) or Aryl hidrocarbon receptor (dioxin-like activity)



SISTEMA-TESTE

Ensaio com leveduras recombinantes





EXEMPLO

sustainability

MDPI

Article

Endocrine Disruption, Cytotoxicity and Genotoxicity of an Organophosphorus Insecticide

Afifa Belaid [1], Nosra Methneni [1], Emna Nasri [1], Sarra Bchir [1], Roel Anthonissen [2], Luc Verschaeve [2], Véronique Le Tilly [3], Vincenzo Lo Turco [4], Giuseppa Di Bella [4], Hedi Ben Mansour [1,*] and Nezar H. Khdary [5,*]

1 Research Unit of Analysis and Process Applied to the Environment, Higher Institute of Applied Sciences and Technology, Mahdia 5100, Tunisia; afifa.belaid@gmail.com (A.B.); methneninosra28@gmail.com (N.M.); emmna.nasri@gmail.com (E.N.); sarra.bchir@yahoo.fr (S.B.)

2 Scientific Direction Chemical and Physical Health Risks, Sciensano, 1050 Brussels, Belgium; Roel.Anthonissen@sciensano.be (R.A.); Luc.Verschaeve@sciensano.be (L.V.)

3 IRDL FRE CNRS 3744, Université Bretagne Sud, 56100 Lorient, France; veronique.le-tilly@univ-ubs.fr

4 BioMorf Department, University of Messina, 98168 Messina, Italy; vincenzo.loturco@unime.it (V.L.T.); giuseppa.dibella@unime.it (G.D.B.)

5 King Abdulaziz City for Science and Technology (KACST), Riyadh 11442, Saudi Arabia

* Correspondence: hdbenmansour@gmail.com (H.B.M.); nkhdary@kacst.edu.sa (N.H.K.)

EXEMPLO

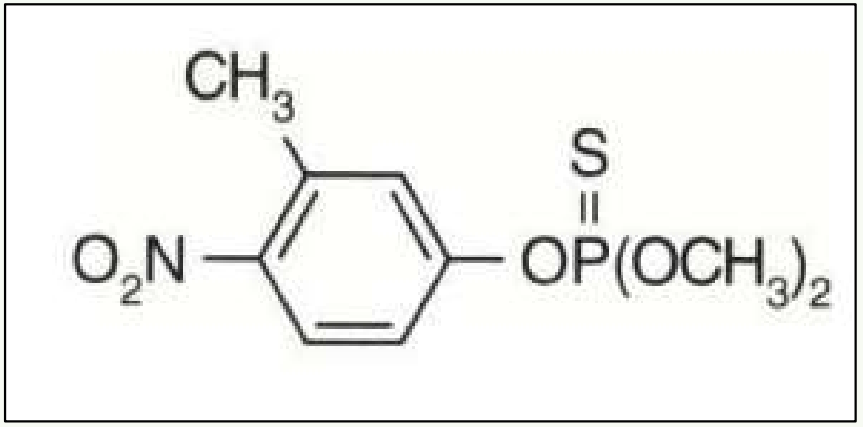
Agrotóxico testado:

- 5 g/L
- 2,5 g/L
- 1,25 g/L

(residuais)

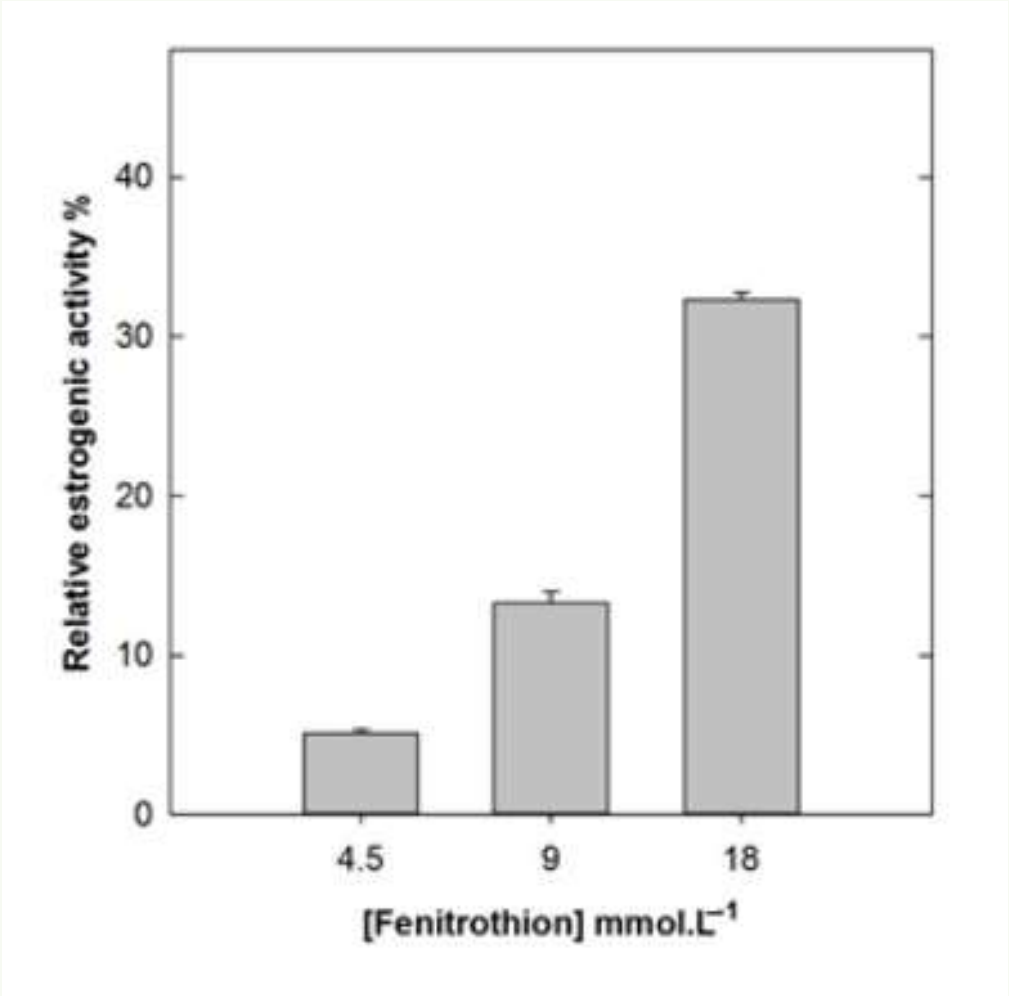
Fenitrotiona

Inseticida



Grupo químico:
Organofosforado

EXEMPLO



18 mmol/L = 1,35 x 10^{-9} mol/L de estradiol (E2) equivalente – 32%

OBRIGRADA!

Profa. Dra. Dânia Elisa Christofoletti Mazzeo Morales

Depto Biotecnologia e Produção Vegetal e Animal

Centro de Ciências Agrárias - UFSCAR

daniamazzeo@ufscar.br