



Departamento de Biología Aplicada



Unidad de Toxicología y Seguridad Química

**CHROMAFFIN CELLS IN CULTURE AS IN  
VITRO ALTERNATIVE MODEL TO STUDY  
THE ORGANOPHOSPHORUS INDUCED  
DELAYED NEUROPATHY**

***Dr. Encarna Quesada***

# Organophosphorus compounds (OPs)

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► Quimical compounds mainly used in agriculture as ***INSECTICIDES***

► Their best known toxic effects are found at ***NERVOUS SYSTEM*** level

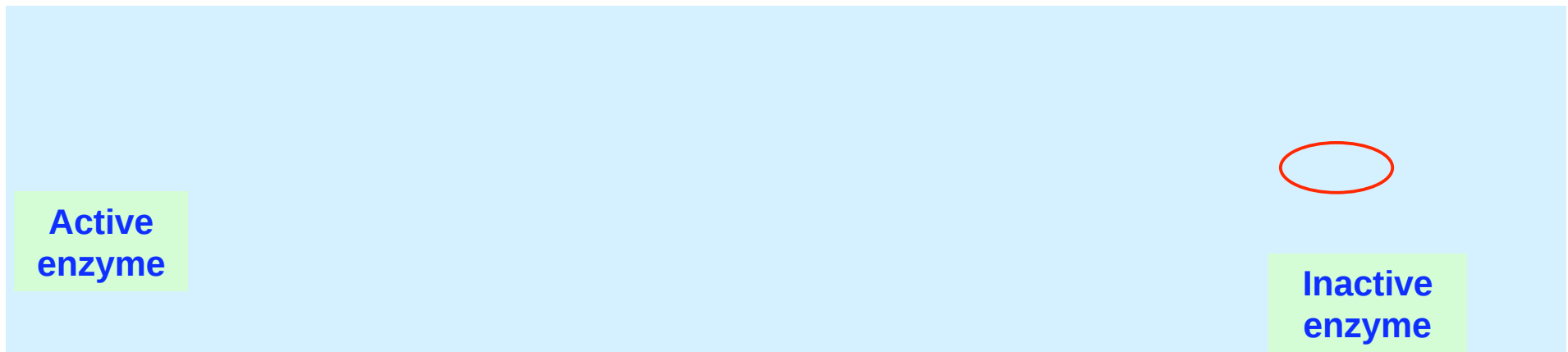


- Lipophilic nature
- Hydrolizable esters
- Pseudo-substrates for esterases

# OPs-esterase interaction

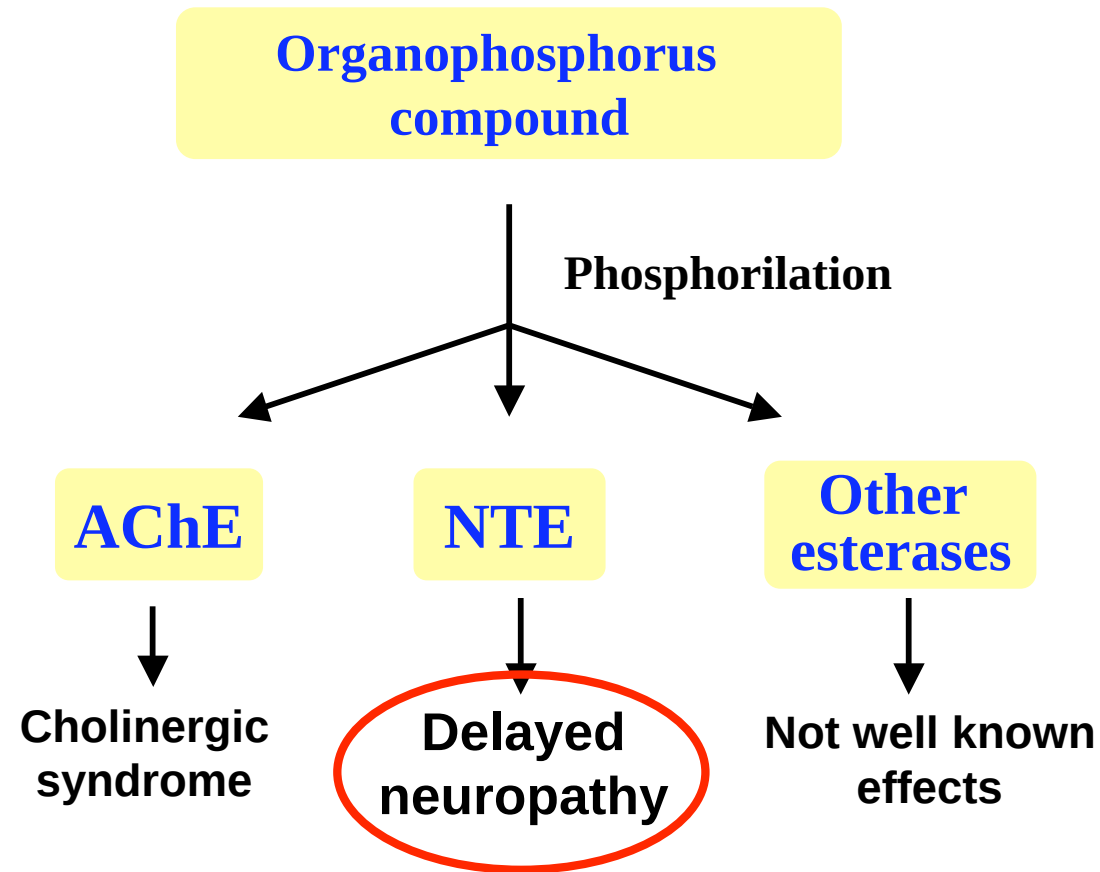


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- (1) Formation of the Michaelis complex
- (2) Phosphorilation of the target enzyme

# OPs-esterase interaction





## Global approach



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Development of a suitable cellular system capable of discriminating in vitro compounds causing delayed neuropathy.

# Material and Methods



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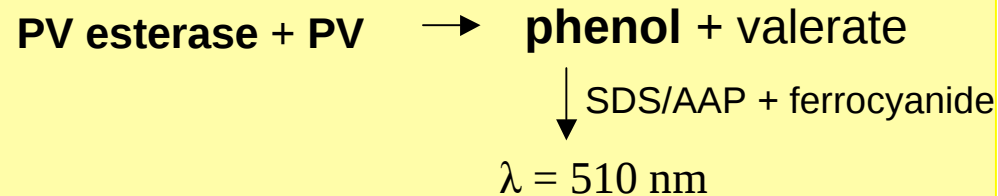
## 1) OPs compounds

**paraoxon**: do not inhibit NTE

**mipafox**: do inhibit NTE

**HDCP**: do inhibit NTE

## 2) Phenyl valerate esterase activity



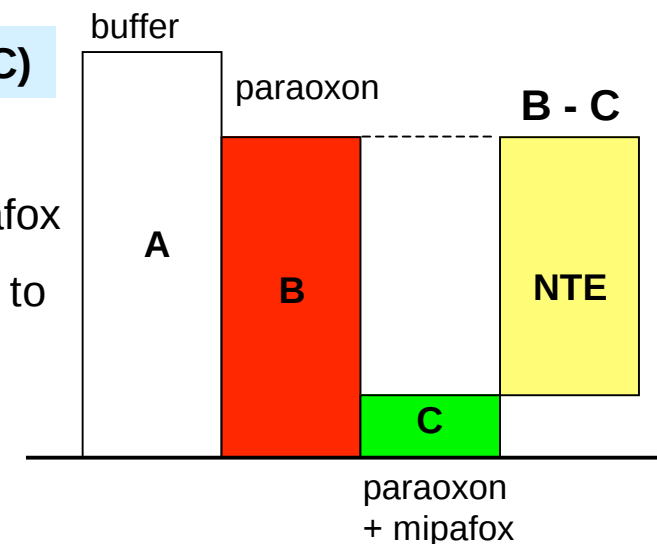
**A**: Total PV esterase activity

(30 min, 37°C)

**B**: PV esterase resistant to 40  $\mu\text{M}$  paraoxon

**C**: PV esterase resistant to 40  $\mu\text{M}$  paraoxon and 250  $\mu\text{M}$  mipafox

**NTE**: PV esterase resistant to 40  $\mu\text{M}$  paraoxon and sensitive to 250  $\mu\text{M}$  mipafox (B-C)





# RESULTS

- I **Characterization of PV esterase activity in adrenal medulla homogenized**
- II **Characterization of PV esterase activity in bovine chromaffin cells in culture**
- III **Modulation of NTE activity by means of adenoviral vector**

## Results

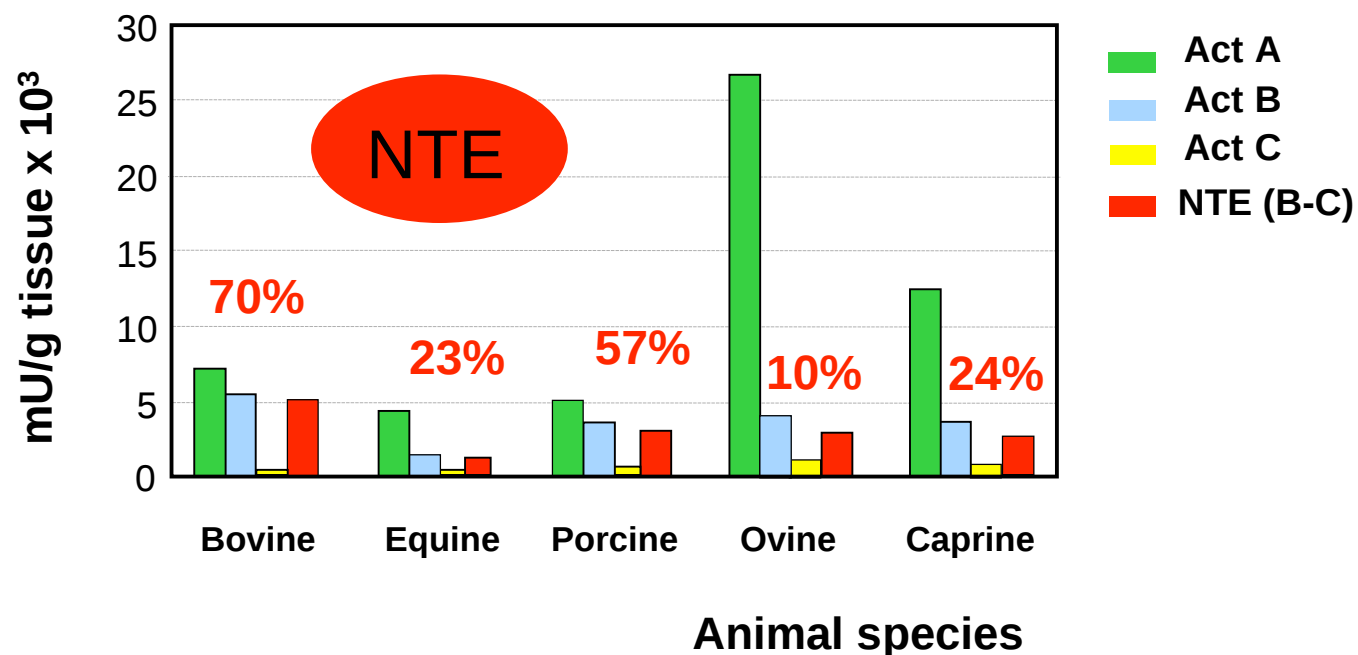


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PV esterase activities in **particulate fraction** of adrenal medulla of several species

A, B, C and NTE activity (mU/g tissue)



D. Romero, E. Quesada, MA. Sogorb, AJ. García-Fernandez, E. Vilanova, V. Carrera (2006). Comparison of chromaffin cells from several animal sources for their use as an *in vitro* model to study the mechanism of organophosphorous toxicity. *Tox. Lett.* 165:221-229.



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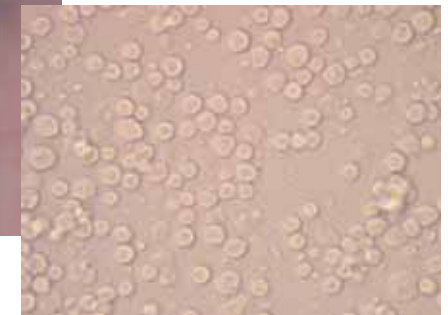
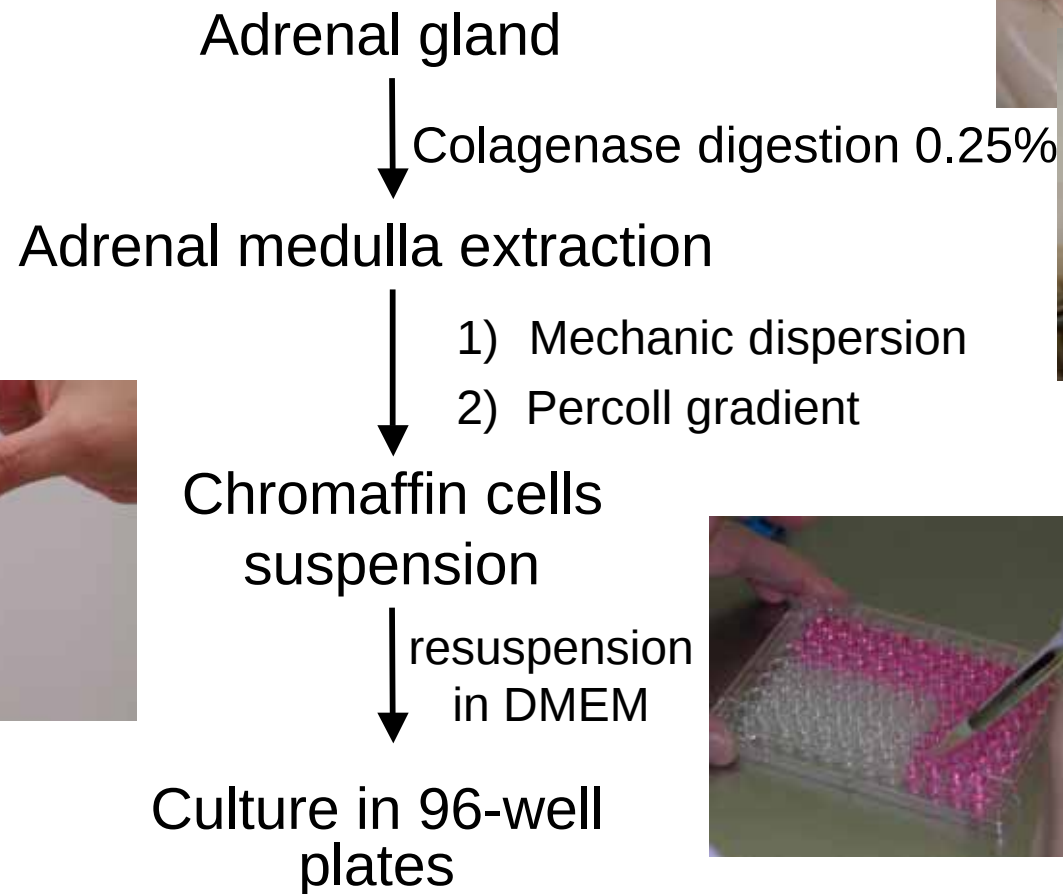
# Material and methods



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## Culture of chromaffin cells of adrenal medulla



## Results

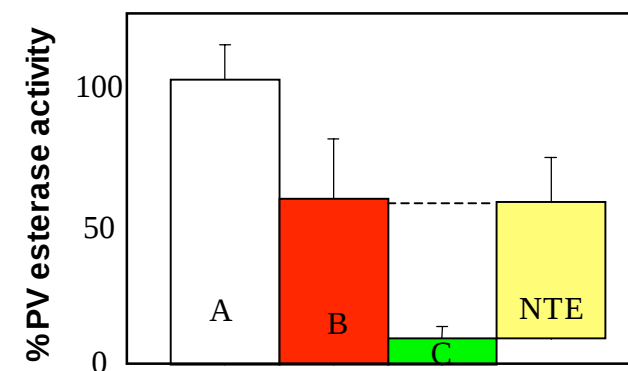


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### PV esterase activity levels in bovine chromaffin cells in culture

| Activity | mU/10 <sup>6</sup> cells | %   |
|----------|--------------------------|-----|
| A        | 22.0 ± 2.7               | 100 |
| B        | 14.2 ± 3.2               | 65  |
| C        | 2.2 ± 0.7                | 10  |
| NTE      | 12.0 ± 3.0               | 55  |



media ± standard deviation for n=4 independent experiments performed by quadruplicate is indicated.

*E. Quesada, M.A. Sogorb, E. Vilanova and V. Carrera (2004). Bovine chromaffin cell cultures as model to study organophosphorus neurotoxicity. Toxicol. Lett. 151: 163-170.*

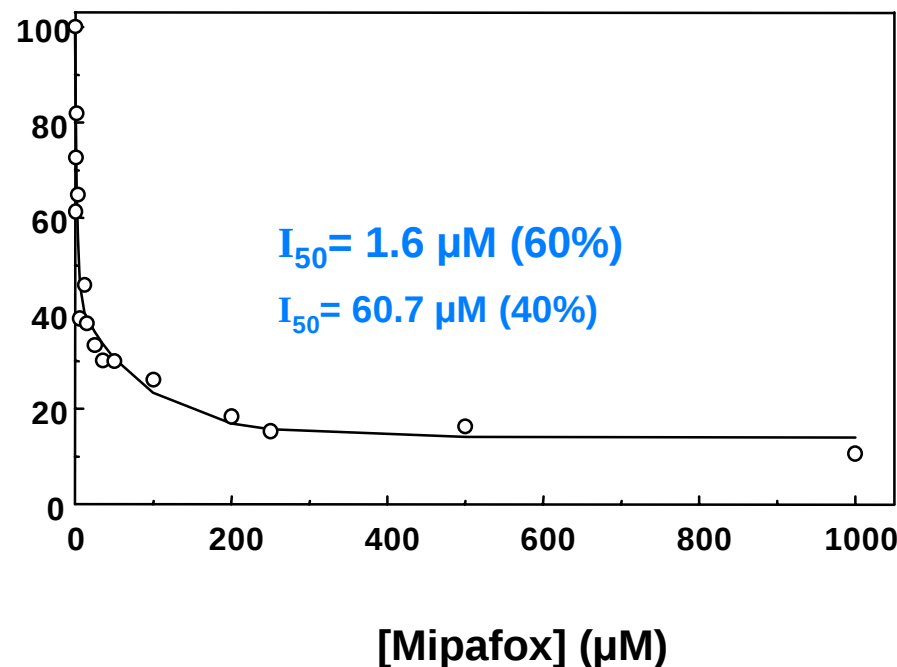
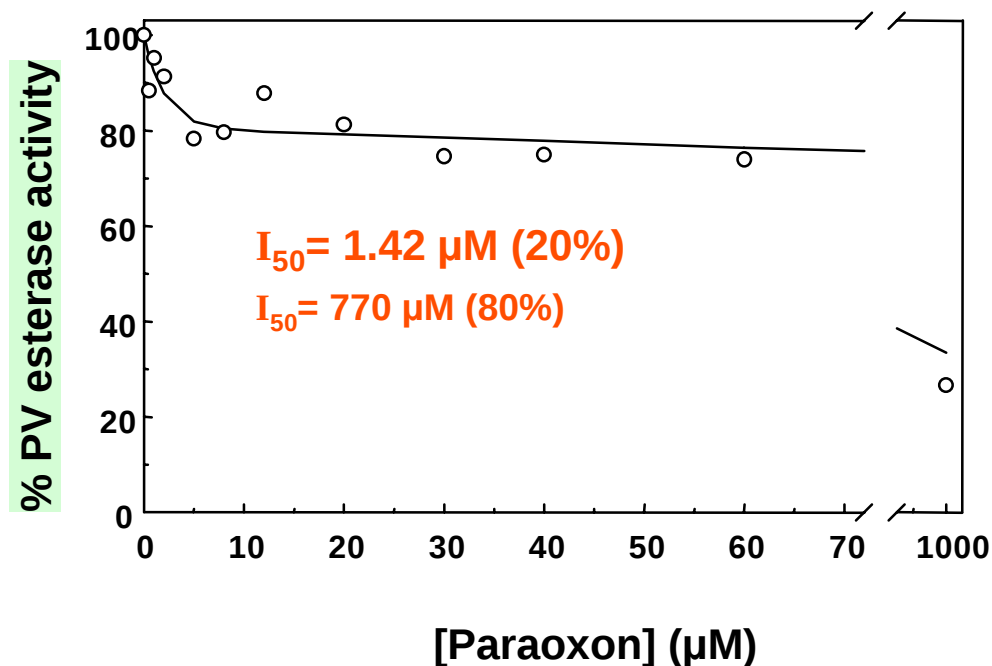
# Results



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Inhibition curves at fixed time (30 min) of **total PV esterase** activity by paraoxon and mipafox in bovine chromaffin cells in culture



$$\% \text{ Actividad} = A_1 e^{-k_1 \cdot I \cdot t} + A_2 e^{-k_2 \cdot I \cdot t} + A_r$$

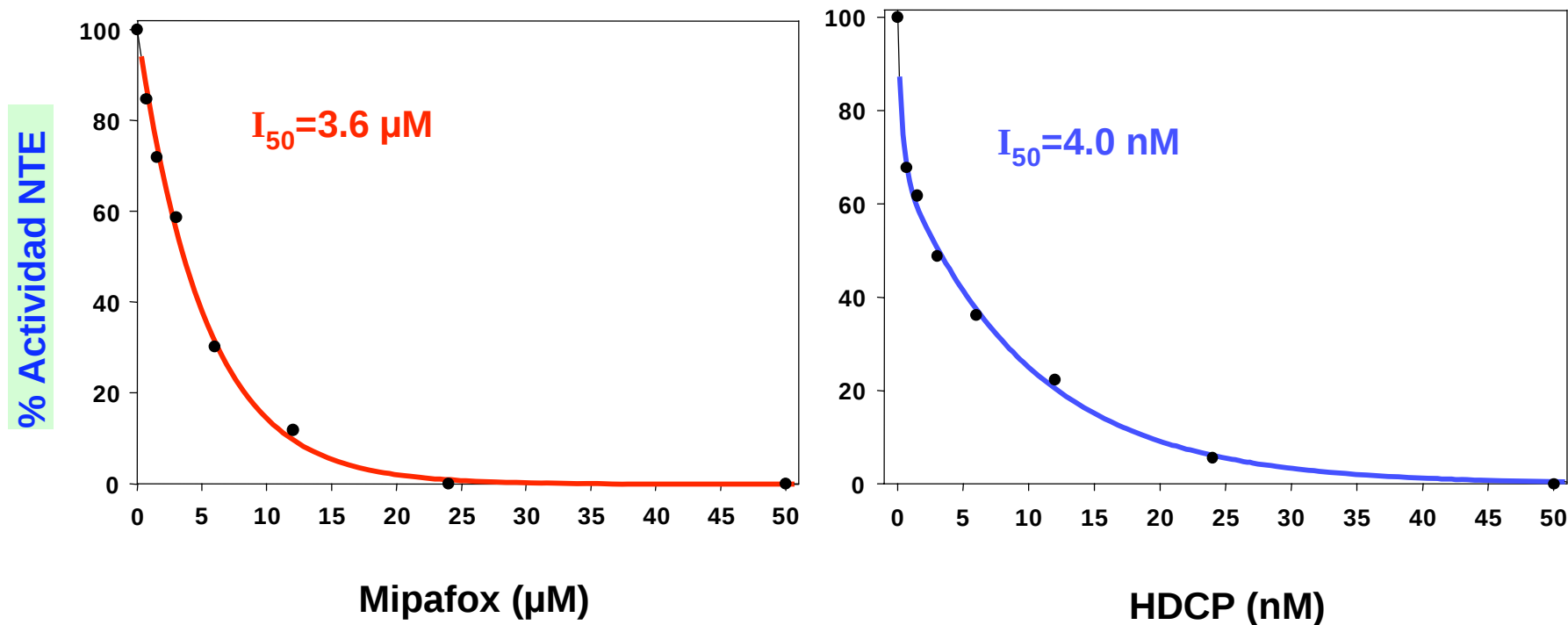
# Resultados



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Inhibition curves at fixed time (60 min) of **NTE** activity by mipafox or HDCP in bovine chromaffin cells in culture



$$\% \text{ Actividad} = A_1 e^{-k_1 \cdot I \cdot t}$$



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I

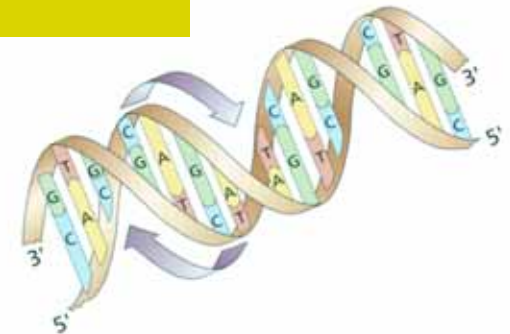
Characterization of PV esterase activity  
in adrenal medulla homogized

II

Characterization of PV esterase activity  
in bovine chromaffin cells in culture

III

**Modulation of NTE activity by means of  
adenoviral vector**



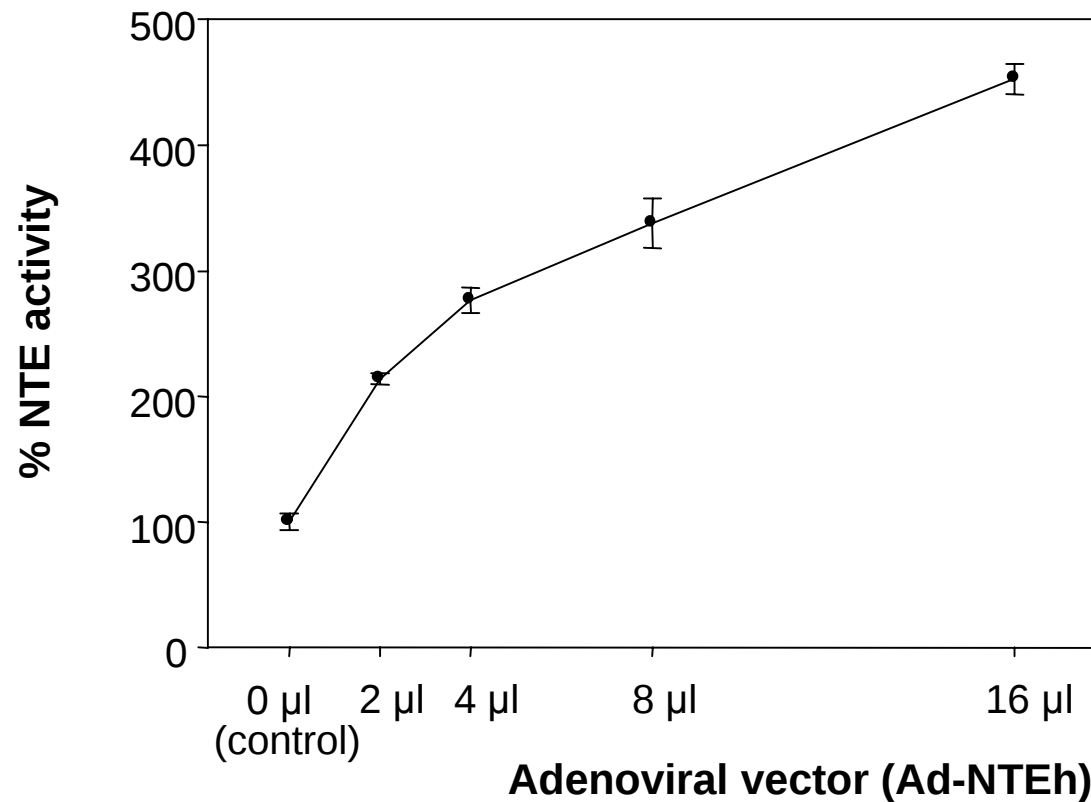
# Results



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**NTE** activity in bovine chromaffin cells in culture after incubation with several volumes of adenoviral vector (Ad-NTEh)



*E. Quesada, J.V. Castell, E. Vilanova, V. Carrera, (2005). "Vector plasmídico de la esterasa diana de neuropatía humana en cultivo primario de células cromafines bovinas". Rev. Toxicol. 22(2):97.*

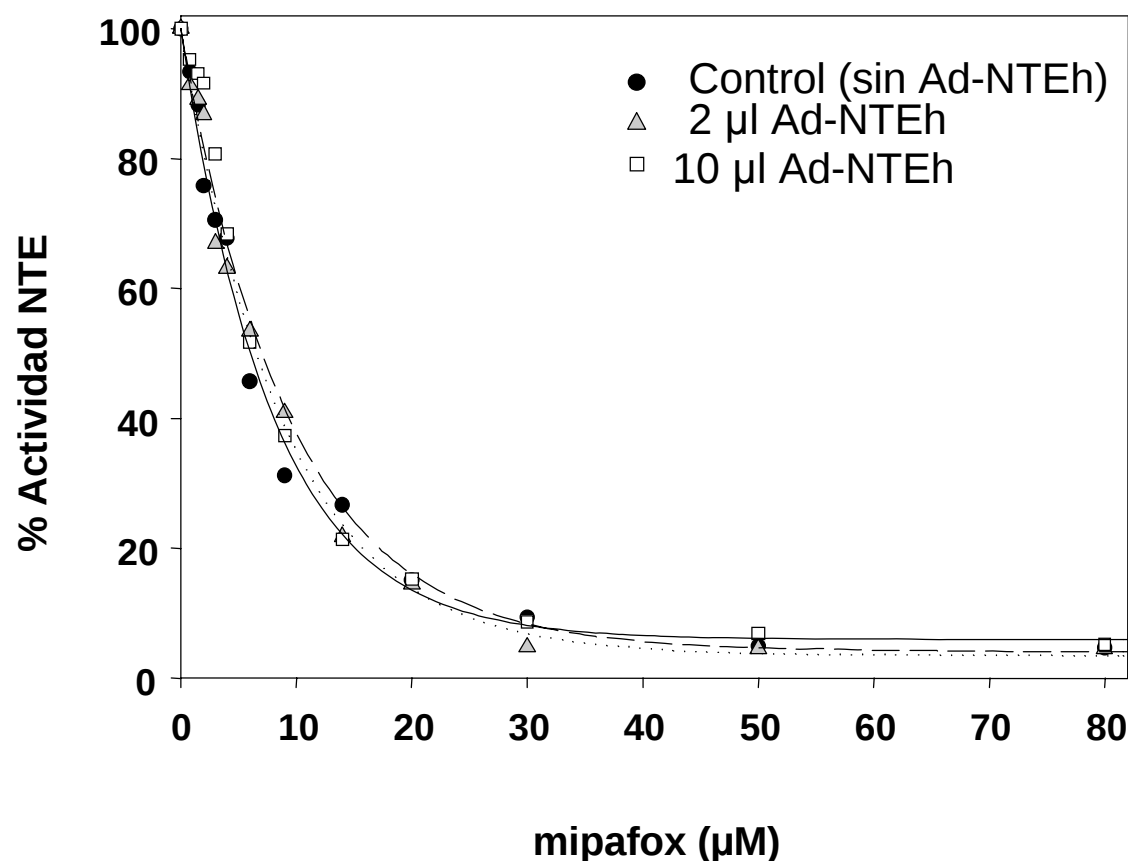
# Resultados



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Inhibition curves at fixed time (60 min) of NTE activity by mipafox in bovine chromaffin cells in culture incubated with Ad-NTEh



| Ad-NTEh        | I <sub>50</sub> (µM) |
|----------------|----------------------|
| 0 µl (control) | 5.5                  |
| 2 µl           | 6.2                  |
| 10 µl          | 6.6                  |

$$\% \text{ Actividad} = A_1 e^{-k_1 \cdot I \cdot t} + A_r$$



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## CONCLUSIONS

Results shown in this work support the use of chromaffin cells as a suitable cellular system for the in vitro assessment of compounds capable of inducing delayed neuropathy

The modulation of NTE activity will provide screening systems to design chemical compounds with a minimal delayed neuropathy potential



*Thank you  
for your attention*

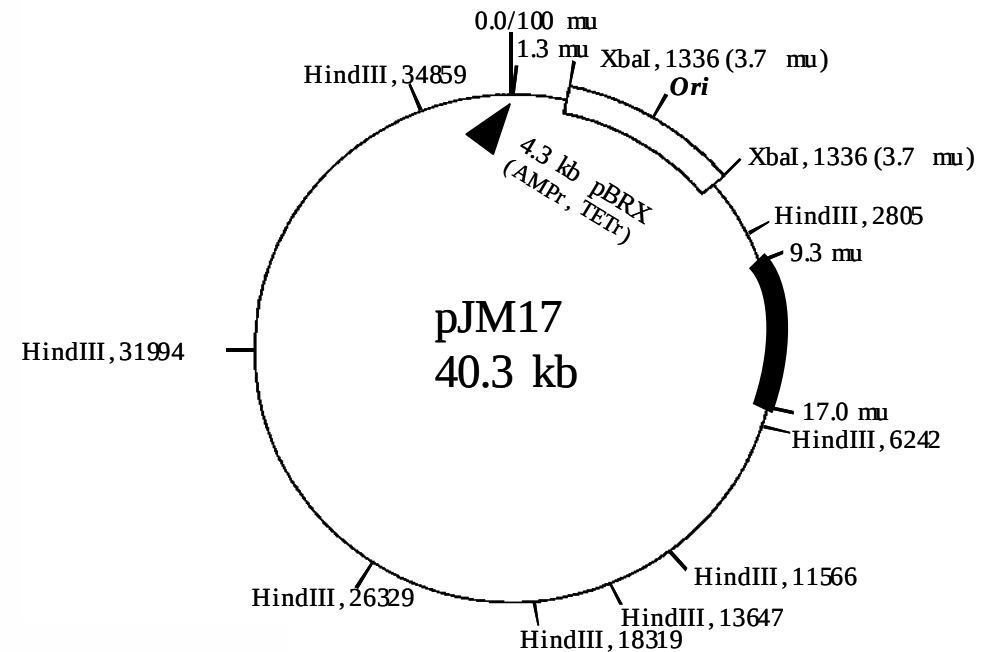
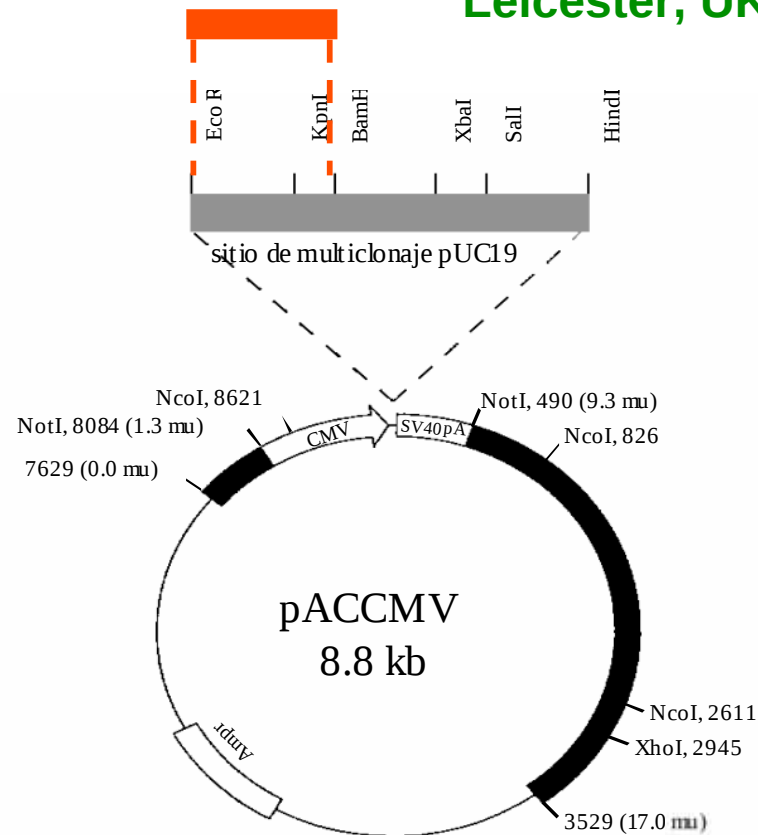
# Homologous recombination



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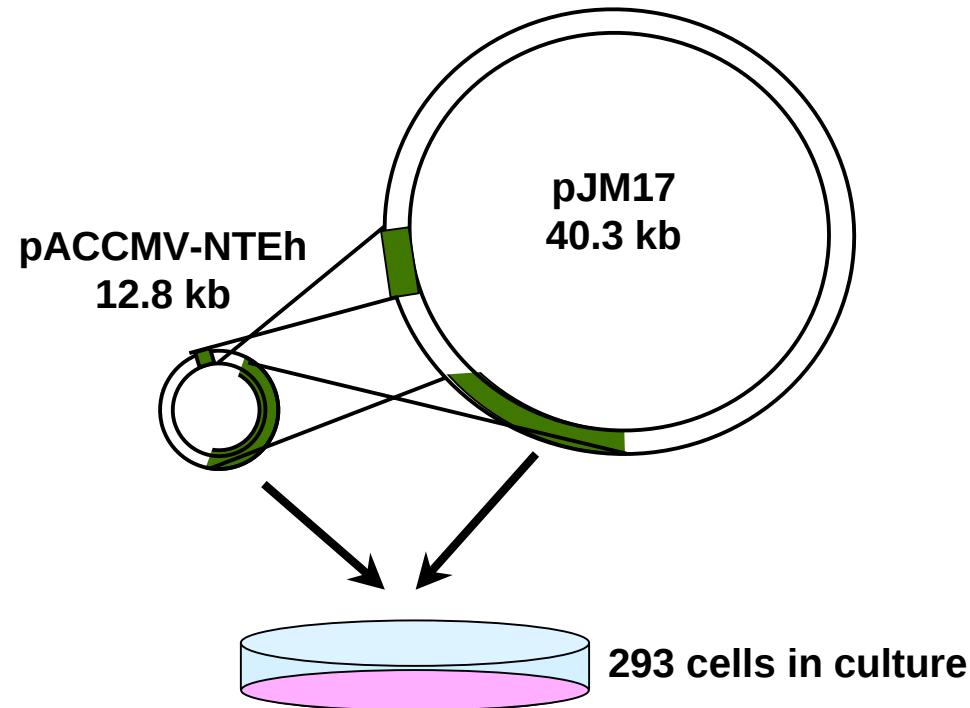
**cDNA NTE h** (Paul Glynn,  
Leicester, UK)



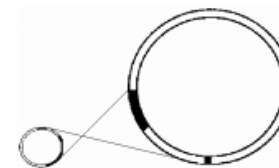
# Homologous recombination



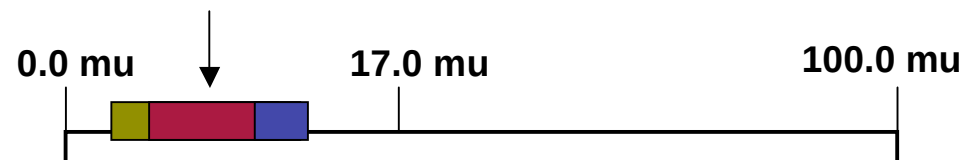
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Transcription cassette with  
CMV promoter containing  
NTEh cDNA



•Recombination event  
•replication



**Recombinant Adenovirus**

