

## Use of EST2 from *A. acidocaldarius* as bioactive part in biosensors for organophosphates detection

Ferdinando Febbraio

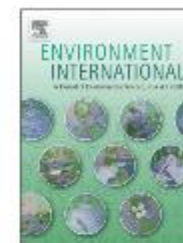
Institute of Protein Biochemistry of CNR, Italy



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# Prenatal exposure to organophosphate pesticides and reciprocal social behavior in childhood<sup>☆</sup>



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### ABSTRACT

Prenatal exposure to organophosphate pesticides (OPs) has been associated with adverse neurodevelopmental outcomes in childhood, including low IQ, pervasive developmental disorder (PDD), attention problems and ADHD. Many of these disorders involve impairments in social functioning. Thus, we investigated the relationship between biomarkers of prenatal OP exposure and impaired reciprocal social behavior in childhood, as measured by the Social Responsiveness Scale (SRS). Using a multi-ethnic urban prospective cohort of mother–infant pairs in New York City recruited between 1998 and 2002 ( $n = 404$ ) we examined the relation between third trimester maternal urinary levels of dialkylphosphate ( $\Sigma$ DAP) OP metabolites and SRS scores among 136 children who returned for the 7–9 year visit. Overall, there was no association between OPs and SRS scores, although in multivariate adjusted models, associations were heterogeneous by race and by sex. Among blacks, each 10-fold increase in total diethylphosphates ( $\Sigma$ DEP) was associated with poorer social responsiveness ( $\beta = 5.1$  points, 95% confidence interval (CI) 0.8, 9.4). There was no association among whites or Hispanics, or for total  $\Sigma$ DAP or total dimethylphosphate ( $\Sigma$ DMP) biomarker levels. Additionally, stratum-specific models supported a stronger negative association among boys for  $\Sigma$ DEPs ( $\beta = 3.5$  points, 95% CI 0.2, 6.8), with no notable association among girls. Our results support an association of prenatal OP exposure with deficits in social functioning among blacks and among boys, although this may be in part reflective of differences in exposure patterns.

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Keywords:

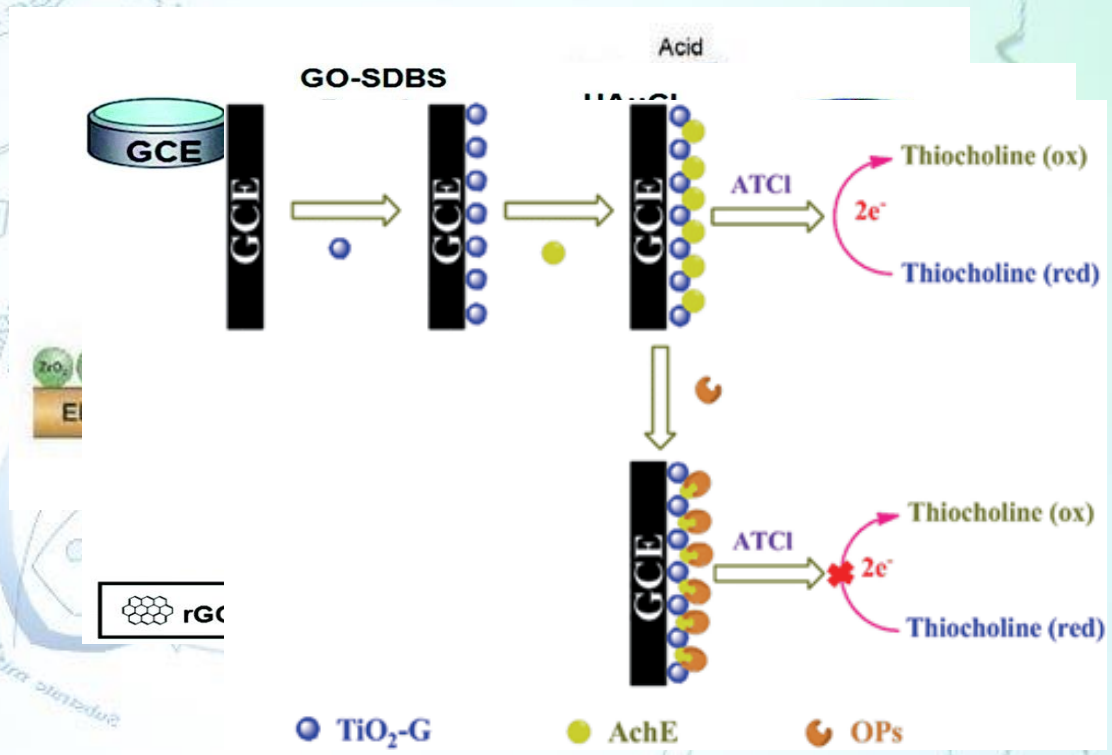
same vehicles. Human skin was mounted in flow through diffusion cells with minimum essential was not associated with ever using any pesticide. Use of individual pesticides or functional or chemical

# Organophosphates detection

## AChE biosensors

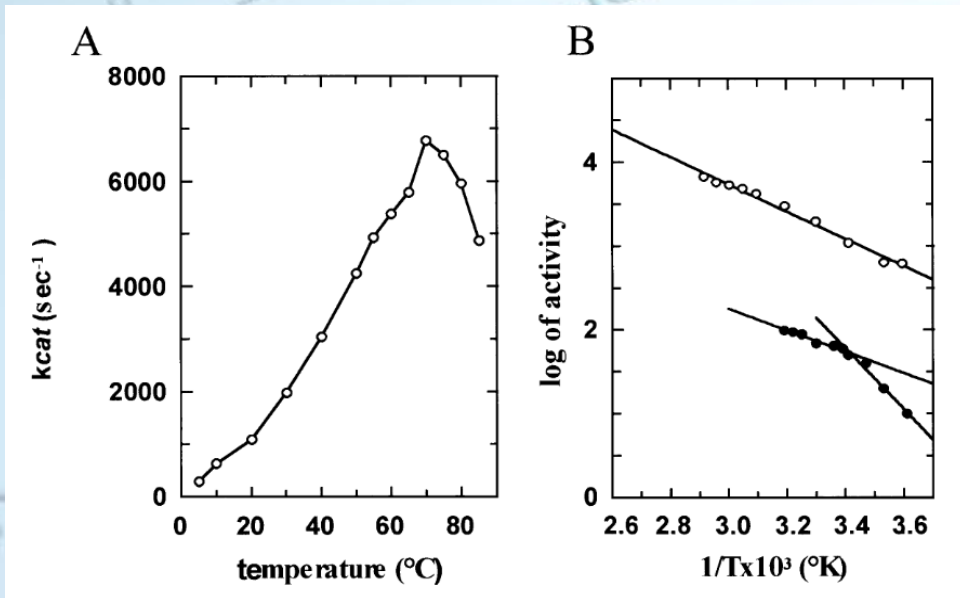


GC-MS  
HPLC-MS  
UPLC-ESMS



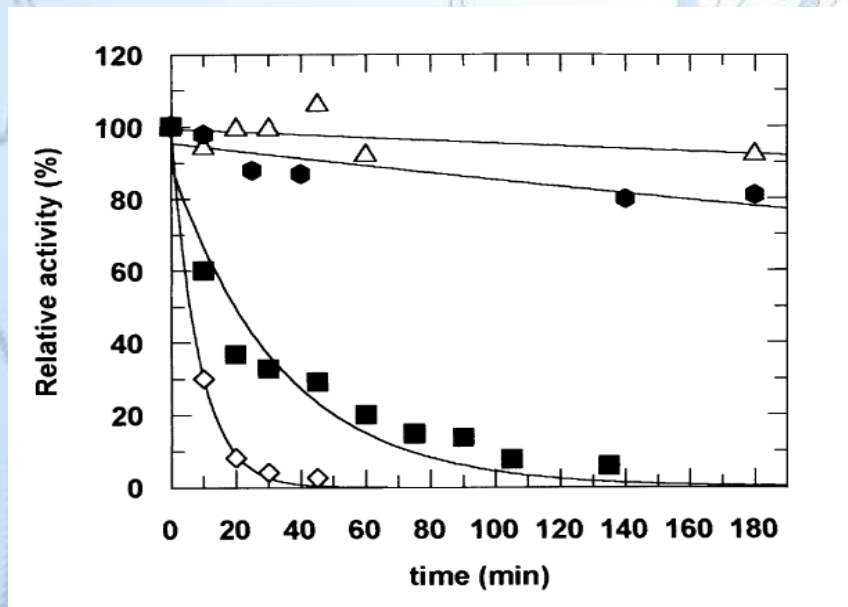
High specificity over a better analytical performance than traditional systems  
 But it has a few disadvantages  
 It is not suitable for large scale detection  
 It is not suitable for large number of samples (e.g. food, water, etc.)  
 Real time detection  
 Requires trained personnel

# Esterase 2 from *Alicyclobacillus acidocaldarius*

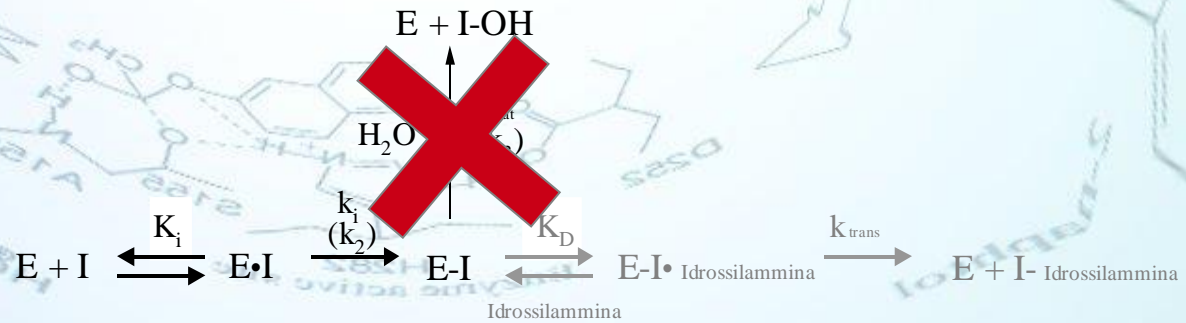
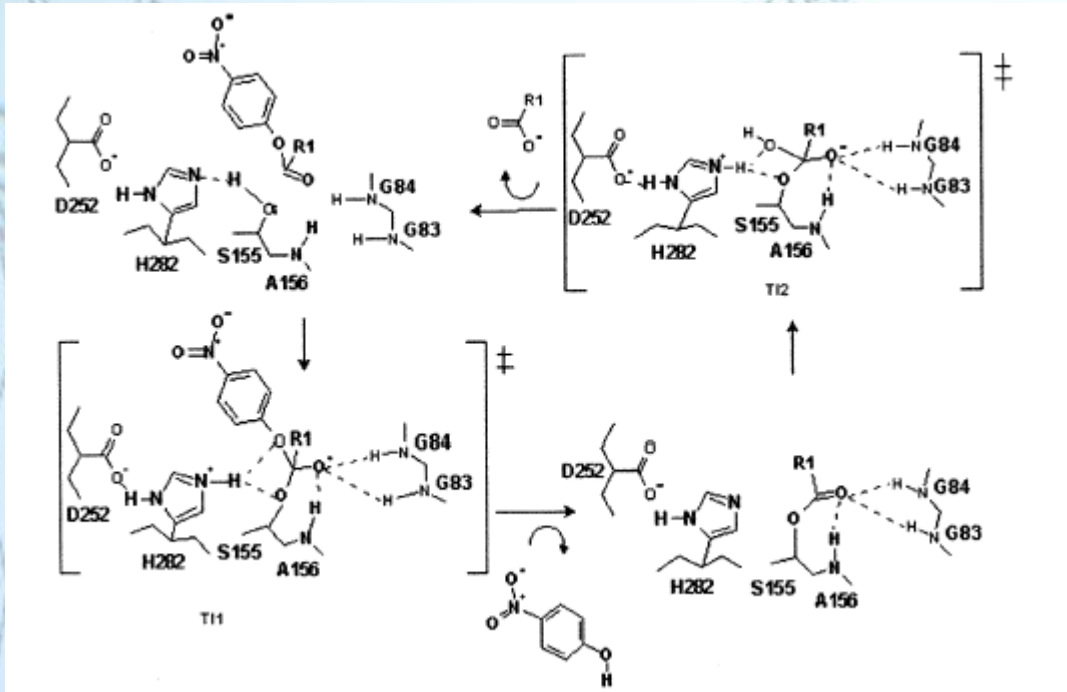


EST2 is a monomeric carboxylesterase of about 34 Kda belonging to the hormone sensitive lipase (HSL) family.

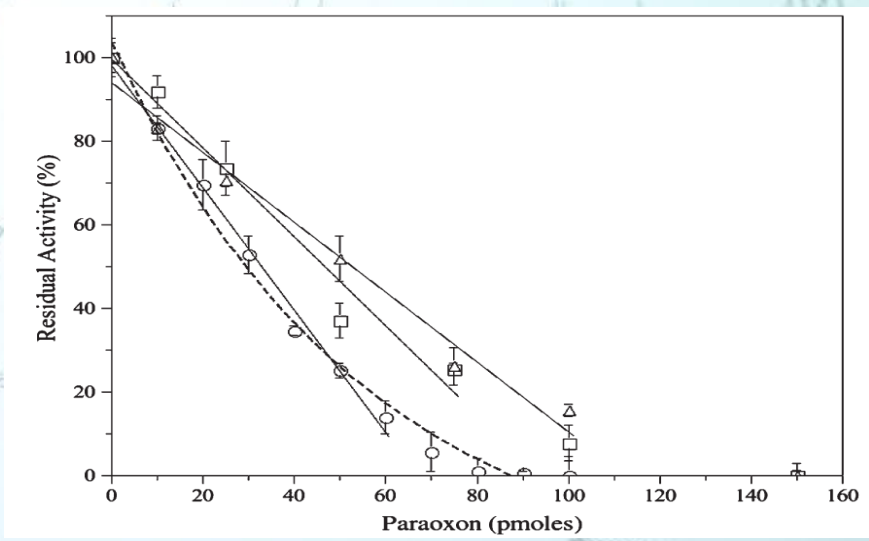
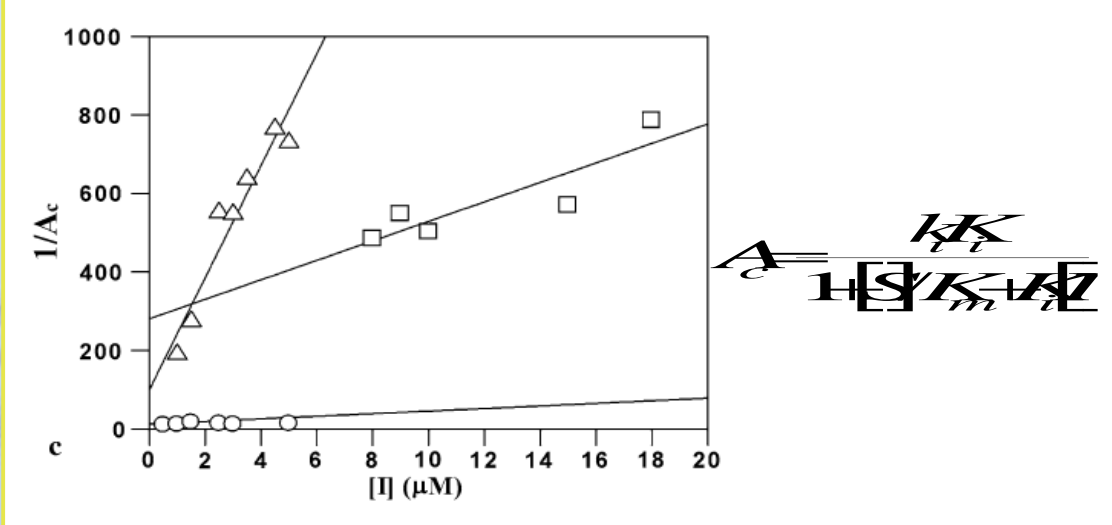
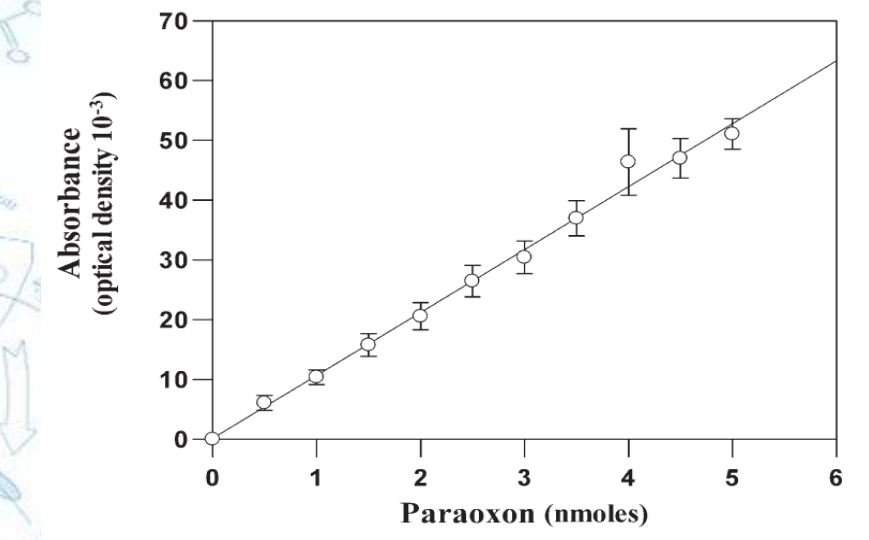
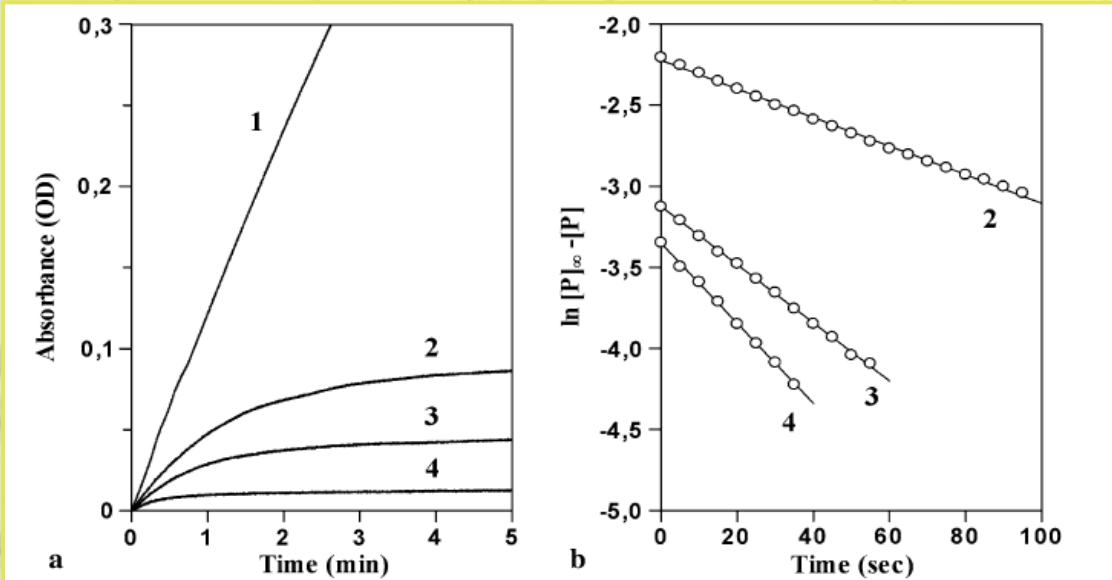
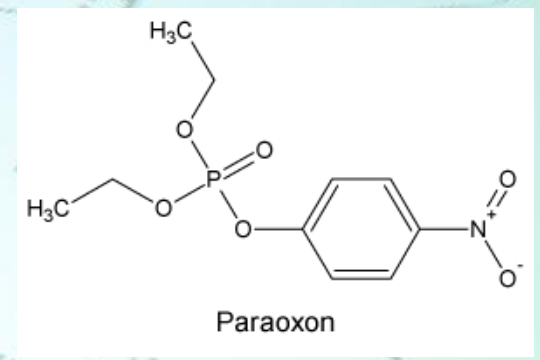
EST2 hydrolyses monoacyl esters of different acyl chain lengths, displaying maximal activity on p-nitrophenyl esters characterized by an acyl chain length of 6-8 carbon atoms, at an optimal temperature of 70  $^{\circ}\text{C}$ .



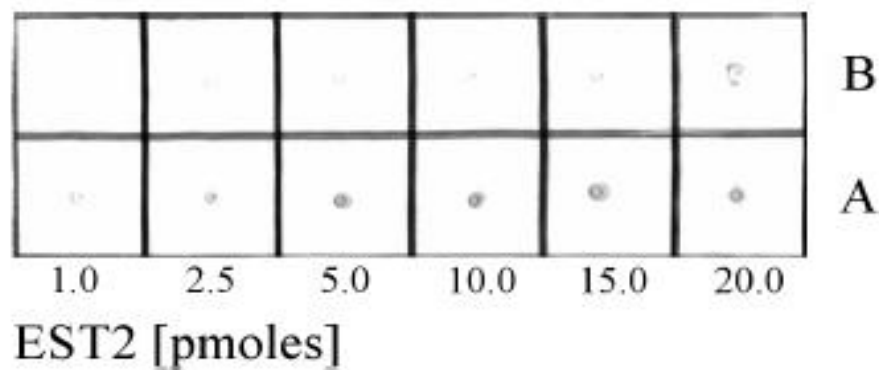
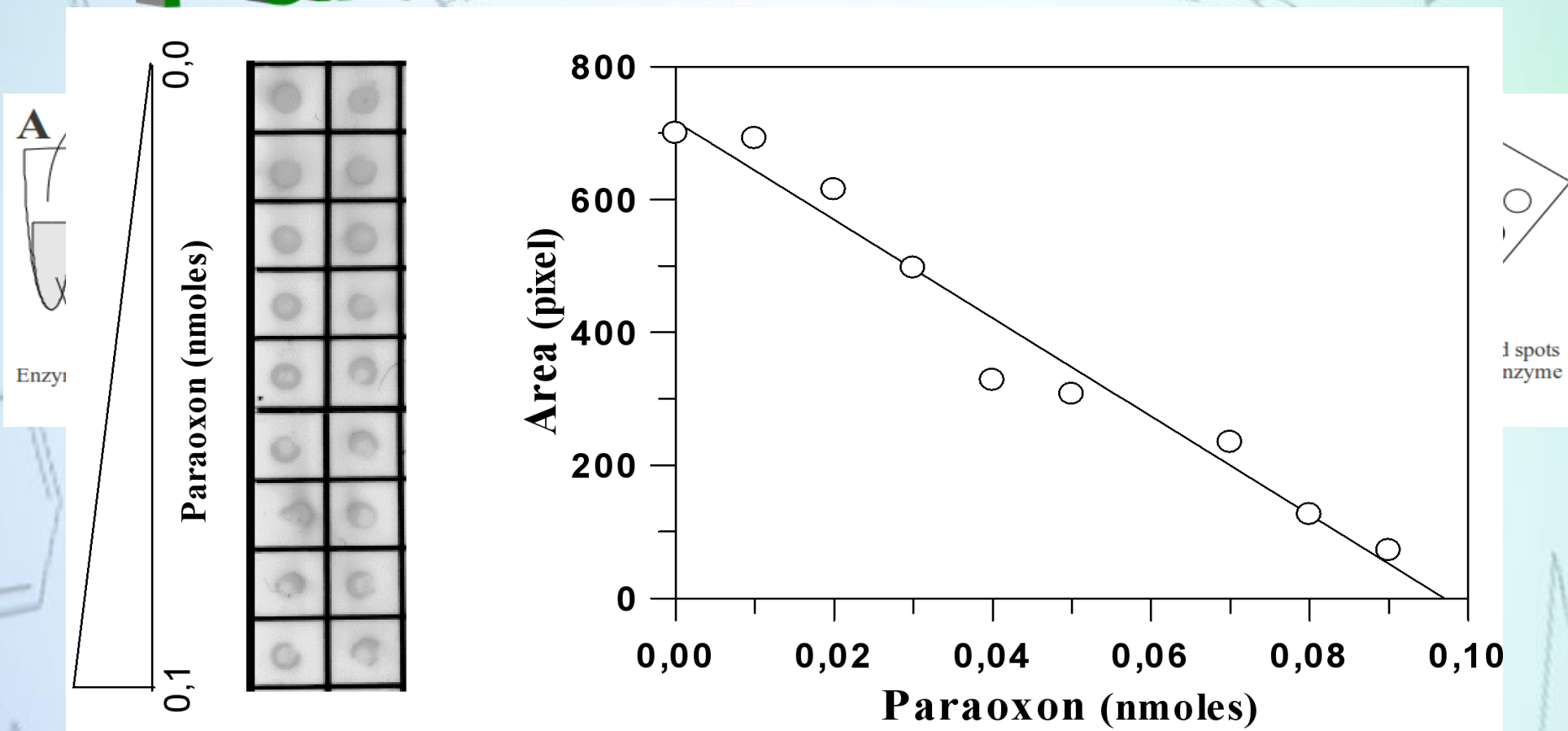
# Paraoxon inhibition



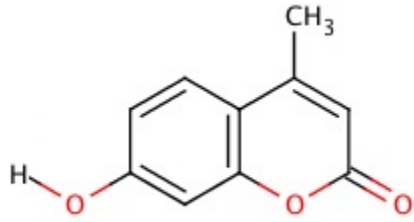
# Paraoxon Inhibition



# Paraoxon detection



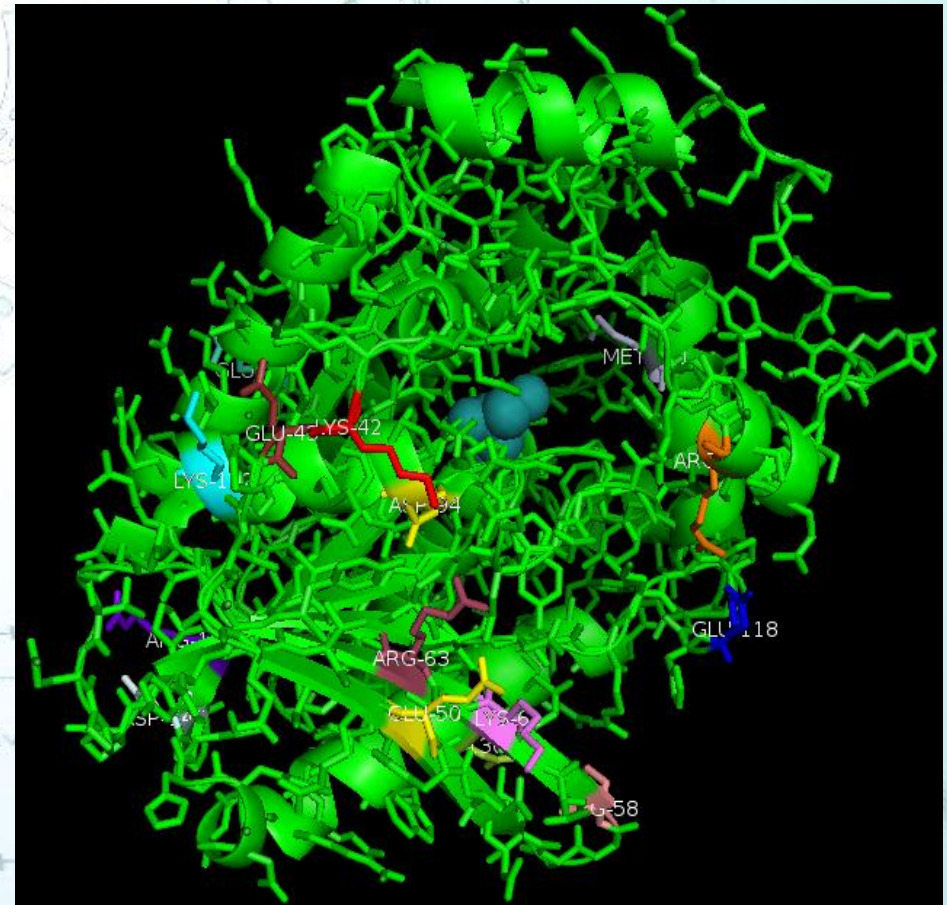
# New assay using fluorescent substrate



**4-Methylumbelliferone**

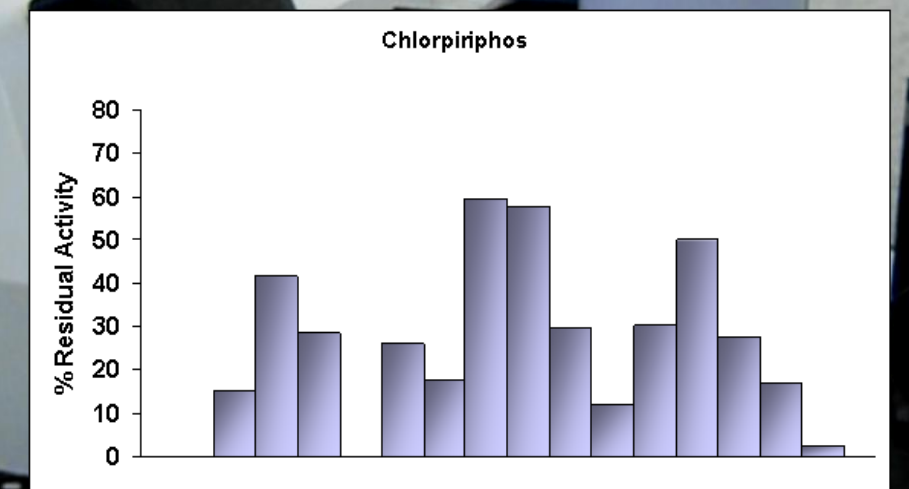
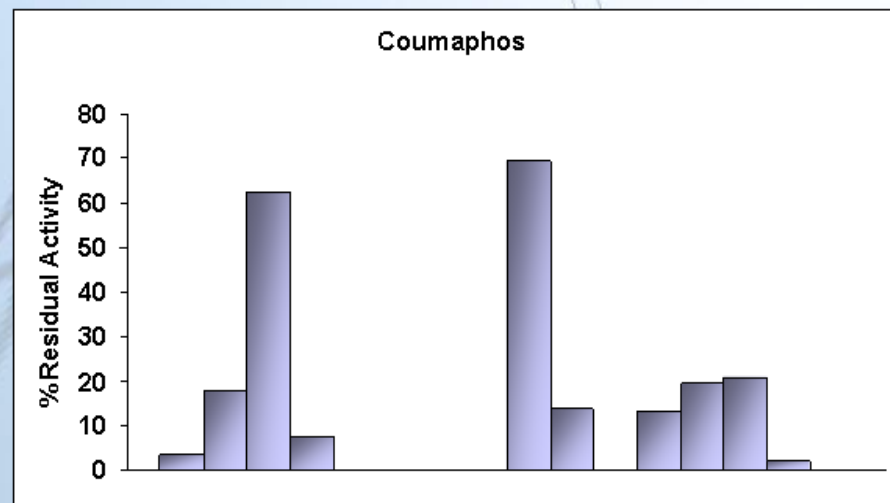
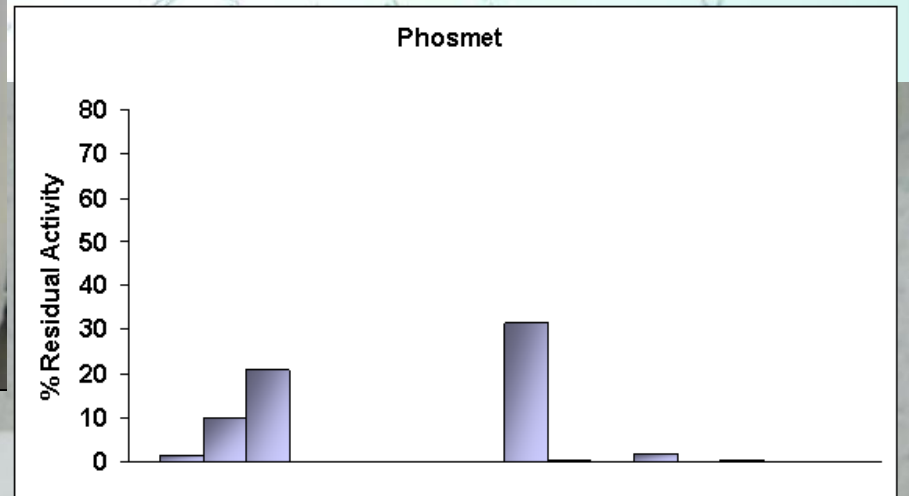
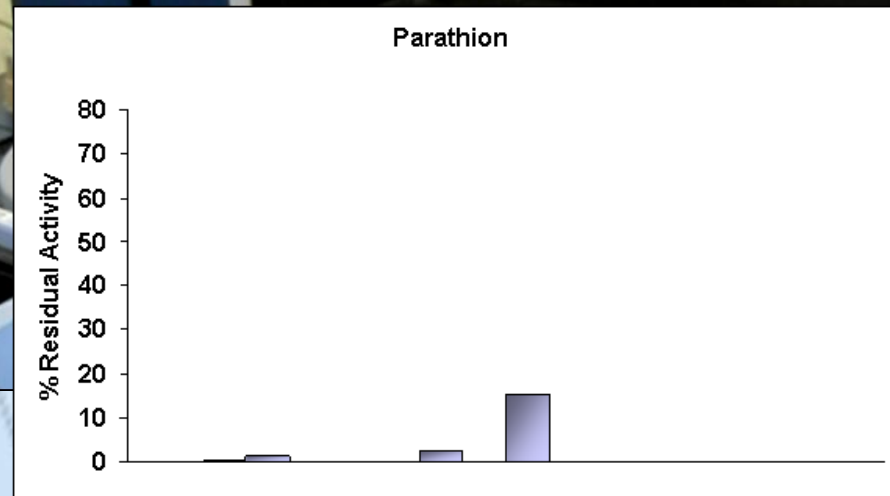
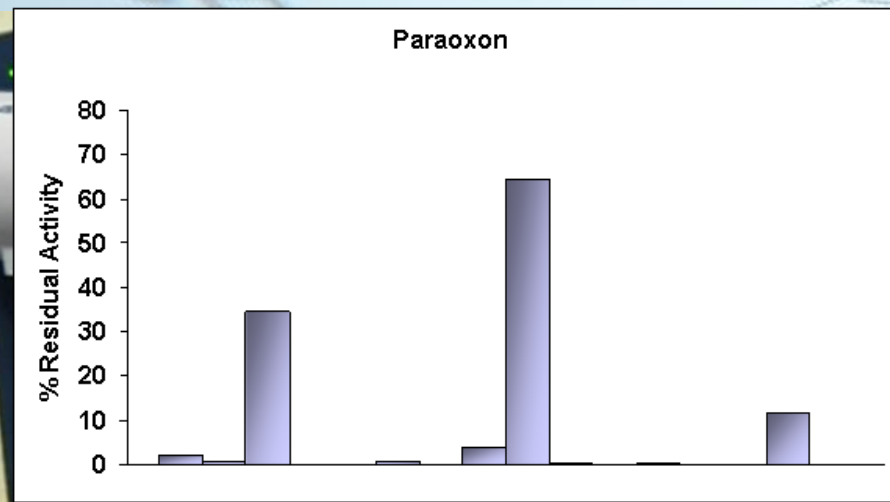
Excitation wavelength: 365 nm

Emission wavelength: 445 nm





# Automated approach with Robotic Station



600

1

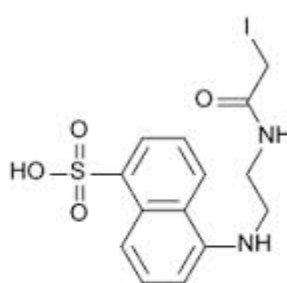
A

## IAEDANS

IAEDANS is an organic fluorophore. It stands for 5-naphthalene-1-sulfonic acid. It is widely used as a marker in fluorescence spectroscopy. 1,5-IAEDANS has a peak excitation wavelength of 336 nm and a peak emission wavelength of 490 nm.

**Formula:** C<sub>14</sub>H<sub>15</sub>IN<sub>2</sub>O<sub>4</sub>S

**Classification:** Sulfonic acids



1,25

20

15

10

05

00

95

## Fluorescein isothiocyanate

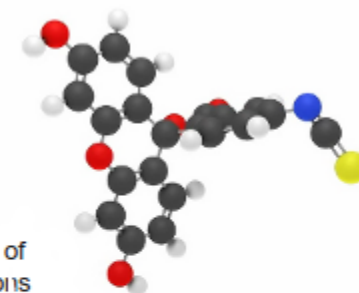
Chemical Compound

Fluorescein isothiocyanate is a derivative of fluorescein used in wide-ranging applications including flow cytometry.

**Molar mass:** 389.382 g/mol

**Formula:** C<sub>21</sub>H<sub>11</sub>NO<sub>5</sub>S

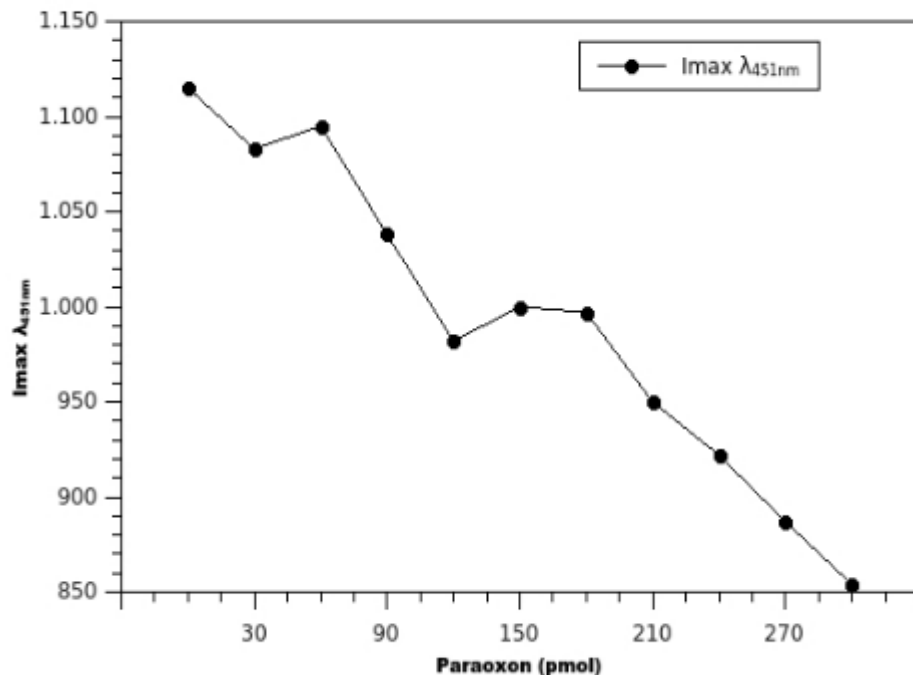
**Melting point:** 359.5 °C



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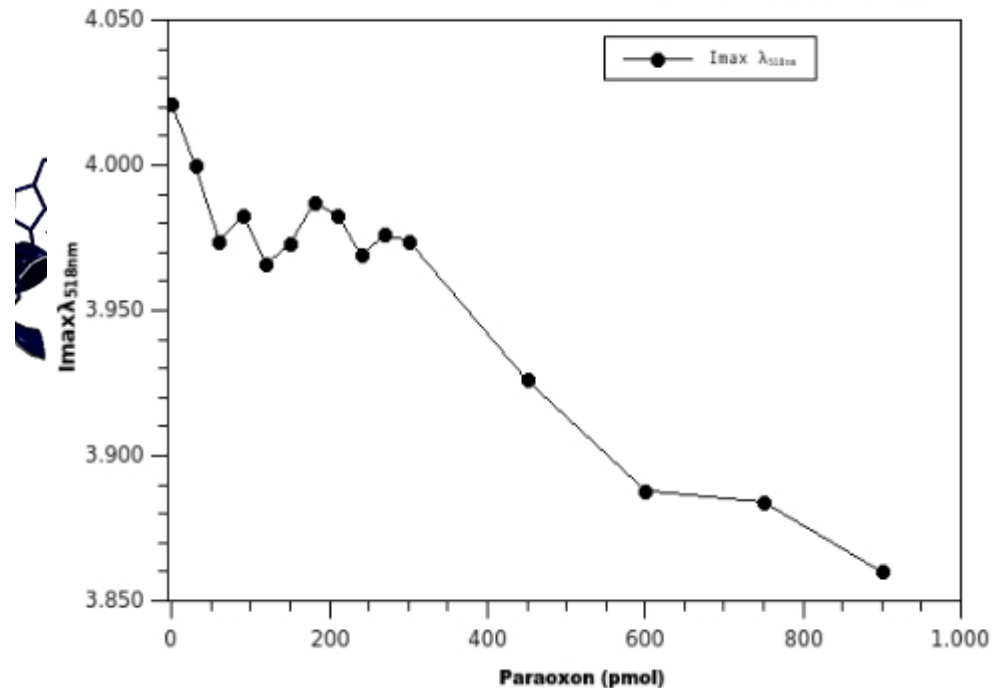
Fluorescence Intensity

EST2-IAEDANS vs Paraoxon



Wavelength (nm)

EST2-FITC vs Paraoxon



**Binding with fluorescent probes**

**Characterization of new EST2 mutants**

**WORK IN**



**PROGRESS**

**Assay on nerve agents**

**Development of principal component analysis software for OP mixture detection**

# Collaborations



## People

Paola Carullo  
Giovanni P. Cetrangolo  
Carla Gori  
Giuseppe Manco  
Luigi Mandrich



## GRANTS



Regione Campania



Ministero dell'Istruzione, dell'Università e della Ricerca

## Industries