united nations
educational, scientific
and cultural
organization

international atomic
energy agency

the
abdus salam
international centre for theoretical physics

SMR 1550 - 12

WORKSHOP ON THE USE OF RECEPTOR BINDING ASSAY (RBA)

1 - 5 September 2003

Co-organized by the International Atomic Energy Agency (I.A.E.A.)

Strategies in the Establishment of Receptor Binding Assay Technology in the Philippines

Elvira Z. SOMBRITO
Philippine Nuclear Research Institute
Diliman, Quezon City, Philippines

Strategies in the Establishment of Receptor Binding Assay Technology in the Philippines

ELVIRA Z. SOMBRITO

Philippine Nuclear Research Institute

Commonwealth Ave., Diliman, Quezon City 1101, PHILIPPINES

ezsombrito@pnri.dost.gov.ph

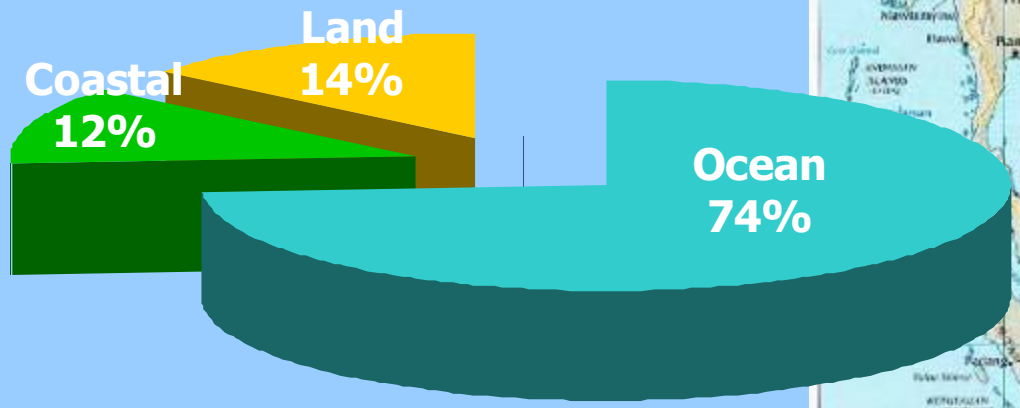
VISION

To provide a quick and sensitive method for PSP detection

TO MINIMIZE RISK TO HUMAN HEALTH

TO MITIGATE ADVERSE ECONOMIC IMPACTS TO THE FISHING INDUSTRY

Territorial area: 222 M ha

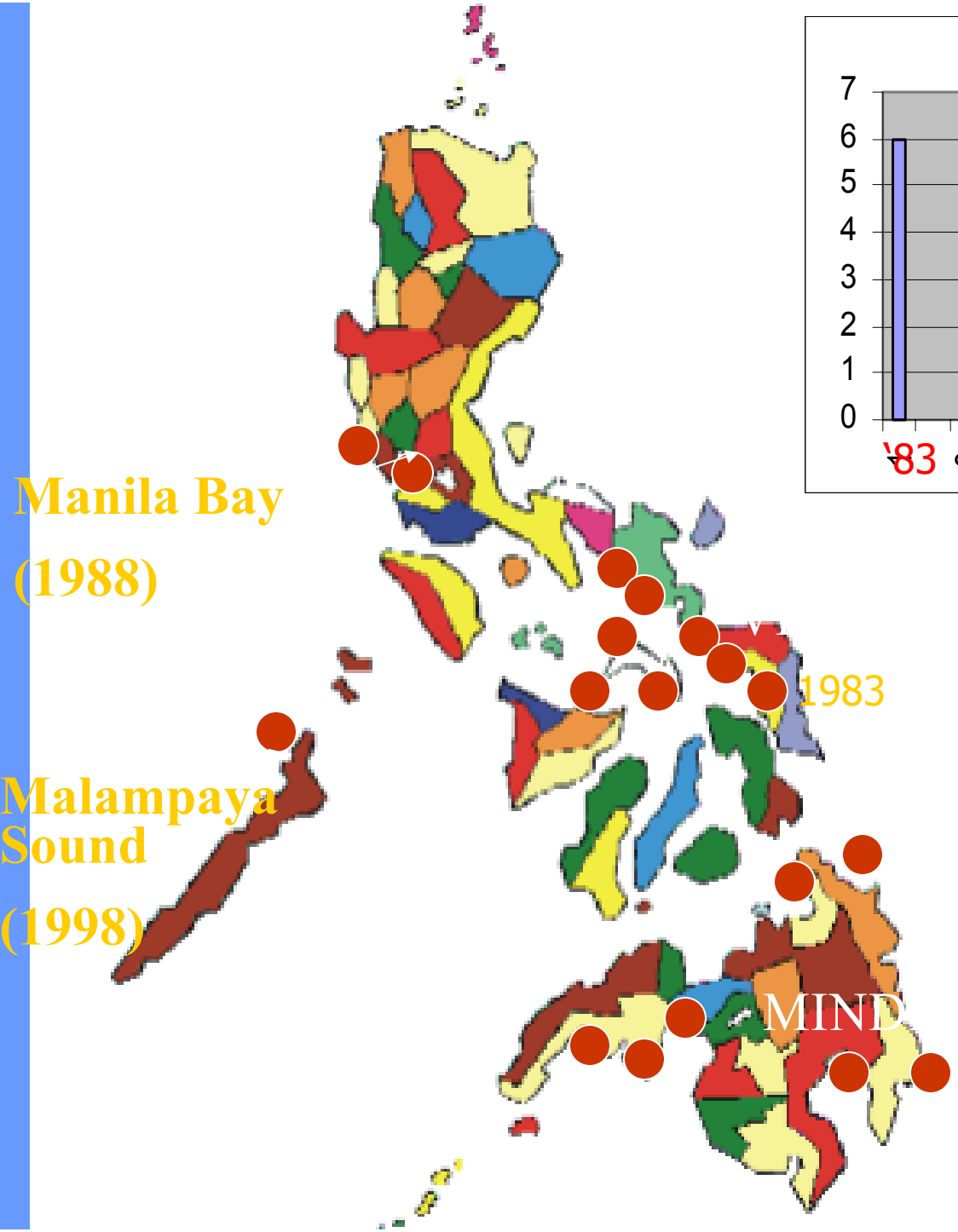


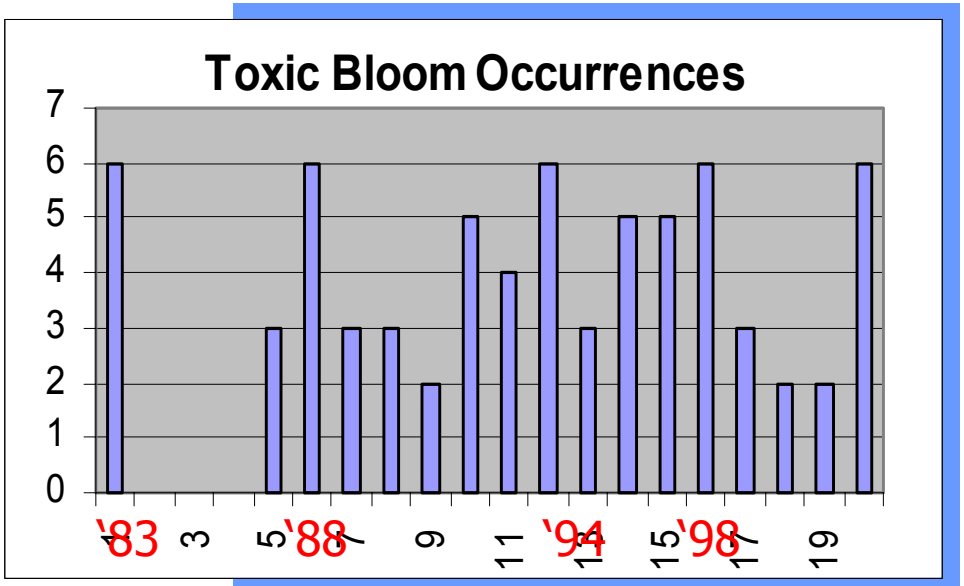
Coastline-36,289 km (5th)

Fisheries- 4% GDP

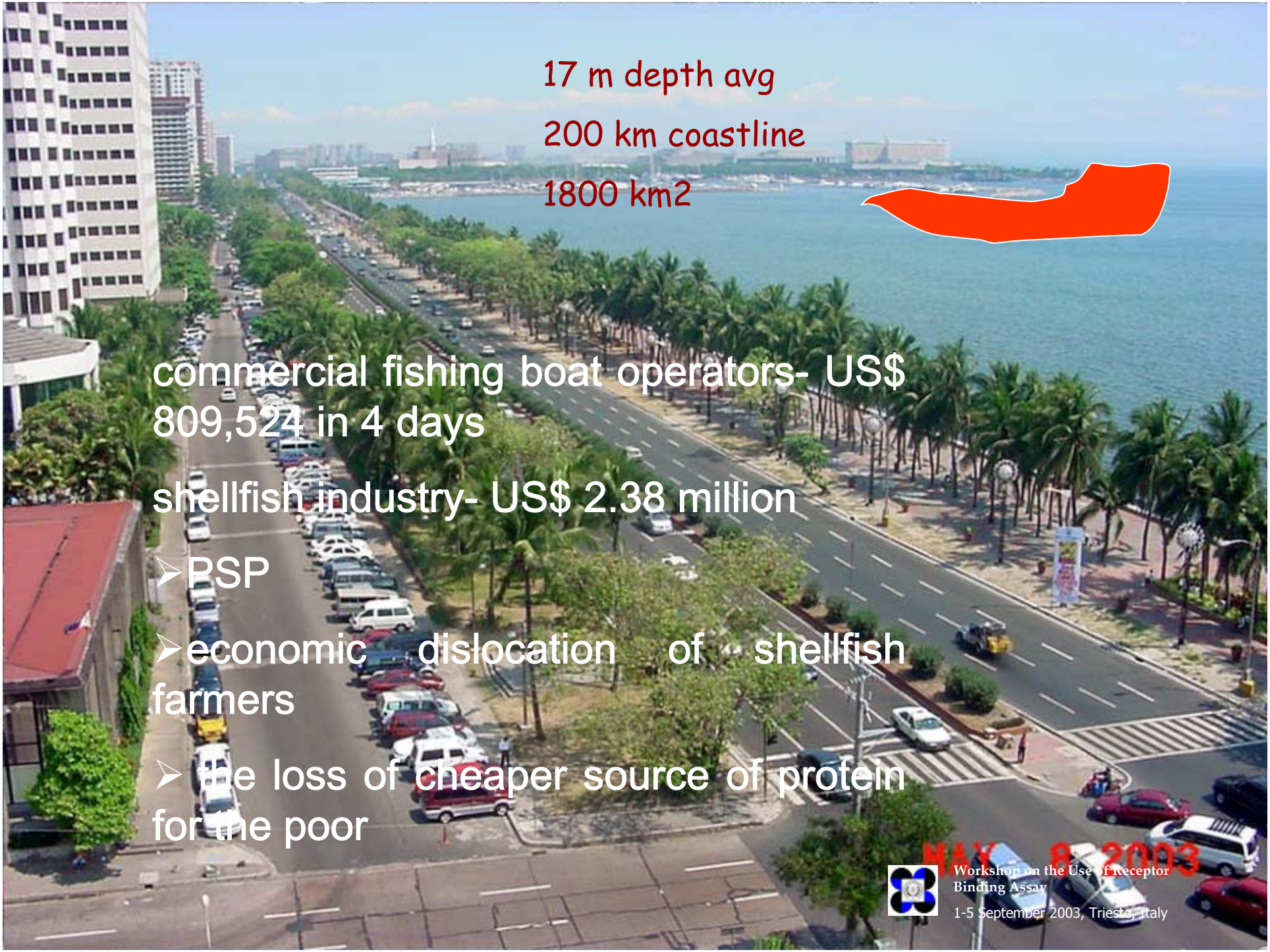
Fish production valued at almost PhP 100B

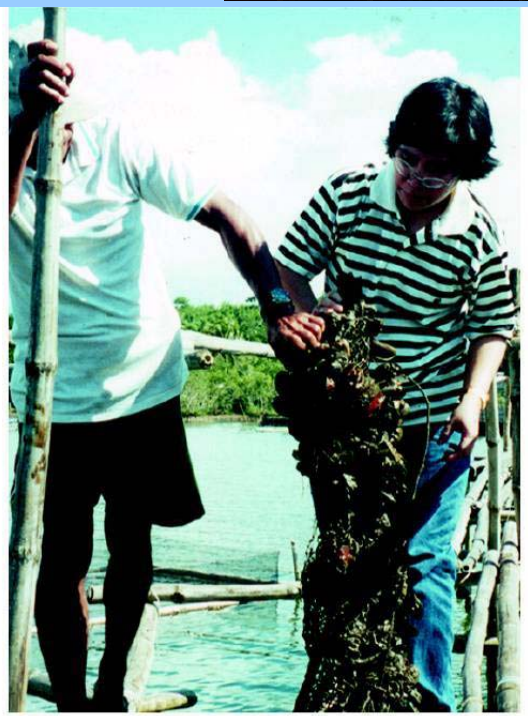
~1M fishermen

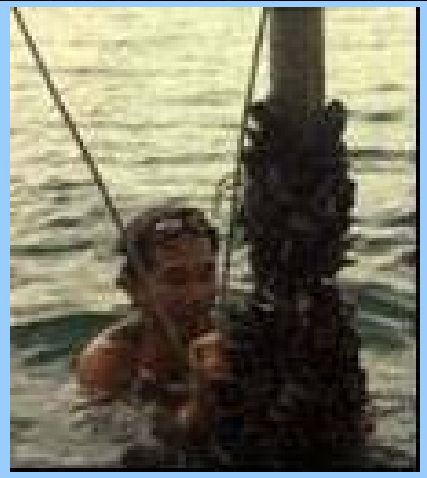


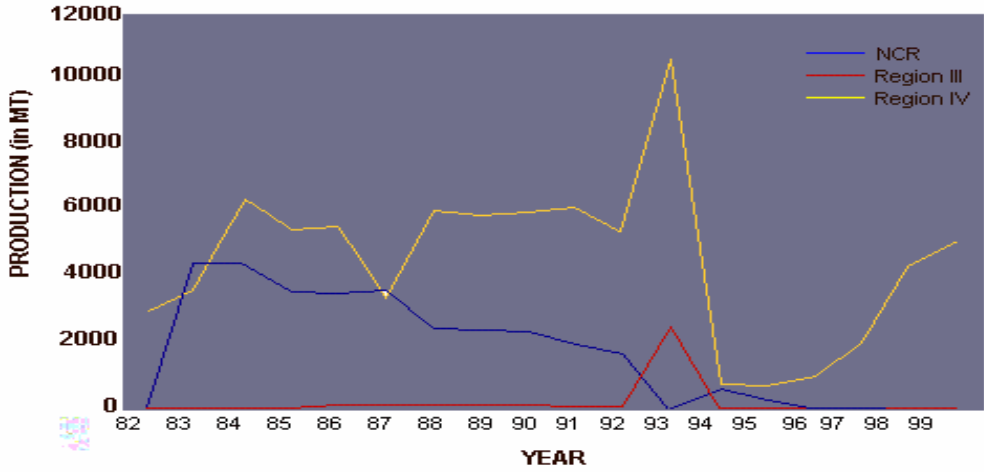


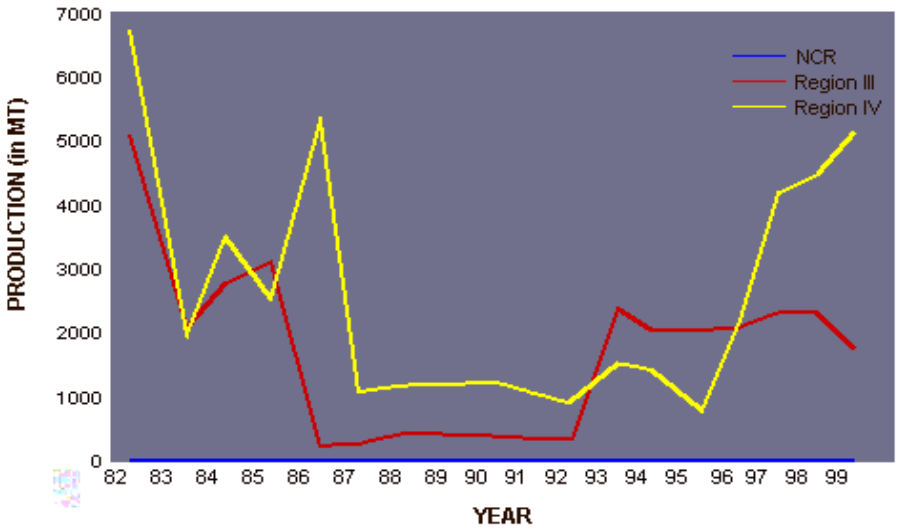
- 22 coastal areas affected
- 2107 PSP cases
- 107 fatalities

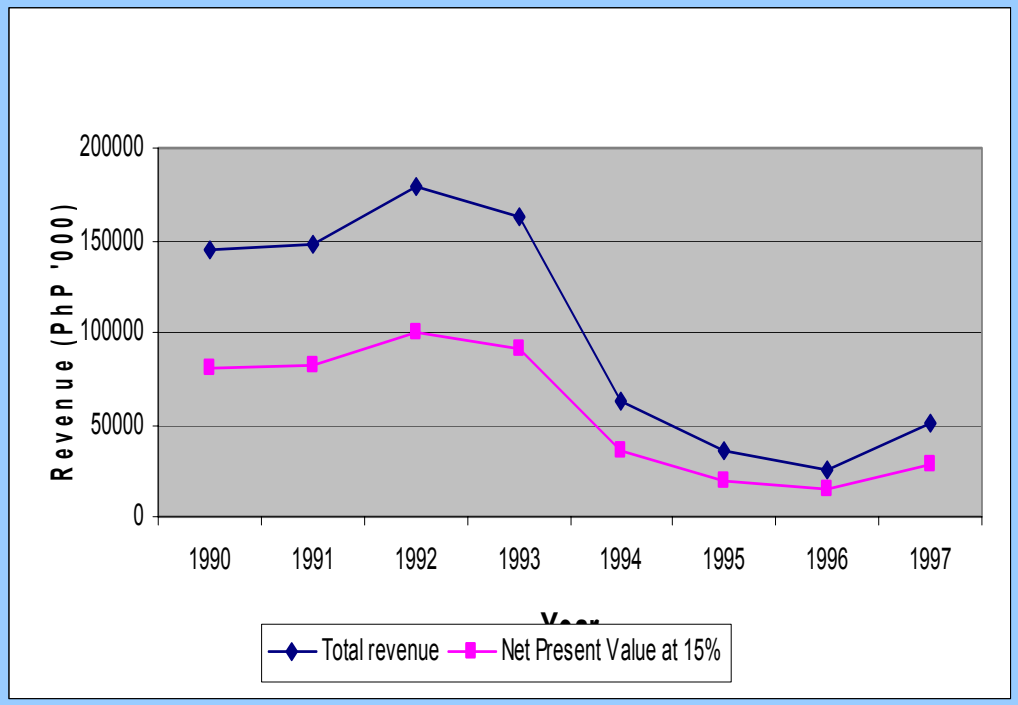

17 m depth avg
200 km coastline
1800 km2
commercial fishing boat operators- US$ 809,524 in 4 days
shellfish industry- US$ 2.38 million
➢PSP
➢economic dislocation of shellfish farmers
➢ the loss of cheaper source of protein for the poor
MAY 8 2003





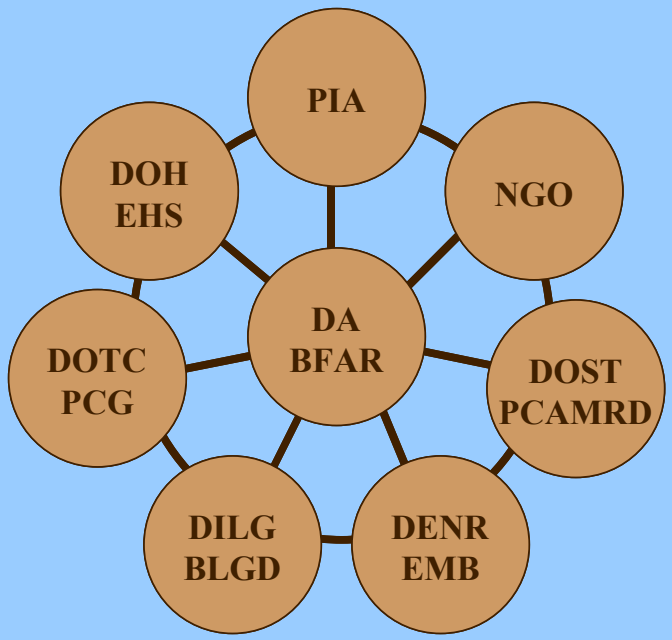

PRODUCTION (in MT)
12000
10000
8000
6000
4000
2000
0
82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99
YEAR
NCR
Region III
Region IV


PRODUCTION (in MT)
7000
6000
5000
4000
3000
2000
1000
0
82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99
YEAR
NCR
Region III
Region IV


Revenue (PhP '000)
200000
150000
100000
50000
0
1990 1991 1992 1993 1994 1995 1996 1997
Total revenue
Net Present Value at 15%

National Red Tide Task Force (NRTTF)

Inter-agency Committee on Environmental Health



Regulatory level in the Philippines: 40 ug/100g

Pyrodinium bahamense var. compressum
Gymnodinium catenatum

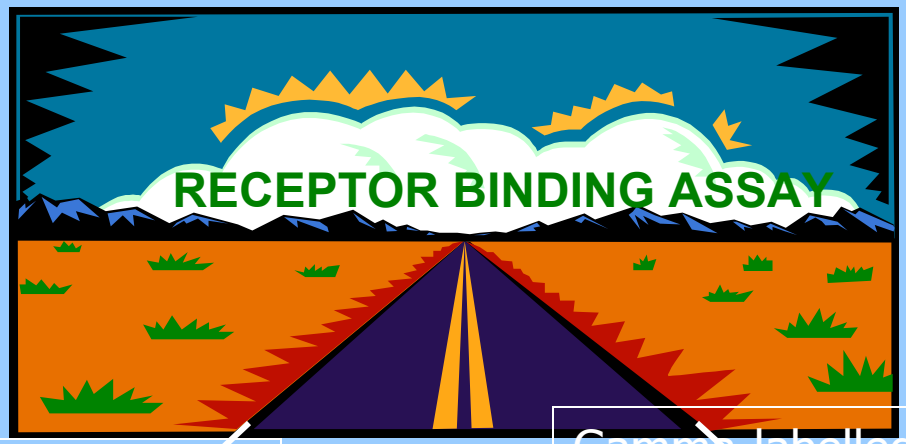
TOXIC RED TIDE SAMPLING SCHEDULE

Period	Toxic organism		Toxin	
	Manila Bay	Other Areas	Manila Bay	Other Areas
Pre red tide	once a week	once a month	once a week	once a month
Detection phase	3x a week	3x a week	Daily	3x a week
Toxic HAB period	3x a week	once a week	3x a week	once a week
Post Bloom	3x a week	once a month	3x a week	once a month
After toxic bloom	once a week	once a month	once a week	once a month

- **Issuance of warning: Presence of toxic HAB organism**
- **Closure: > 40 ug/100g or confirmed PSP case**
- **Lifting of ban: < 40 ug/100g, organism not in active phase once a week sampling for 3 consecutive weeks; no PSP case**

- Identification of alternative protocol for PSP assay
- Facility and Expertise
- Interlaboratory and procedure intercomparison
- Capability for the production of the radiolabelled tracer
- Capability for the isolation, purification and characterization of saxitoxin
- Inclusion into the national red tide program

Strategies



AOAC

Gamma-labelled STX

Production of 3HSTX

Commercial 3H-STX

Inclusion into the national program

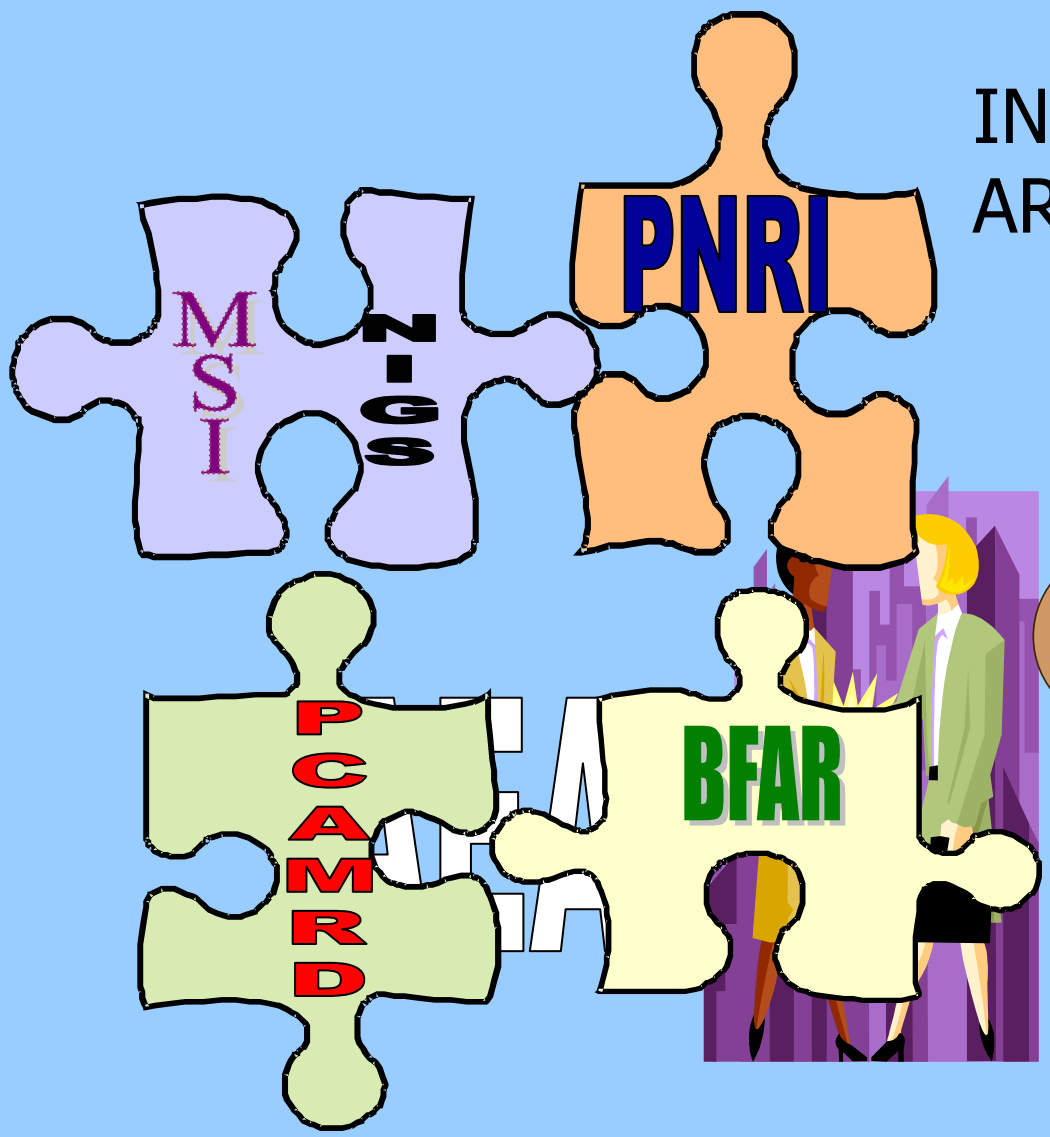
tritium-labelling of STX

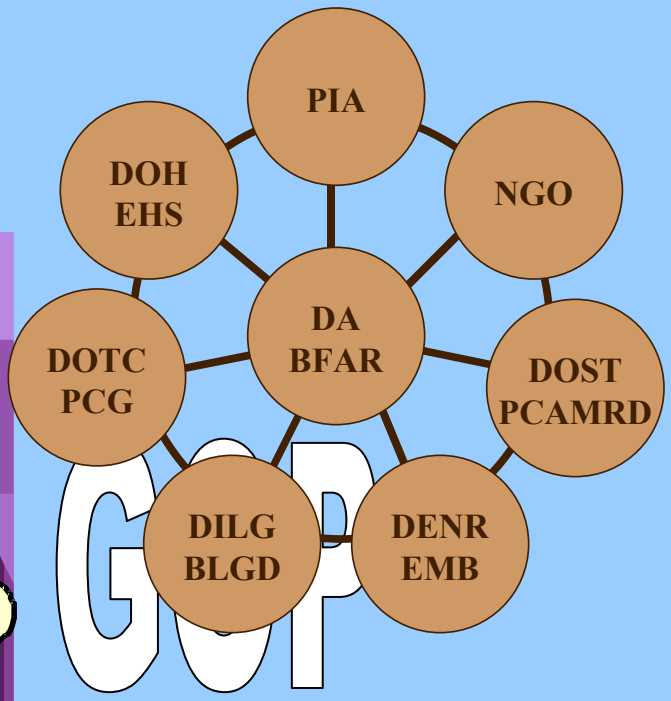
RBA

Isolation/purfication of saxitoxin

Building facilities, technical skills and relationships

INSTITUTIONAL ARRANGEMENTS





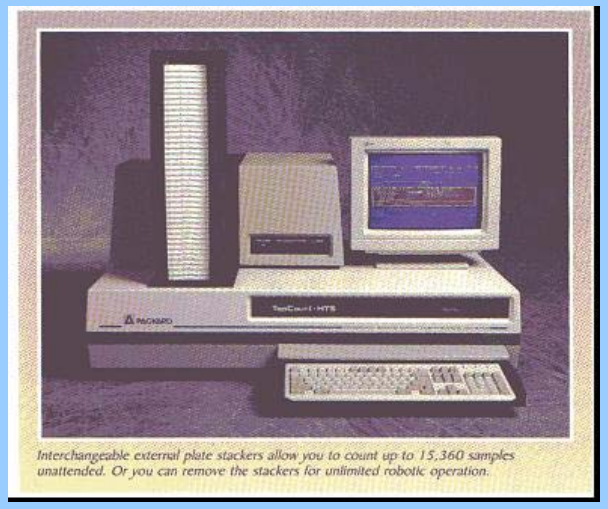
PROGRAM:

APPLICATIONS OF NUCLEAR TECHNIQUES TO ADDRESS SPECIFIC RED TIDE (HARMFUL ALGAL BLOOM) CONCERNS

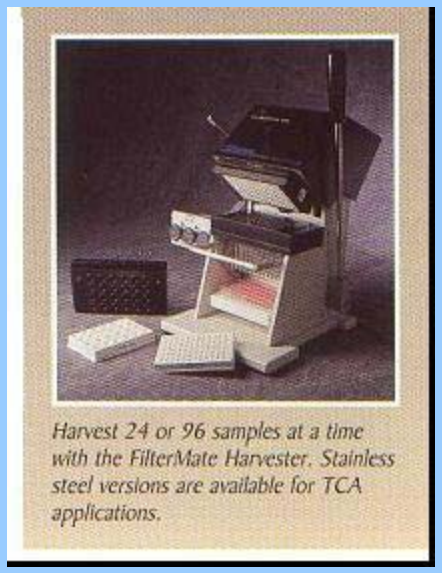
Rapid Assays for the Detection of Paralytic Shellfish Poisons

Role of Sediments in Toxic Algal Blooms and Record of Bloom Occurences in Bay Sediments

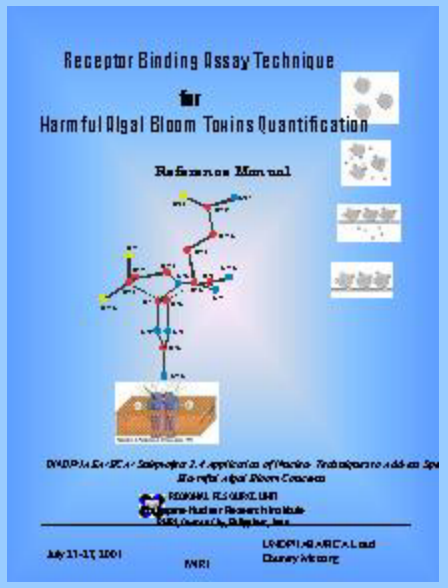
Modelling of Toxic Red Tides in the Philippines



Interchangeable external plate stackers allow you to count up to 15,360 samples unattended. Or you can remove the stackers for unlimited robotic operation.



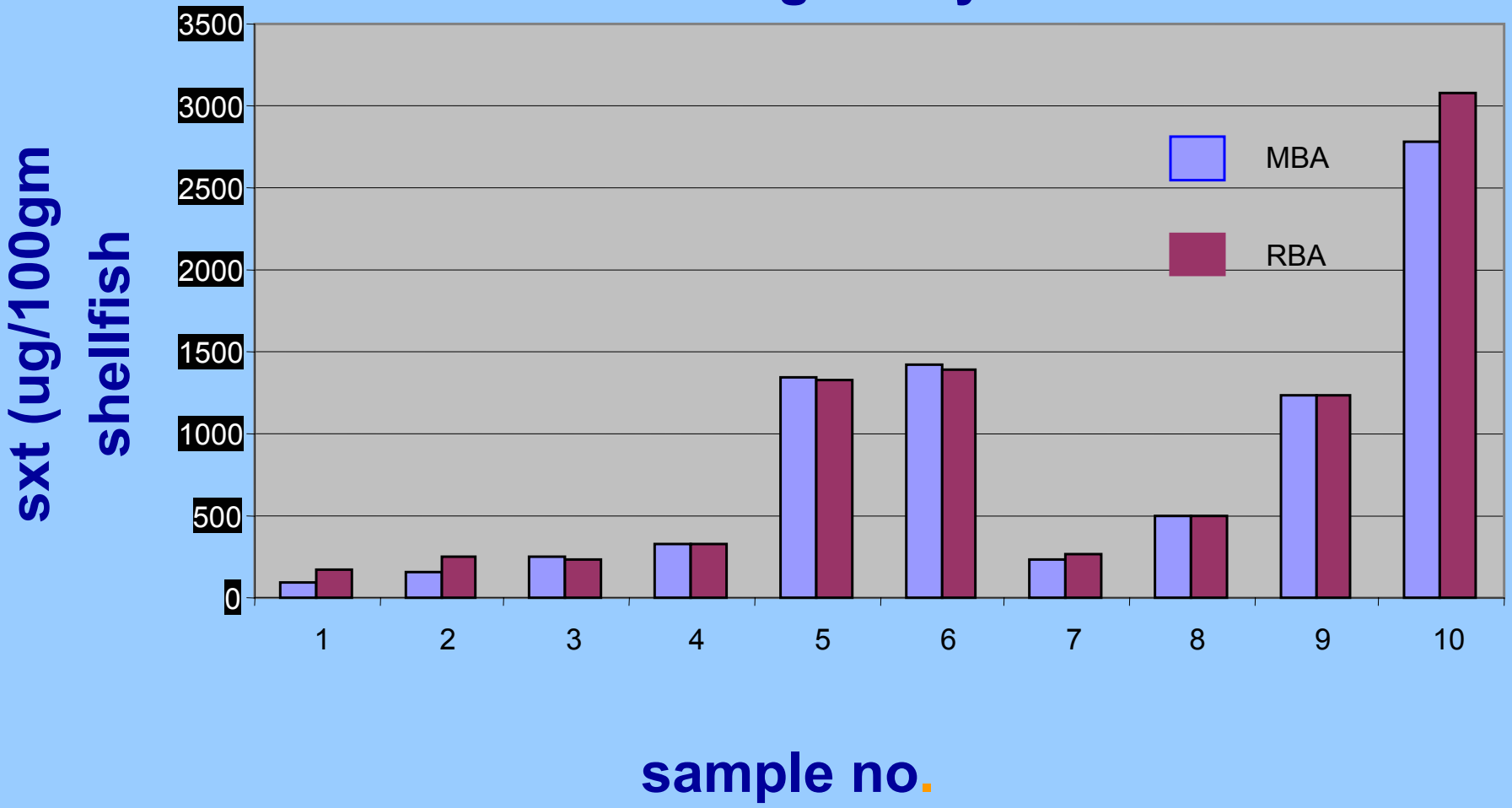
Harvest 24 or 96 samples at a time with the FilterMate Harvester. Stainless steel versions are available for TCA applications.



Workshop on the Use of Receptor Binding Assay

1-5 September 2003, Trieste, Italy

Comparison of Mouse Bioassay vs Receptor Binding Assay



Linear correlation of different methods for PSP quantitation*

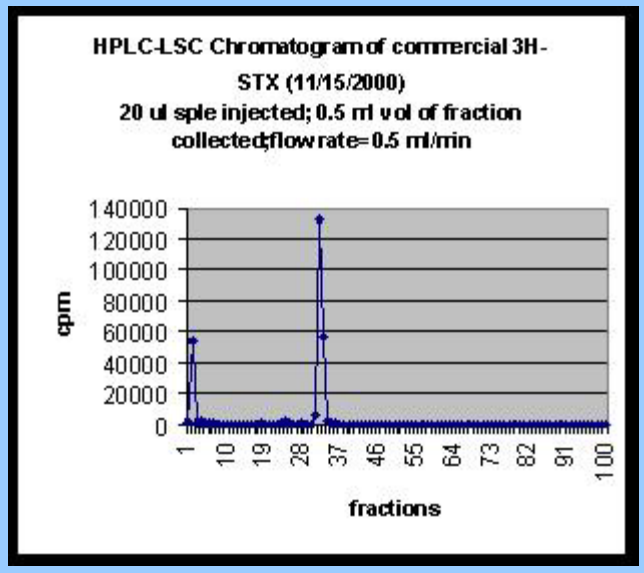
24 shellfish extracts

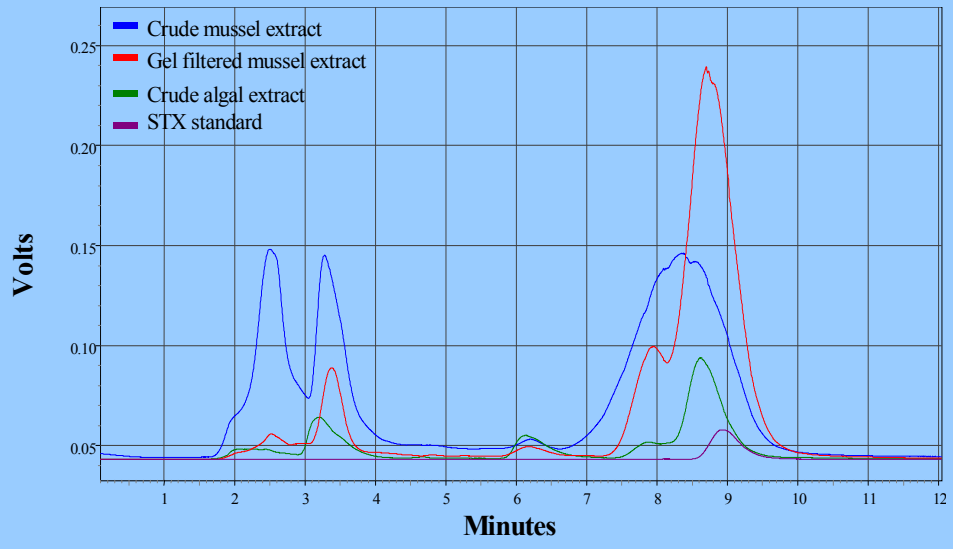
10 samples > 40ug/100

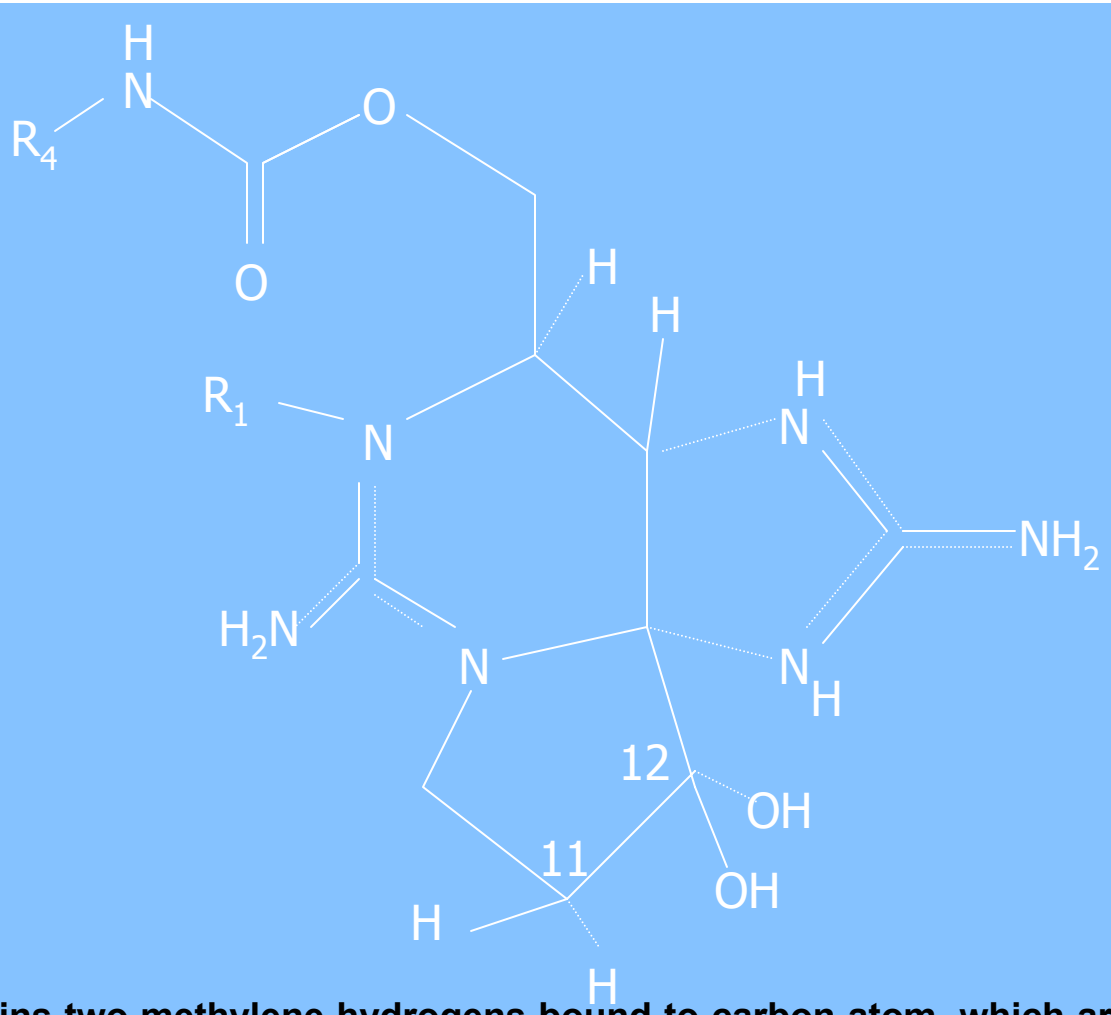
4 samples at 1500 ug/100g avg

PCO-HPLC vs Mouse Bioassay	0.8476
PCO-HPLC vs R BA	0.8281
PCO-HPLC vs ECOS-HPLC	0.9490
Mouse Bioassay vs RBA	0.7681
Mouse Bioassay vs ECOS-HPLC	0.9226
RBA vs ECOS-HPLC	0.8673

*UPMSI Project terminal Report (unpublished data)


HPLC-LSC Chromatogram of commercial 3H-STX (11/15/2000)
20 ul sple injected; 0.5 ml vol of fraction collected;flow rate=0.5 ml/min
cpm
140000
120000
100000
80000
60000
40000
20000
0
1
10
19
28
37
46
55
64
73
82
91
100
fractions


Crude mussel extract
Gel filtered mussel extract
Crude algal extract
STX standard
Volts
0.25
0.20
0.15
0.10
0.05
1
2
3
4
5
6
7
8
9
10
11
12
Minutes



Saxitoxin contains two methylene hydrogens bound to carbon atom, which are slowly exchangeable with solvent hydrogen. The functional group shown at C-12 is a hydrated ketone, a carbonyl group with which water has formed a reversible covalent bond, resulting in a gem-diol, $C(OH)_2$. Despite its tendency to hydrate, C-12 retains the properties of a ketone, particularly the ability to enolize, which renders protons at C-11 readily exchangeable. Since these hydrogens are exchangeable, it is possible to label saxitoxin with tritium, using exchange reaction. The reaction conditions can be manipulated to permit exchange labeling with tritium to produce tritium labeled saxitoxin of high specific activity.

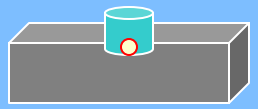
3H-STX

Specific Activity	14.9 Ci/mmol
	35.5 mCi/mg
Molecular Wt.	419 g/mol
Radioactive Conc.	250 uCi/ml
Pack size	50 uCi
Mmole in pack	3.36 mmol
STX conc.	16.78 uM
Amt of STX /pack	1.41 ug
In assay well	2.2 nM
	0.19 ng
Activity per well	7 nCi

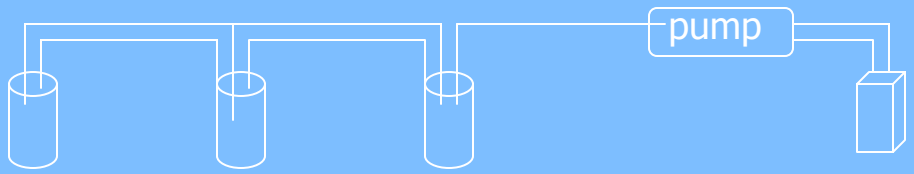
Tritiation of STX

Incubation

Temperature, pH, time

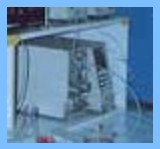


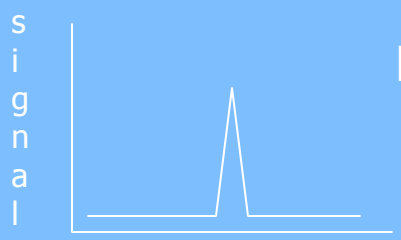
Removal of excess tritium



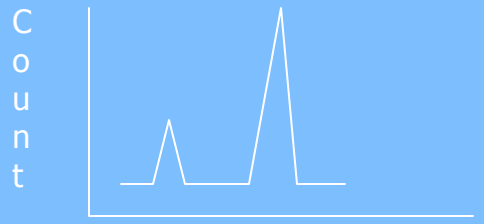
Final Purification &Characterization

HPLC

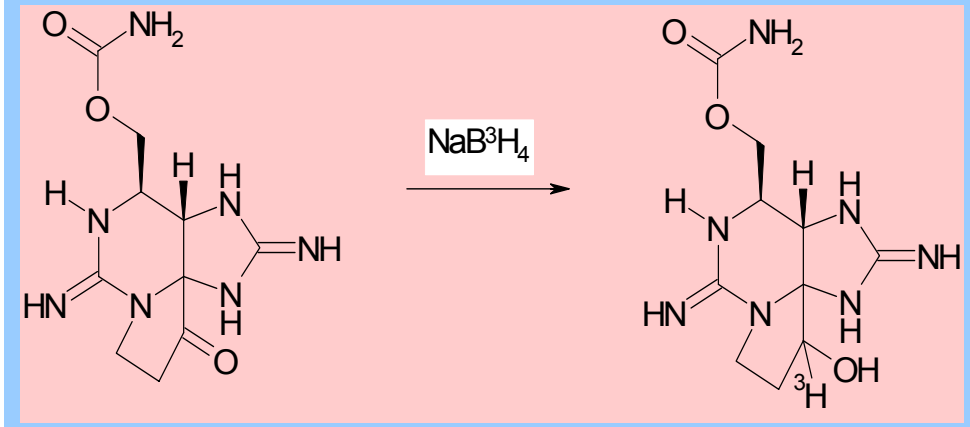




LSC



OPTIONS

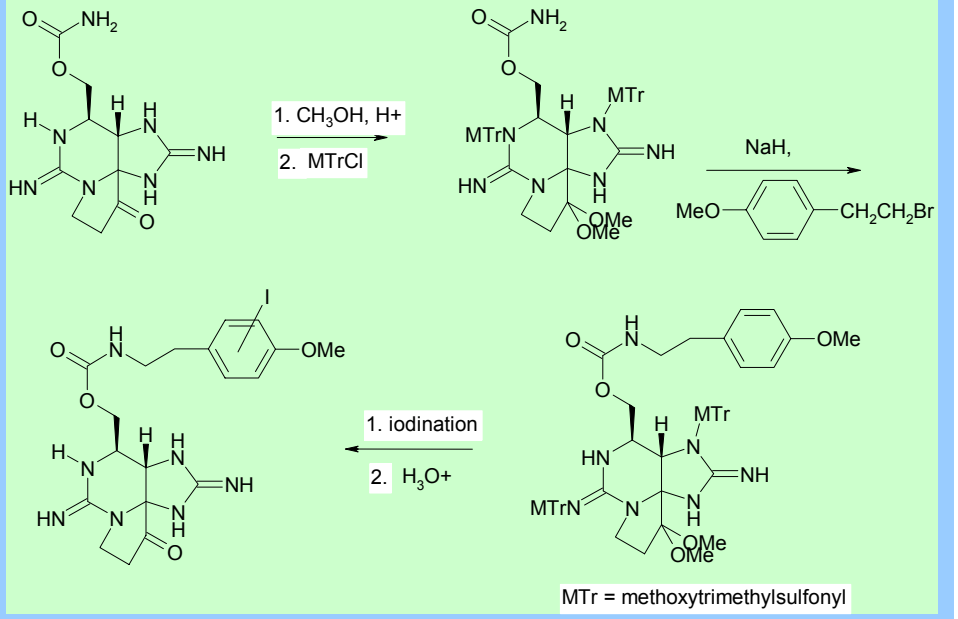


OTHER TOXINS:

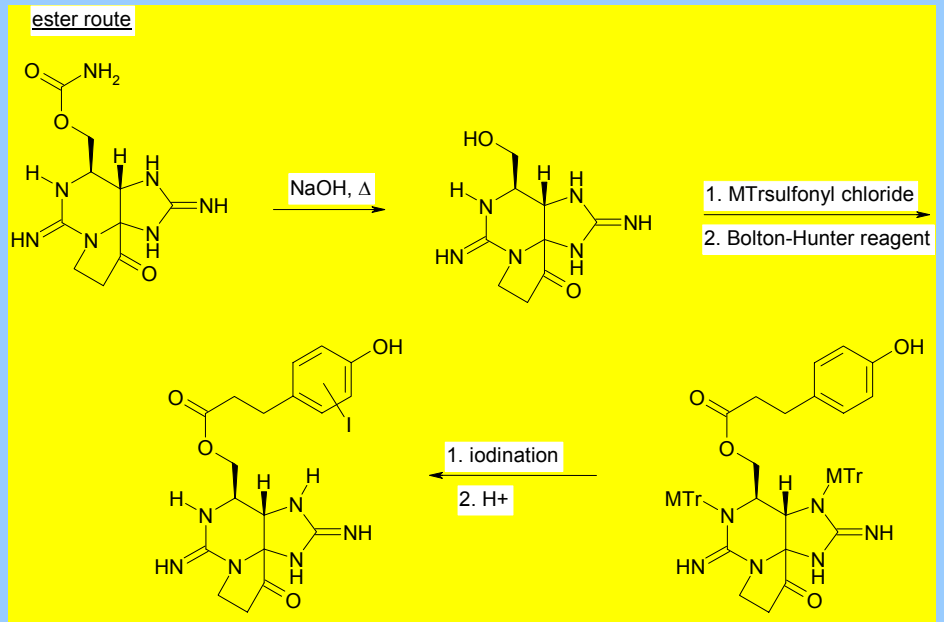
TETRODOTOXIN

CONOTOXIN

carbamate route



ester route



Thank you!!!

MERRIAN TANGONAN
MARICEL HONRADO
AZUCENA DE VERA
LUCILLE ABAD

harbour tour boat at sunset, Manila Bay