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SMR 1550 - 10

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.)

High Throughput Receptor Assay for Paralytic Shellfish Poisoning Toxins

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High Throughput Receptor Assay for Paralytic Shellfish Poisoning Toxins

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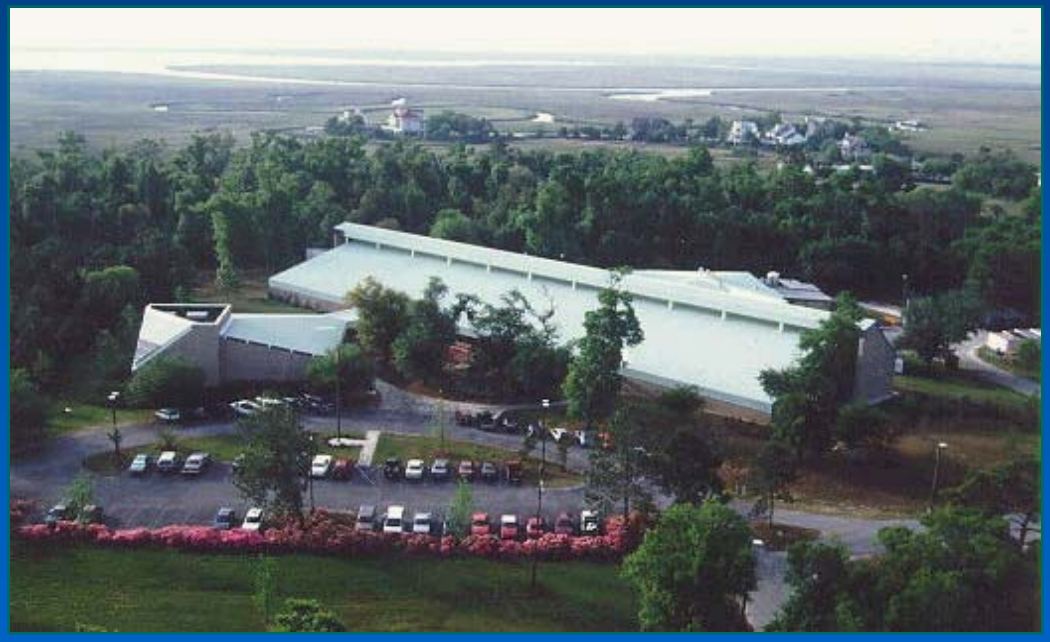

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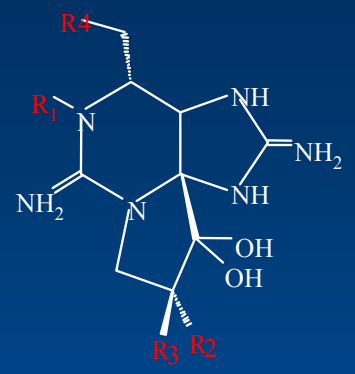
Detection of PSP Toxins



Challenges

- Multiple toxin forms
- Biotransformation
- Complex sample matrices

		R1	R2	R3	R4	MU /μ mol
Carbamate	STX	H	H	H	$OCONH_2$	2483
	Neo STX	OH	H	H	$OCONH_2$	2295
	GTX1	OH	OSO3-	H	$OCONH_2$	2468
	GTX2	H	OSO3-	H	$OCONH_2$	892
	GTX3	H	H	OSO3-	$OCONH_2$	1584
	GTX4	OH	H	OSO3-	$OCONH_2$	1803
Sulfocarbamoyl	GTX5 (B1)	H	H	H	OCONHSO3-	160
	GTX6 (B2)	OH	H	H	OCONHSO3-	-
	C1	H	OSO3-	H	OCONHSO3-	15
	C2	H	H	OSO3-	OCONHSO3-	239
	C3	OH	OSO3	H	OCONHSO3-	33
	C4	OH	H	OSO3-	OCONHSO3-	143
Decarbamoyl	dcSTX	H	H	H	OH	1274
	dcNeoSTX	OH	H	H	OH	-
	dcGTX1	OH	OSO3-	H	OH	-
	dcGTX2	H	OSO3-	H	OH	1617
	dcGTX3	H	H	OSO3-	OH	1872
	dcGTX4	OH	H	OSO3-	OH	-
Deoxydecarbamoyl	doSTX	H	H	H	H	-
	doGTX2	H	H	OSO3-	H	-
	doGTX3	H	OSO3-	H	H	-

[1]Oshima, 1995

Detection Methods: Approaches

Assays

- *provide integrated measure of toxin congeners present*

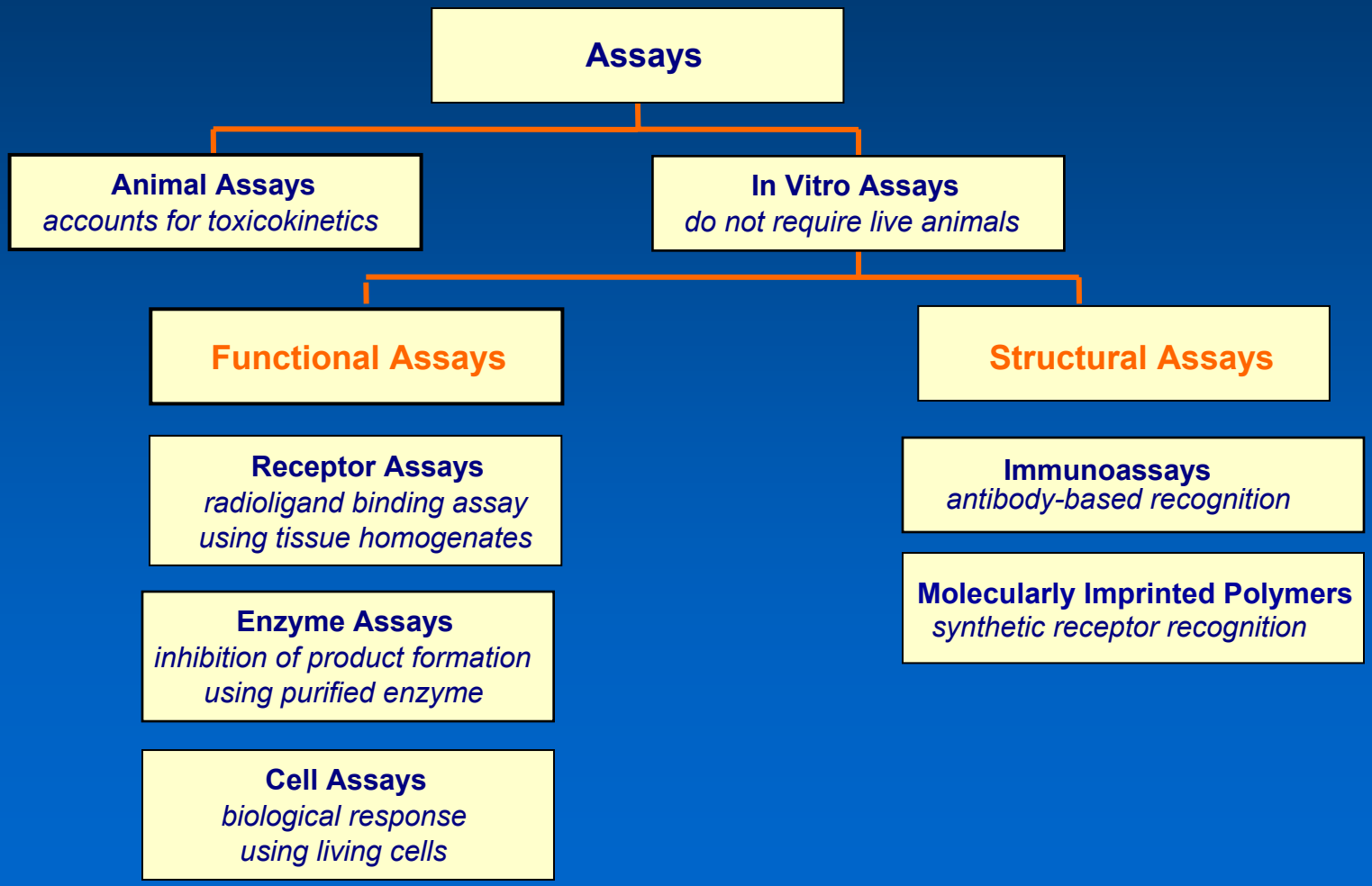
- *rapid*
- *sensitive*
- *high throughput*
- *low cost*
- *little technical expertise*

Analytical Methods

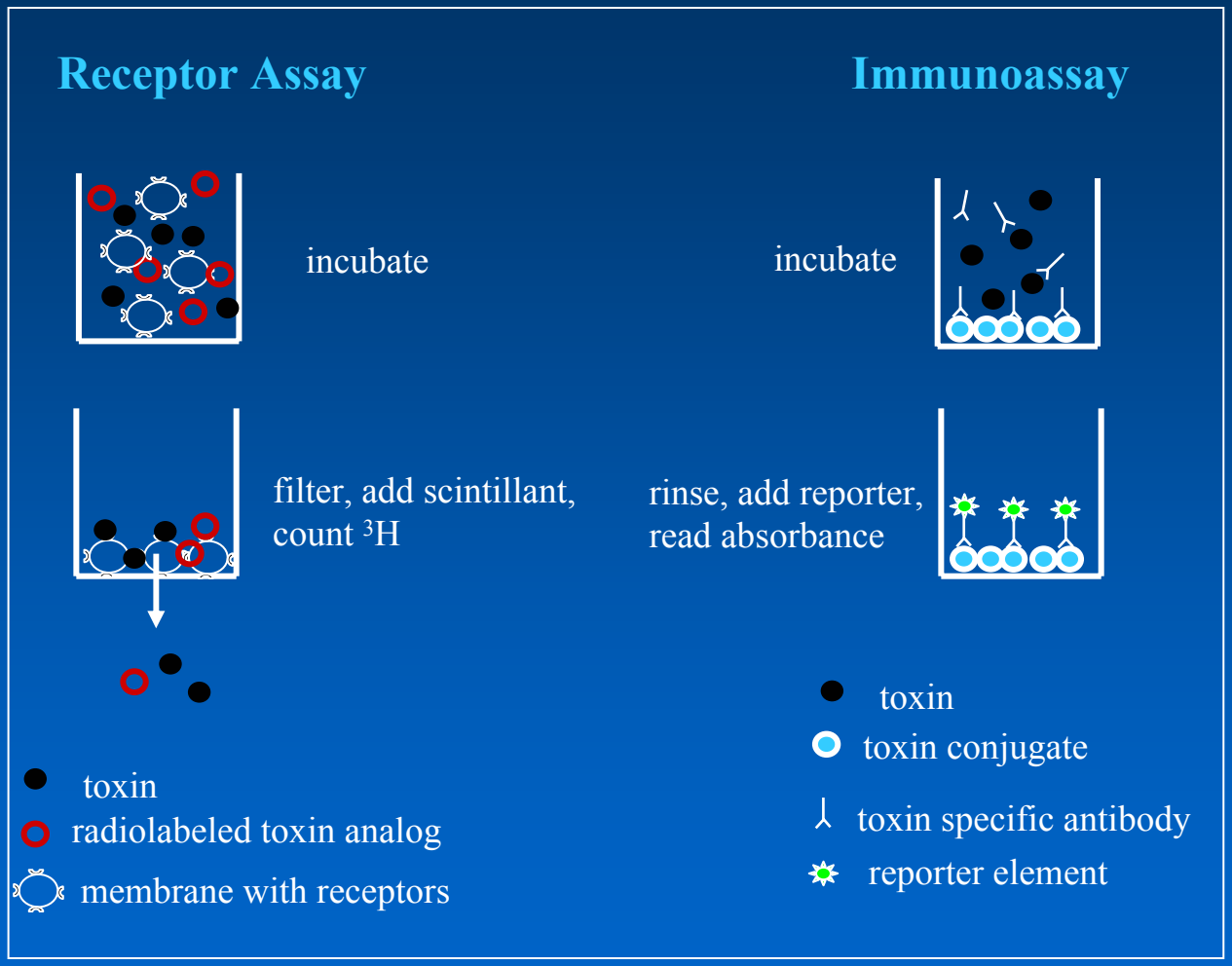
- *provide quantitative measure of individual toxin congeners present*

- *sensitive*
- *quantitative*
- *definitive*
- *expensive*
- *technical expertise*

Assay Methods for Marine Biotoxins



Comparison of Two Workhorse Assays: Receptor vs ELISA



Measures integrated toxic potency of all PSP congeners based on relative affinity to the receptor

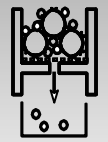
Measures PSP toxin analogs based on degree of structural recognition by the antibody

Receptor Assays for Marine Algal Toxins

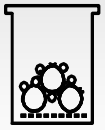
Toxin Classes: saxitoxin, brevetoxin, ciguatoxin, domoic acid

1. Membrane preparation containing receptors

2. Incubate ^{3}H ligand with standard or sample with membrane preparation

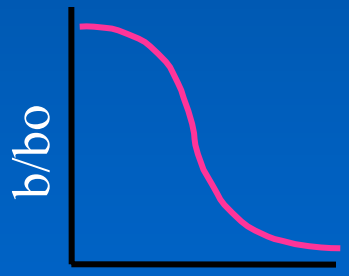


3. Remove unbound ^{3}H ligand by washing and filtation



4. ^{3}H ligand bound to receptor sites determined by liquid scintillation counting

$$T + R \rightarrow TR$$
$$+$$
$$T^{*}$$
$$\downarrow$$
$$T^{*}R$$

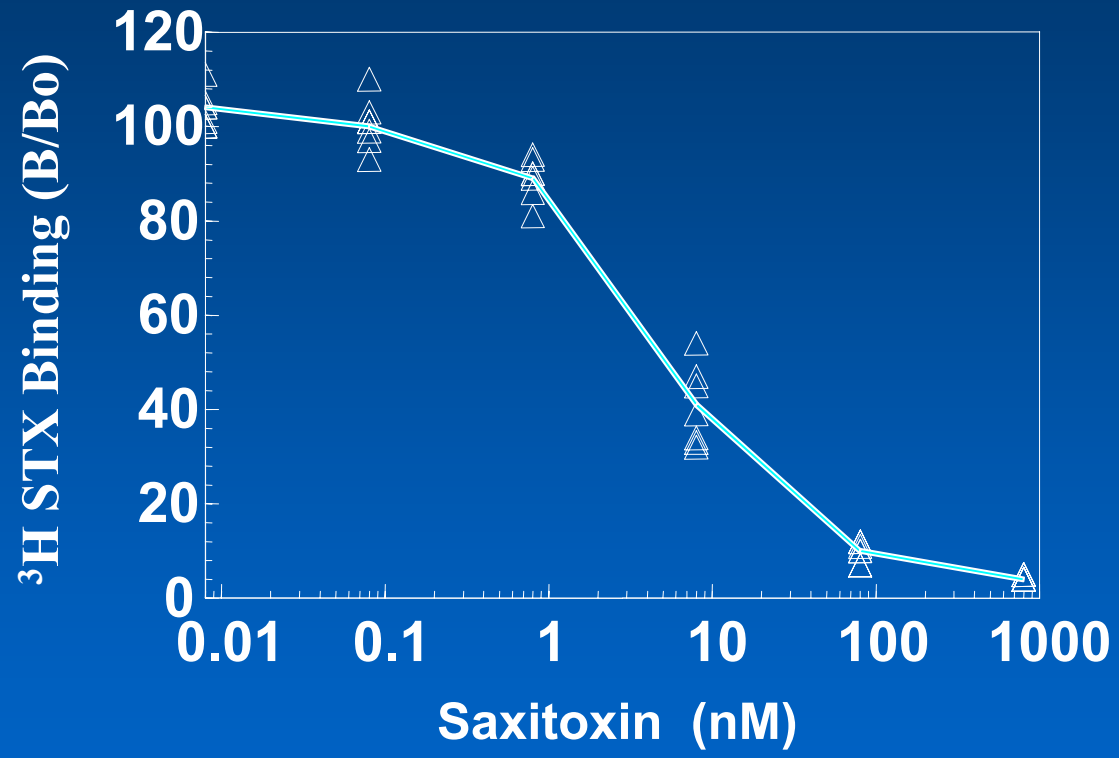


- detection limits in nM range
- assay response is proportional to the binding affinity of each toxin congener
- provides measure of integrated toxic potency of all toxin congeners present

Receptor Assays: High Throughput Format

- total assay time 3 h
- 13 samples/plate in duplicate at 3 dilutions
- minimal technical training

^{3}H STX Binding Competition Curve

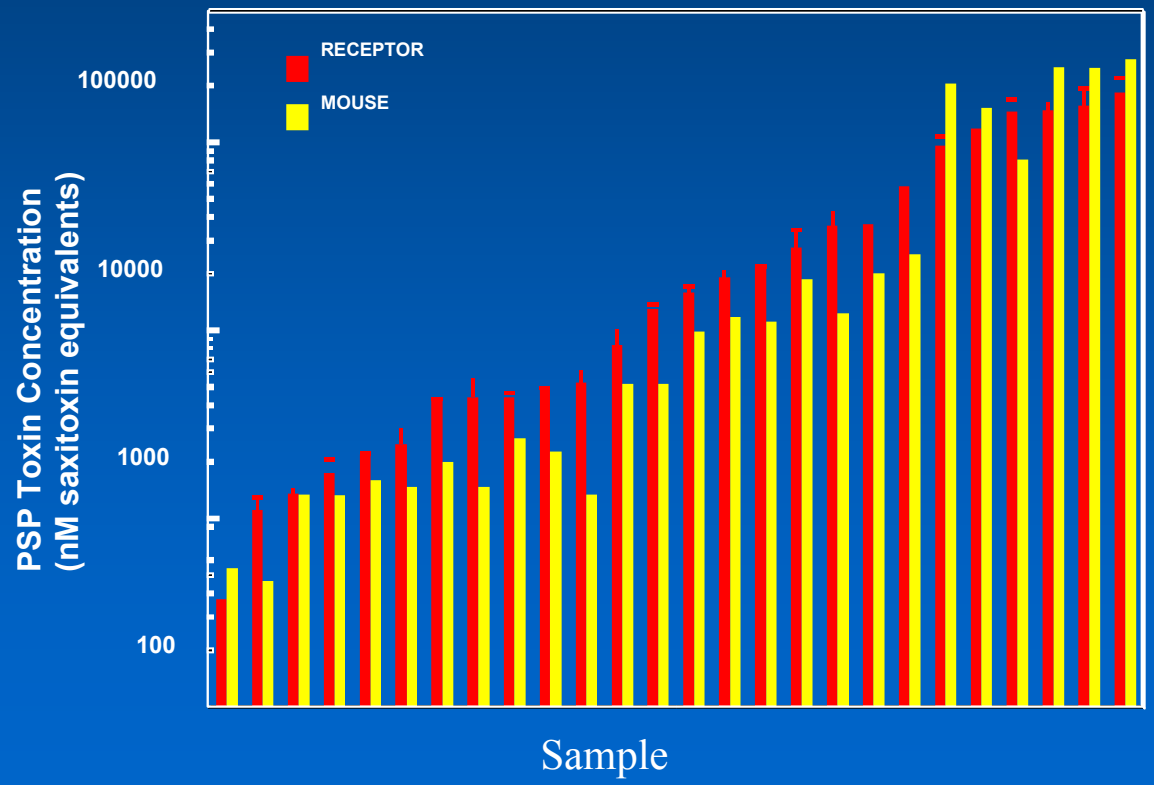


$K_i = 3.66 \pm 0.86$ nM in 7 independent assays run of different days

Limit of detection ~5 ng/ml in a sample extract

PSP Receptor Assay: Potential as a Regulatory Tool

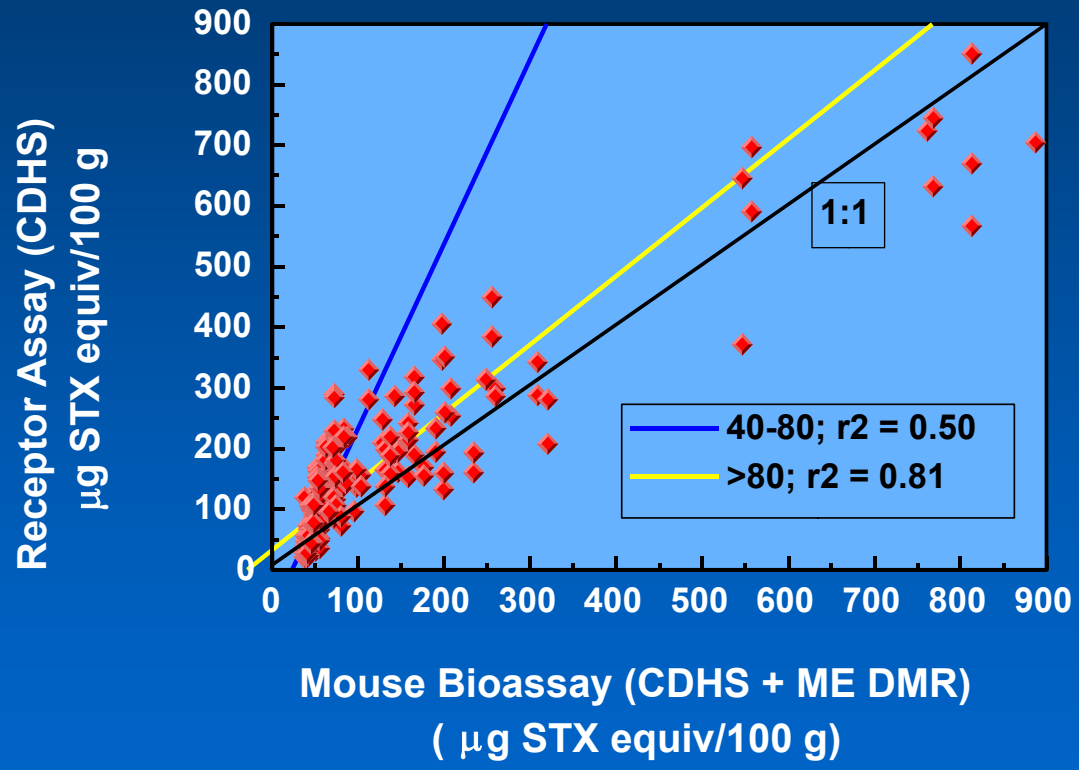
Method Comparison of Receptor Assay vs AOAC Mouse Bioassay for Determining PSP in Shellfish Extracts



Correlation coefficient r = 0.9464

Interlaboratory Comparison: Mouse Bioassay vs Receptor Assay

(NOAA Charleston, California Dept. Health Services, Maine Dept. Marine Resources)



** Assays agree well above the regulatory limit of 80 µg/100 g shellfish

Interlaboratory Comparison: Mouse Bioassay vs Receptor Assay

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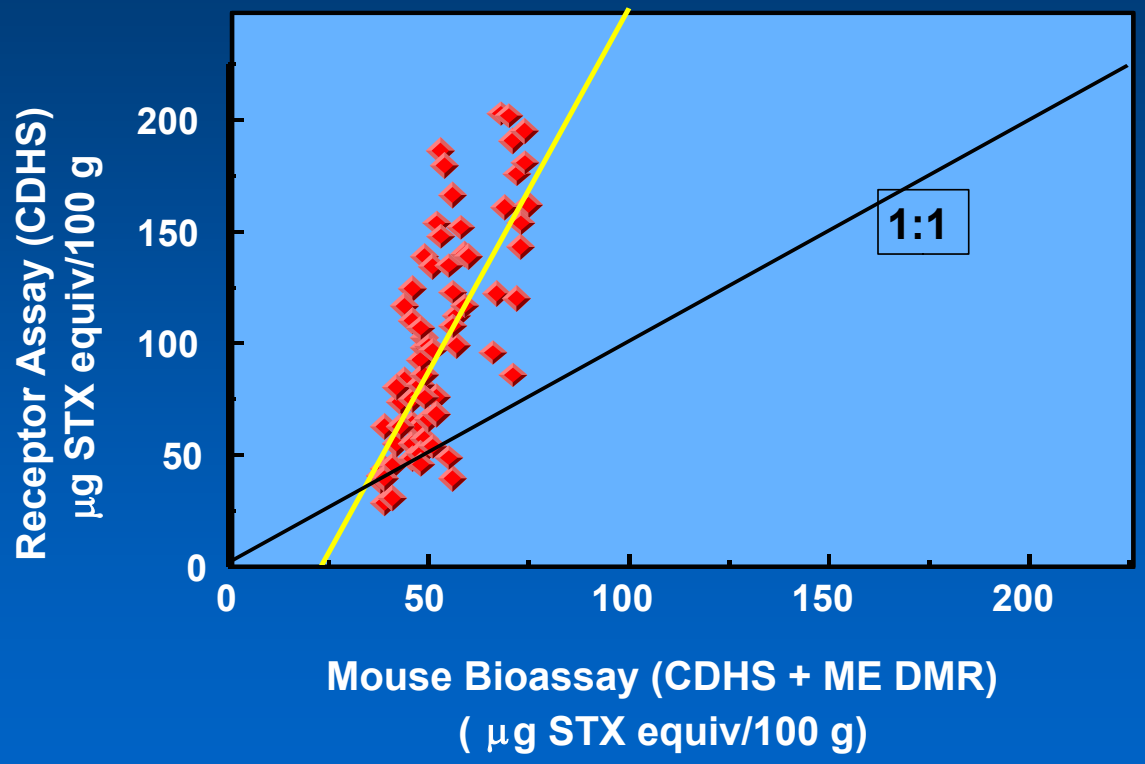


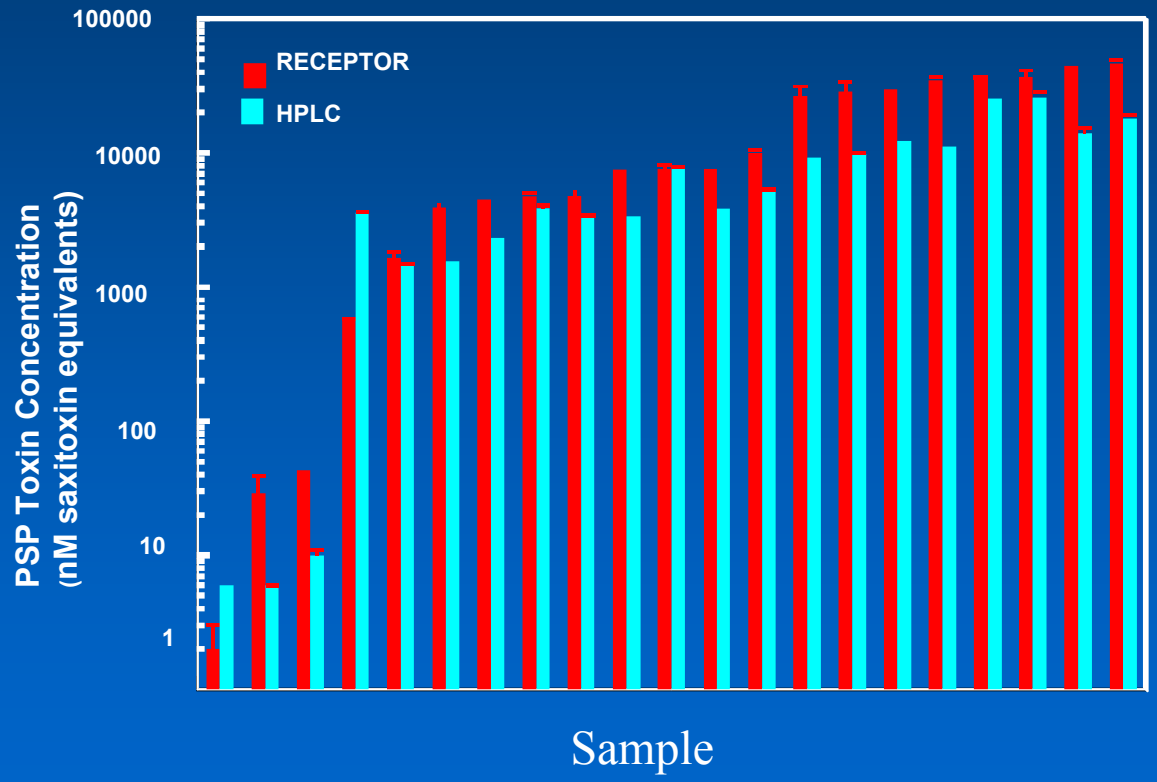
** undiluted sample matrix may cause underestimate in mouse bioassay < 80 mg/100 g shellfish

NOTE: Similar discrepancy observed HPLC vs mouse bioassay

(Study currently pending availability of ^{3}H STX)

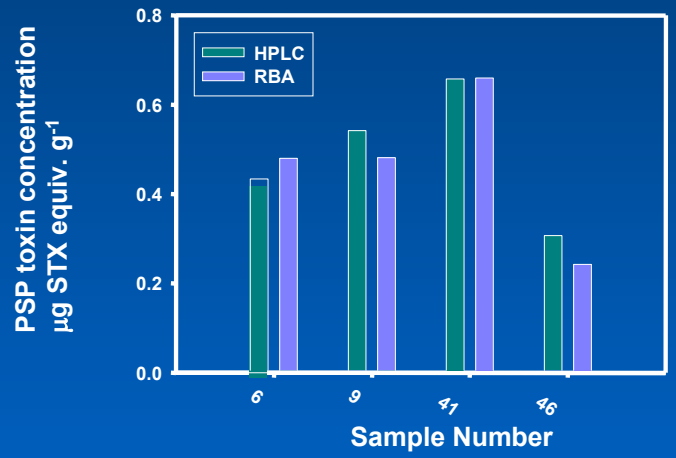
PSP Receptor Assay: Environmental Applications

Method Comparison between Receptor Assay and HPLC Analysis of Phytoplankton Extracts

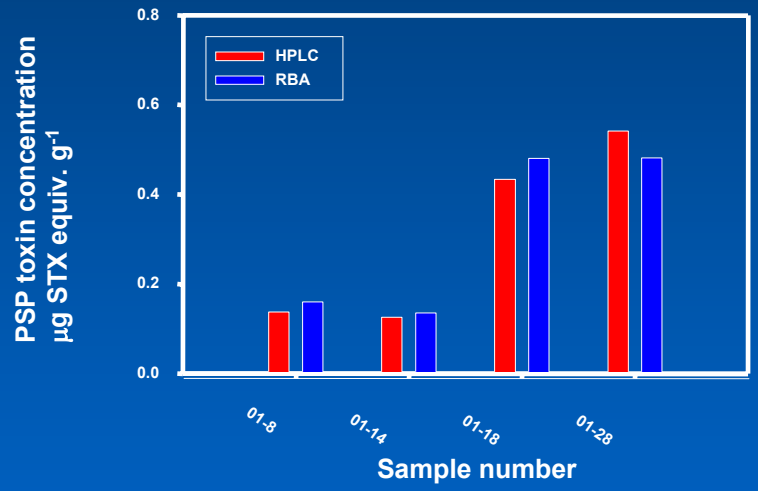


Correlation coefficient r = 0.8781

PSP Receptor Assay: Utility in Identification of Trophic Transfer of Saxitoxins to Endangered Right Whales

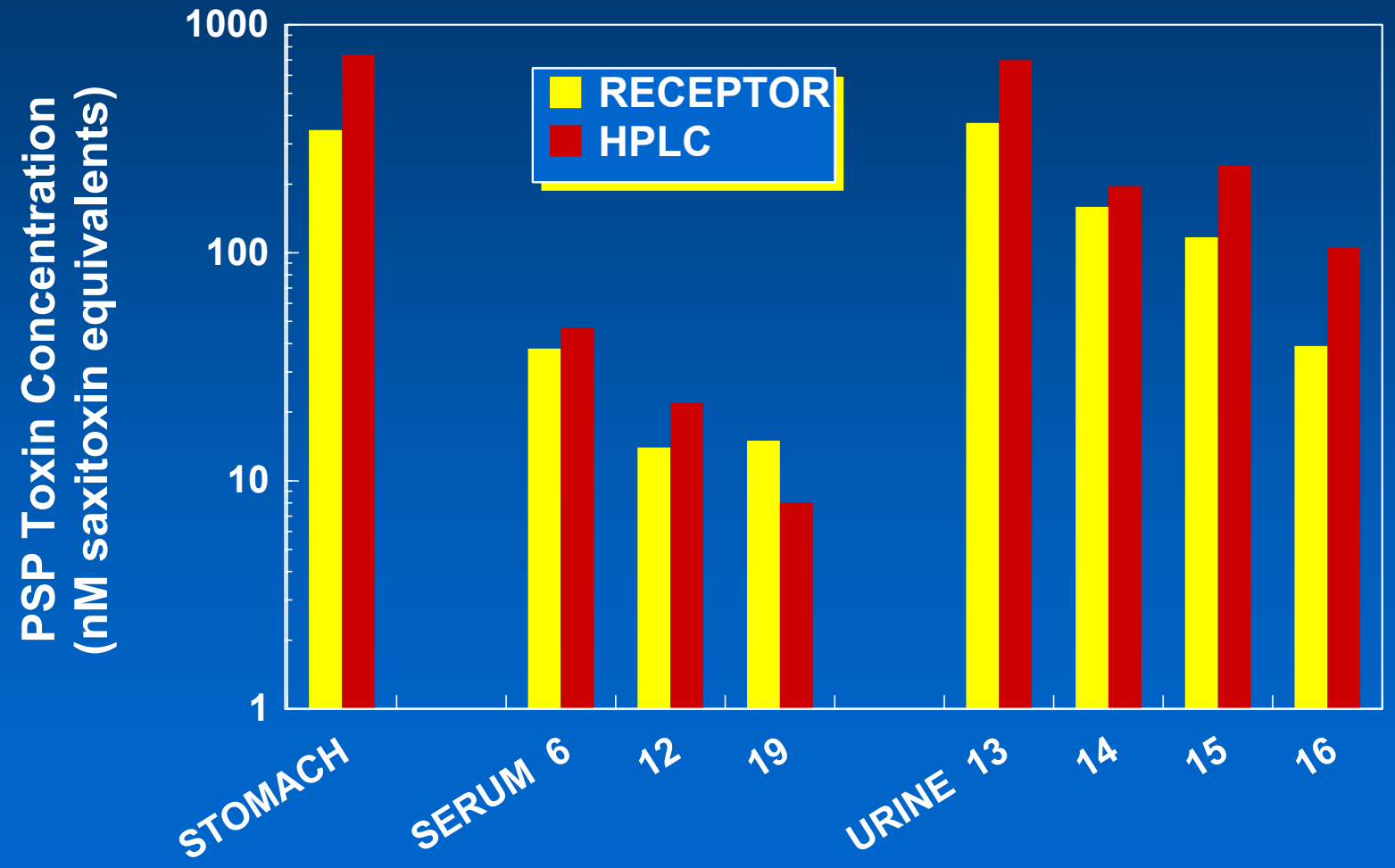


PSP toxin concentration in zooplankton net haul samples showing close agreement between values as determined by RBA and HPLC-FD.



PSP toxin concentration in right whale fecal samples showing close agreement between values as determined by RBA and HPLC-FD.

PSP Receptor Assay: Application to Exposure Assessment in Humans



Summary

- Receptor assays are advantageous over other testing methods when a measure of total toxic potency is desired

- The PSP receptor assay has become a robust, high throughput "workhorse" assay, with demonstrated utility in:
  - shellfish testing
  - environmental monitoring
  - human exposure assessment

- Acceptance of the PSP receptor assay for regulatory purposes requires formal interlaboratory calibration and provision of adequate long-term supply of reagents, both at the international level

Conclusion

The NOAA Marine Biotoxins Program strongly supports the role of the IAEA in leading the effort to bring the PSP receptor assay to international acceptance as a monitoring/regulatory tool for PSP toxin testing in seafoods and environmental samples

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