

EROD as bioindicator/Biomarker for monitoring of marine contaminants along the Dalian coasts



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Outline

1. Brief introduction to Biomarker
2. *EROD* as biomarker for monitoring marine contaminants along the Dalian coasts
3. Study on other biomarkers (*AHH*, *BPH*)
4. Work in the future



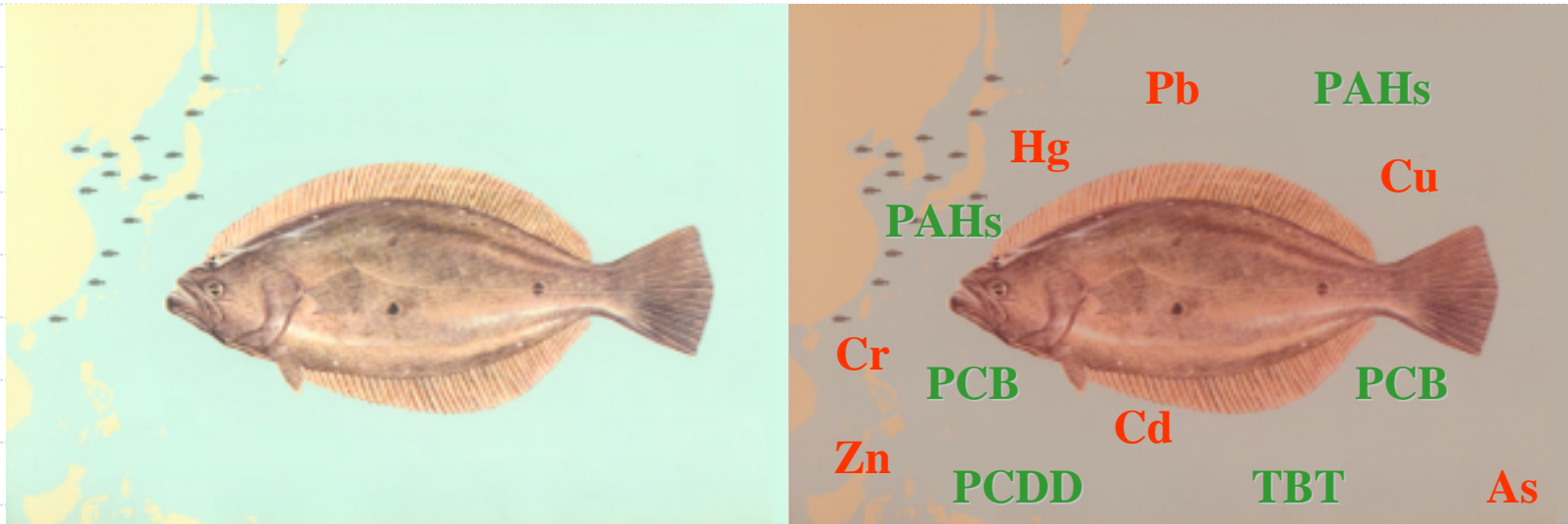
1 Brief Introduction to Biomarker

Biomarker, Biological marker

-  **What is biomarker?**
-  **Why biomarker is hot?**
-  **What biomarkers are available?**



What is biomarker?



Indicator for detecting 'internal changes' induced by exterior stresses

Idea from Dr. Seonock Woo



High

ECOLOGICAL SIGNIFICANCE

‘bioindicator organisms’ or ‘bioindicator species’ is used

*Loss of Habitat (1)

Ecosystem

*Reduced Diversity (2)

*Altered Abundance (2)

*Distributional Changes (2)

Bioindicators

community

*Reproductive Failure (2)

population

*Degenerative Diseases (2)

*Reduced Fecundity (3)

*Size Reduction (1)

individual

Biomarkers

*Reduced Growth Rate (3)

*Tissue Resorption (1)

*Negative Energy Balance (3)

sub-organismic

changes

*Abnormal Physiology (1-3)

*Cellular Pathology (1)

*Behavioural Changes (1)

Biomarkers

*Biochemical and Cellular Biomarkers (1-2)

Low

Early

DISTRESS SIGNALS DAMAGE

Late

“biomarkers” or “biomarker response”: sub-organismic changes occurring at cellular, biochemical, molecular, or physiological levels, that can be measured in cells, body fluids, tissues, or organs within an organism and are indicative of xenobiotic exposure and/or effect.

The Final Design Plan for the HOTO Module of GOOS, 2001

high

Ecological significance

*Loss of habitats(1) **

** Biodiversity decrease (2)*

** Species distribution change (2)*

**breeding capacity loss (2)*

** Immunity decrease (2)*

** Bearing lowering (2)*

** Individual size decrease (1)*

** Growth rate decrease (3)*

** Tissues mutation (3)*

** Negative energy balance (3)*

** Physiological abnormality (1-3)*

** Cell pathology (1)*

** Behavior change (1)*

** Biochemistry & cytology indexes (1-2)*

(1) → (3)
difficulty increasing for
measurement

early

late

Distress signals damage



Why biomarker is hot?

Characteristics:

- ⊕ SPECIFICITY
- ⊕ EARLY-WARNING
- ⊕ UNIVERSALITY

Advantage:

Some biomarkers are very specific in helping to establish **cause-and-effect relationships** between an exposure to contaminants and biological responses.

- Fast
- Micro level
- Low-priced







Biomarkers using in marine biomonitoring

- ⊕ **Acetylcholinesterase (AChE)**
- ⊕ **Cytochrome P450-1A1 (CYP4501A1)**
 - **7-ethoxyresorufine-O-deethylase (EROD)**
- **DNA Adduct formation**
- **Other biomarkers such as Metallothionein (MT)**



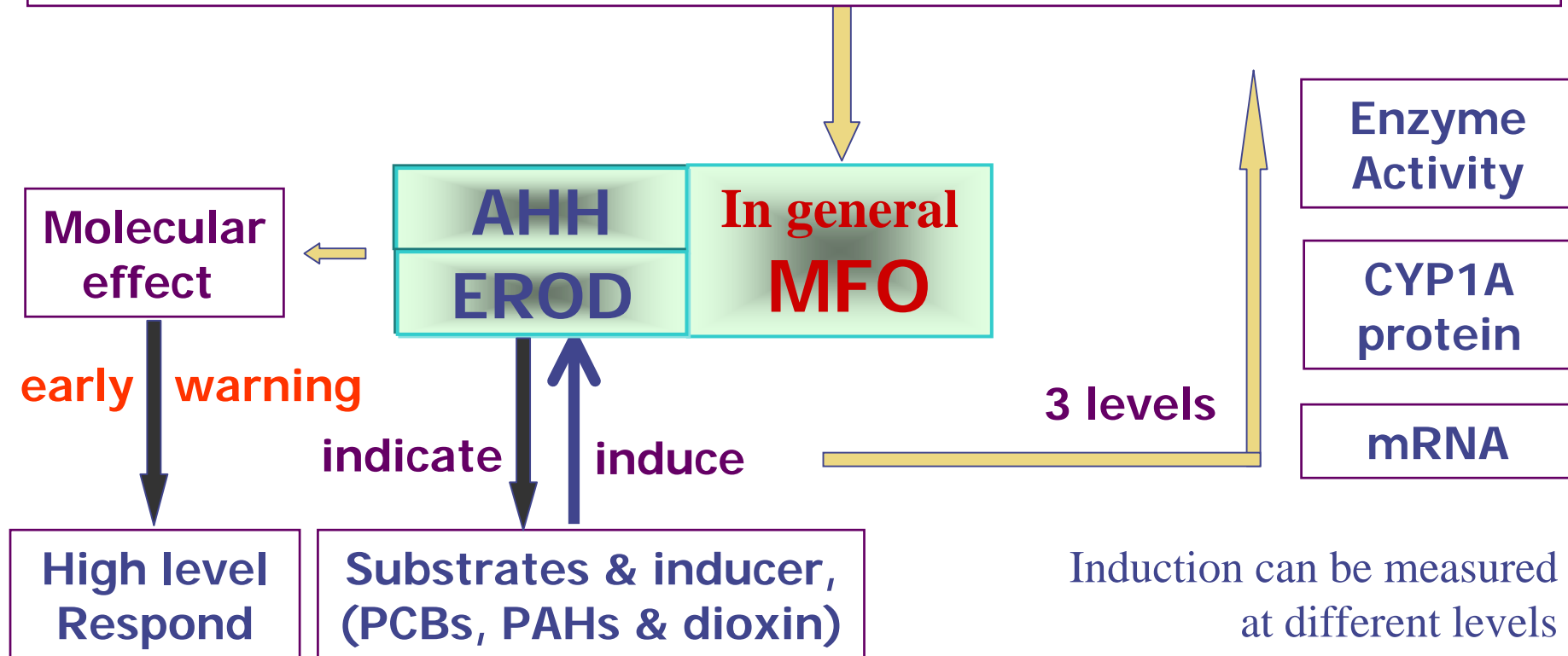
2 *EROD* as biomarker for monitoring marine contaminants along the Dalian coasts

-  **The study on EROD induction by organic contaminants in the liver of marine benthic fish *Paralichthys olivaceus***
-  **Investigation using EROD activity as biomarker and chemical analysis**
-  **The 3-year biomonitoring by measurement of EROD activities in fish**
-  **The hepatic EROD activities analysis by using a fluorescence plate-reader**



Studies on biomarkers focus on MFO of fish

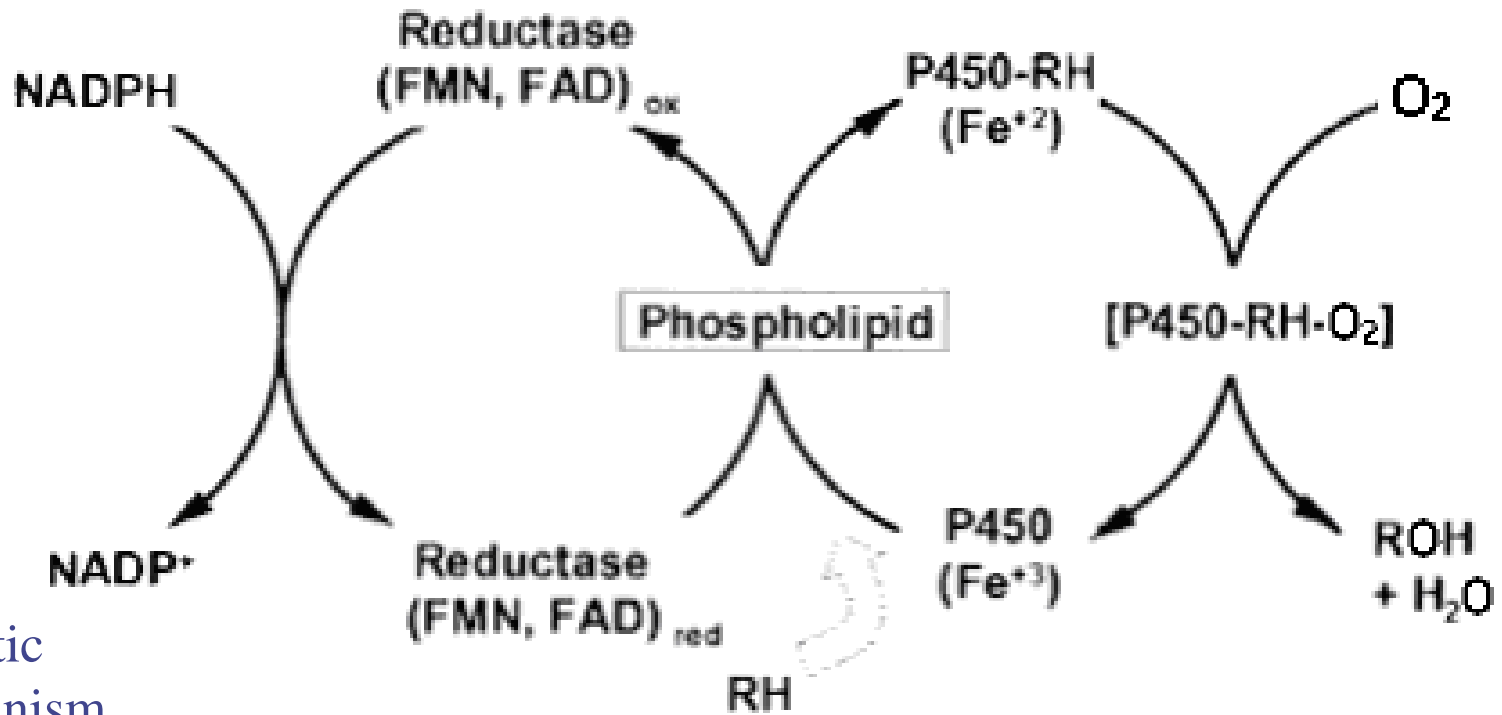
Mixing Function Oxygenase (MFO), an electron transport chain, including CYP-450, NADPH CYP-450 reductase & lecithoid



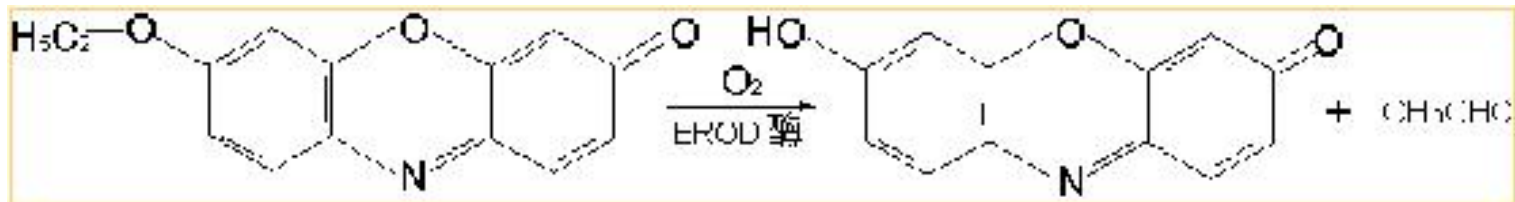
less reversible and more detrimental



Electron transfer and metabolism of substrates by microsomal CYP system



catalytic
mechanism



Bandiera S.M. (2001)

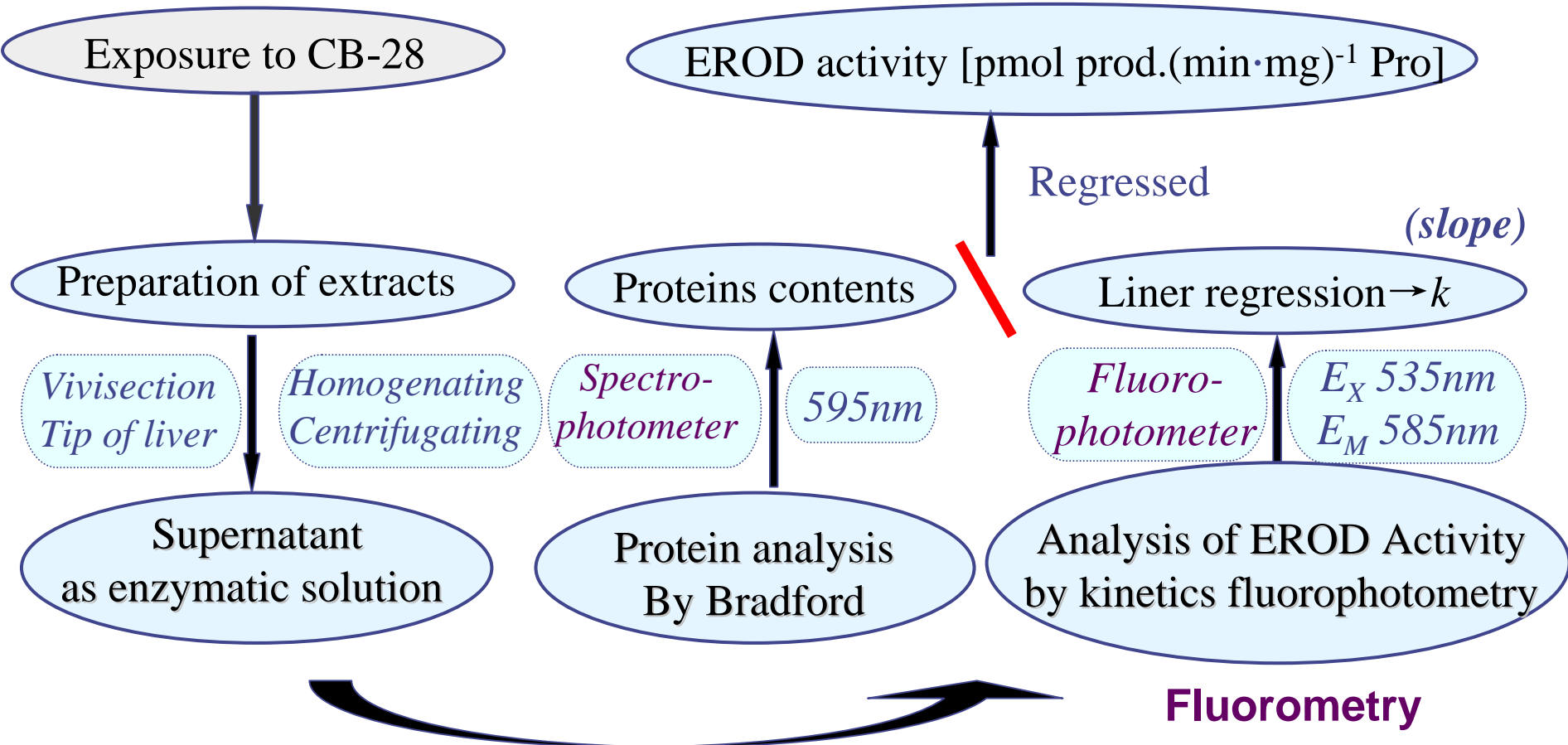


The study on EROD induction by organic contaminants in the liver of marine benthic fish *Paralichthys olivaceus*



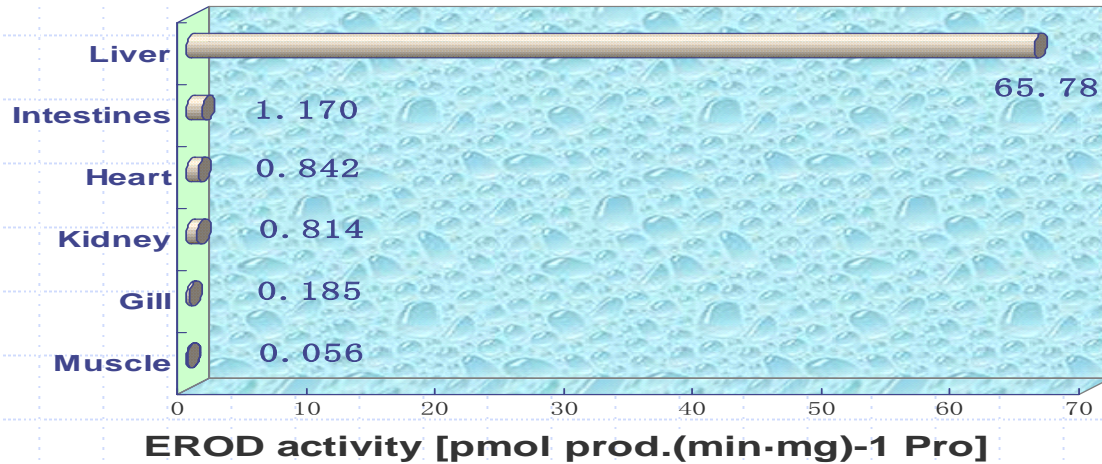
Resorufin

CB28



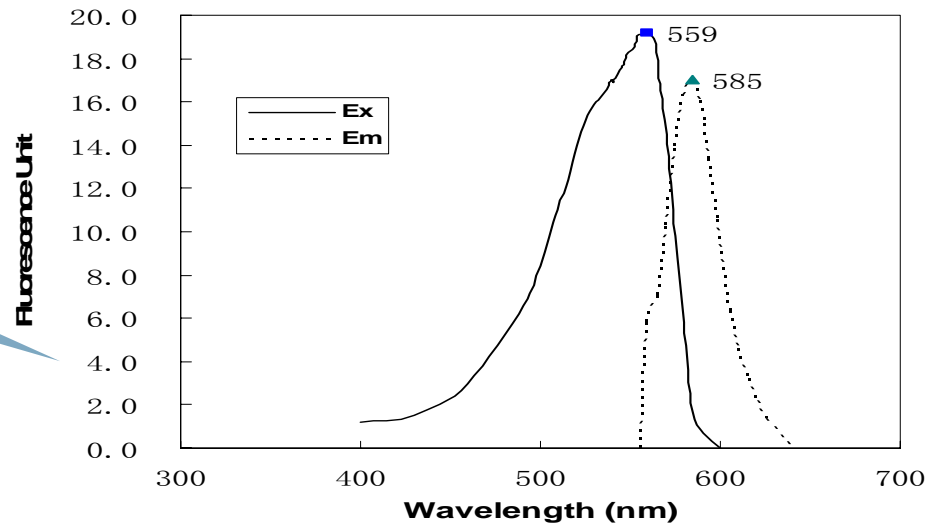


Results

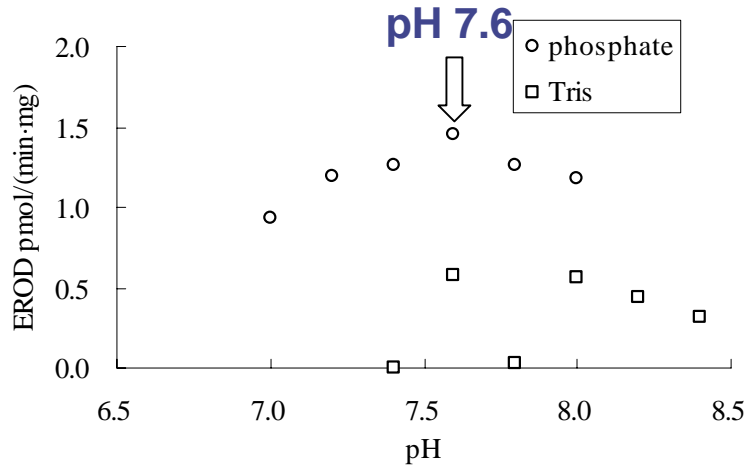


Test organ
Kirby et al. (1999)

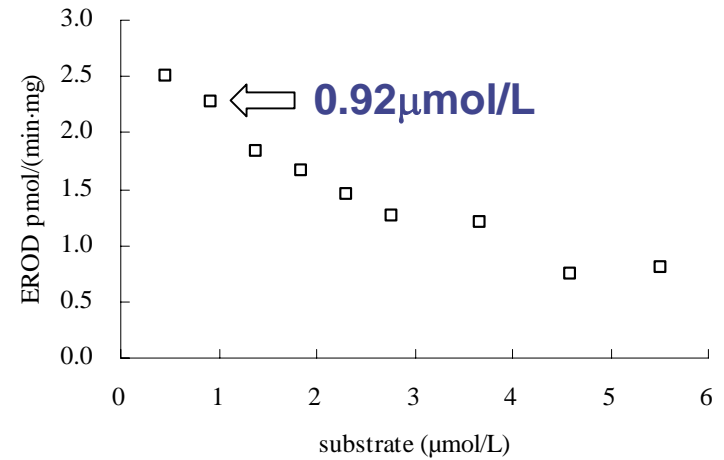
Selection of
Ex & Em



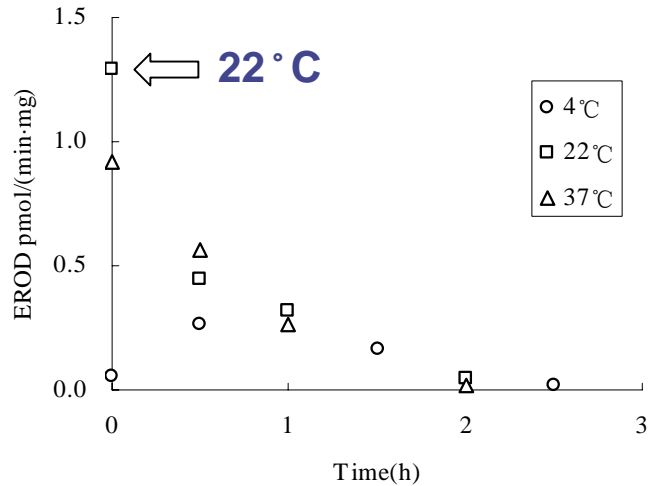
Influence of butter solution pH on EROD activities



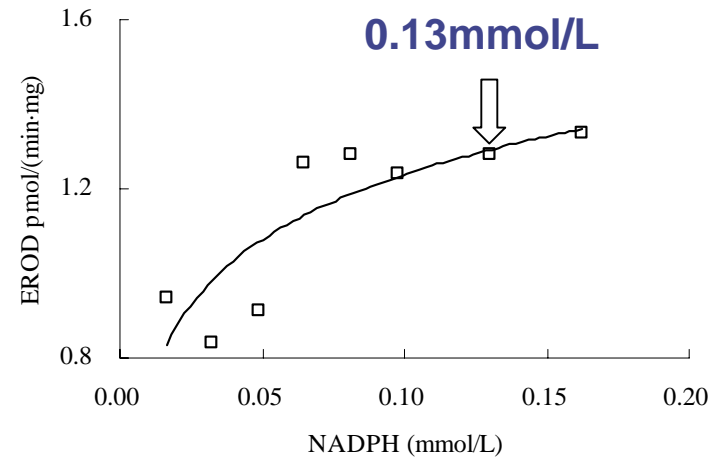
Influence of substrate content on EROD activities



Temperature effect on the stability of EROD activities



Dependence of EROD activities for NADPH



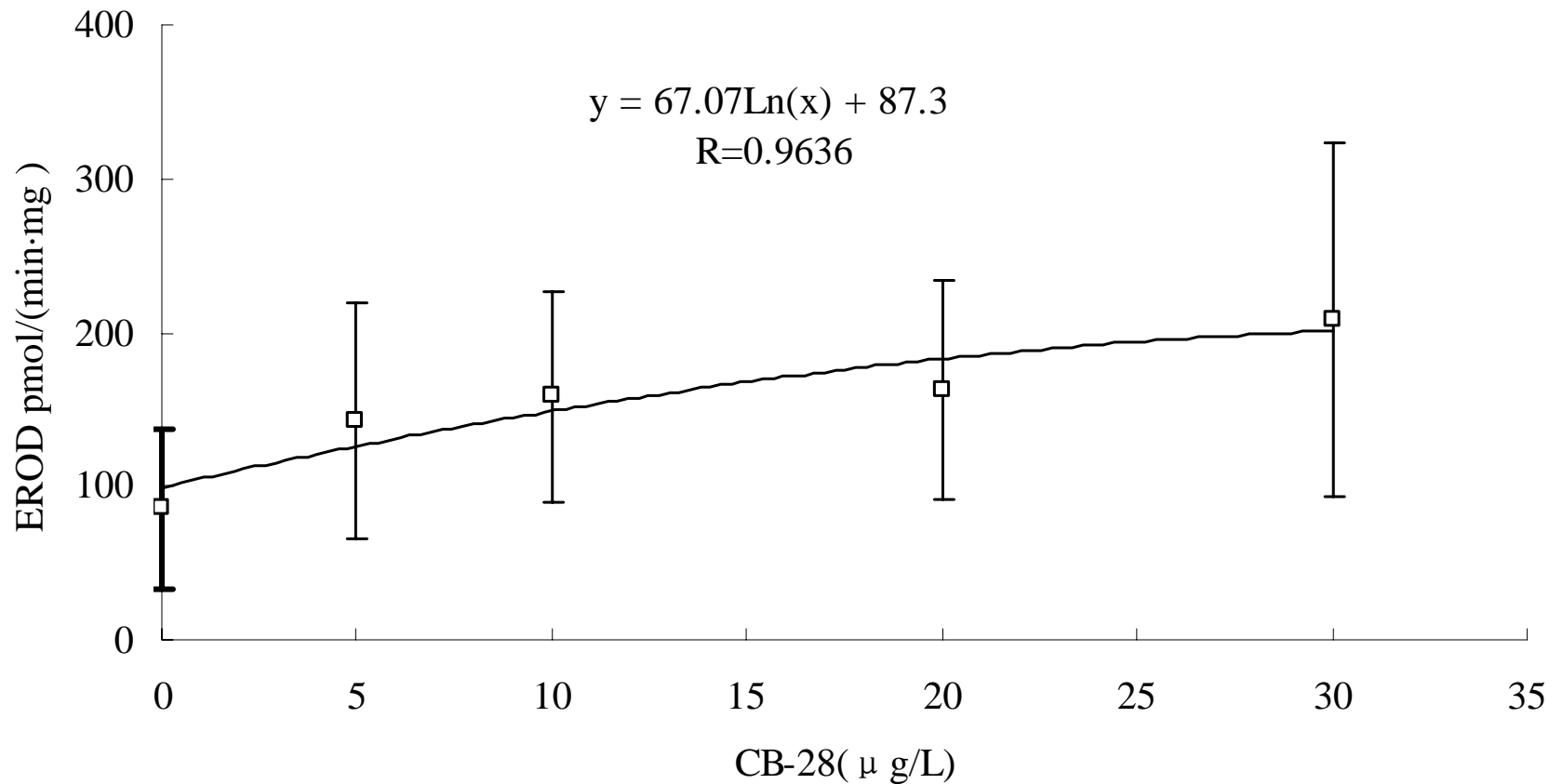


The relationship between some pollutants and response of EROD activities in *Limanda limanda*

Pollutants	Relationship r	Pollutants	Relationship r	Pollutants	Relationship r
Trace Metal		Lindane	0.630	CB-31	0.971
		Aldrin	0.405	CB-52	0.550
Arsenic	0.561	o.p' - DDE	0.278	CB-101	0.961
Cadmium	0.241	Dieldrin	0.012	CB-105	0.975
Copper	0.093		0.012	CB-118	0.966
Mercury	0.060	o.p' - DDD	0.192	CB-128	0.998
Zinc	0.007	p.p' - DDD	0.580	CB-138	0.939
		p.p' - DDT	0.002	CB-149	0.997
Pesticide				CB-153	0.981
		PCBs		CB-156	0.977
α-HCH	0.272			CB-170	0.995
HCB	0.046	CB-28	0.991	CB-180	0.986



Relationship between CB-28 contents and response of EROD activity (dose-effect/dose-response)





Investigation using EROD activity as biomarker and chemical analysis



 Investigation Area



Test species:
Hexagrammos otaki

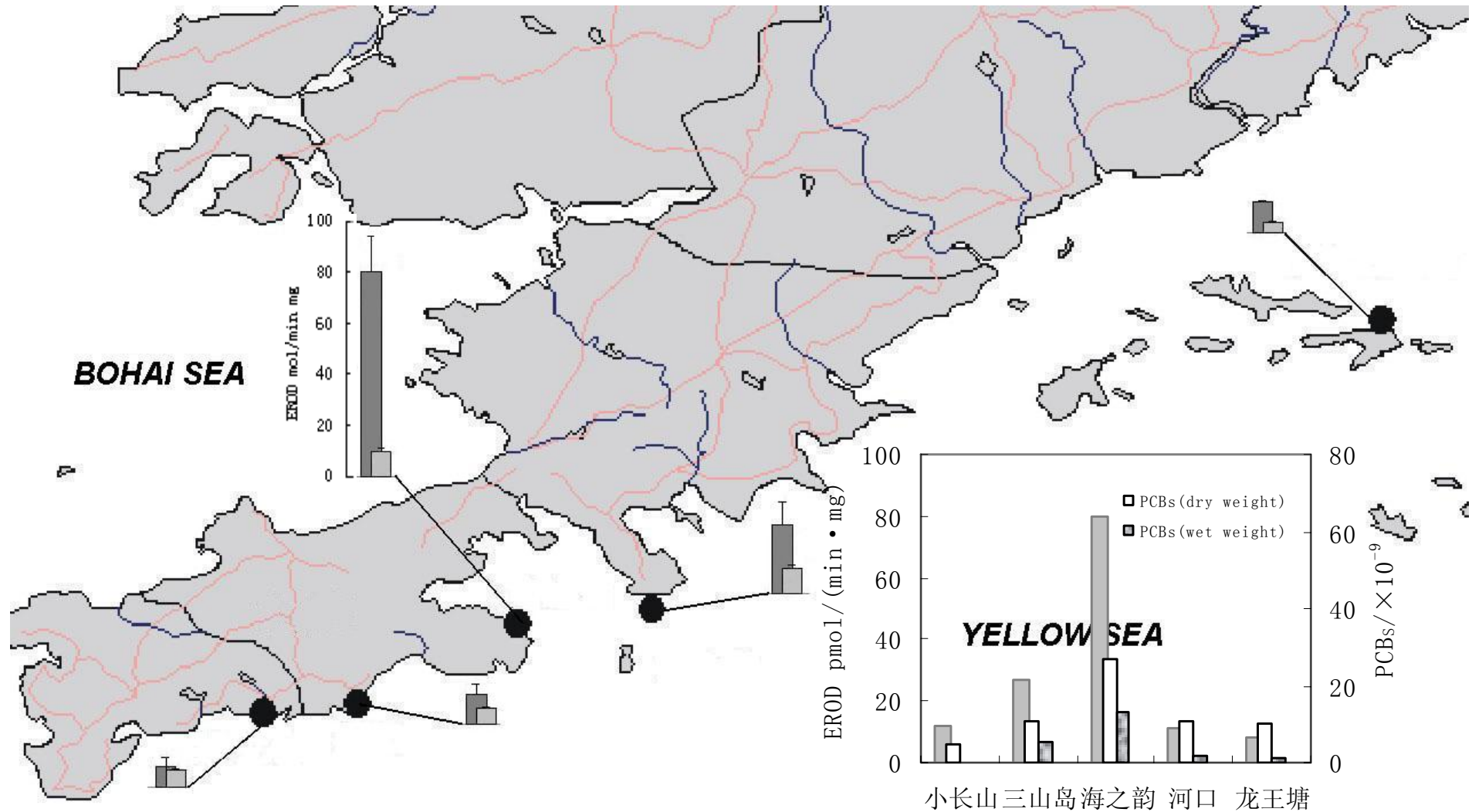
Sampling and pretreatment







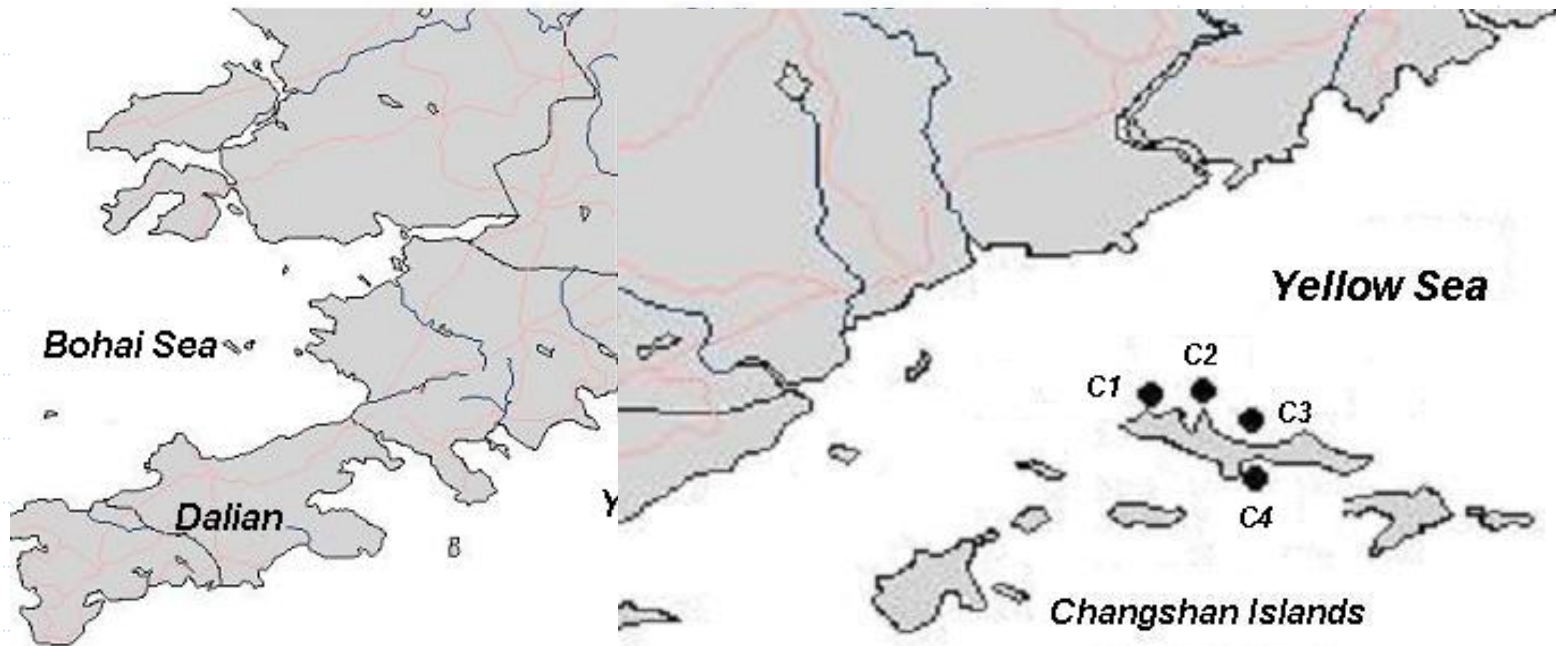
Investigation area and Results

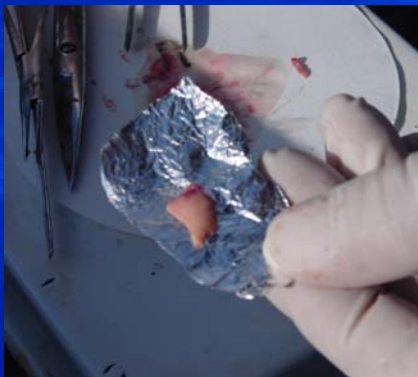
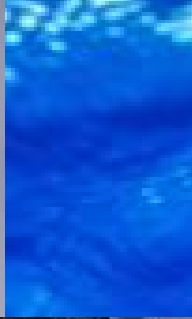




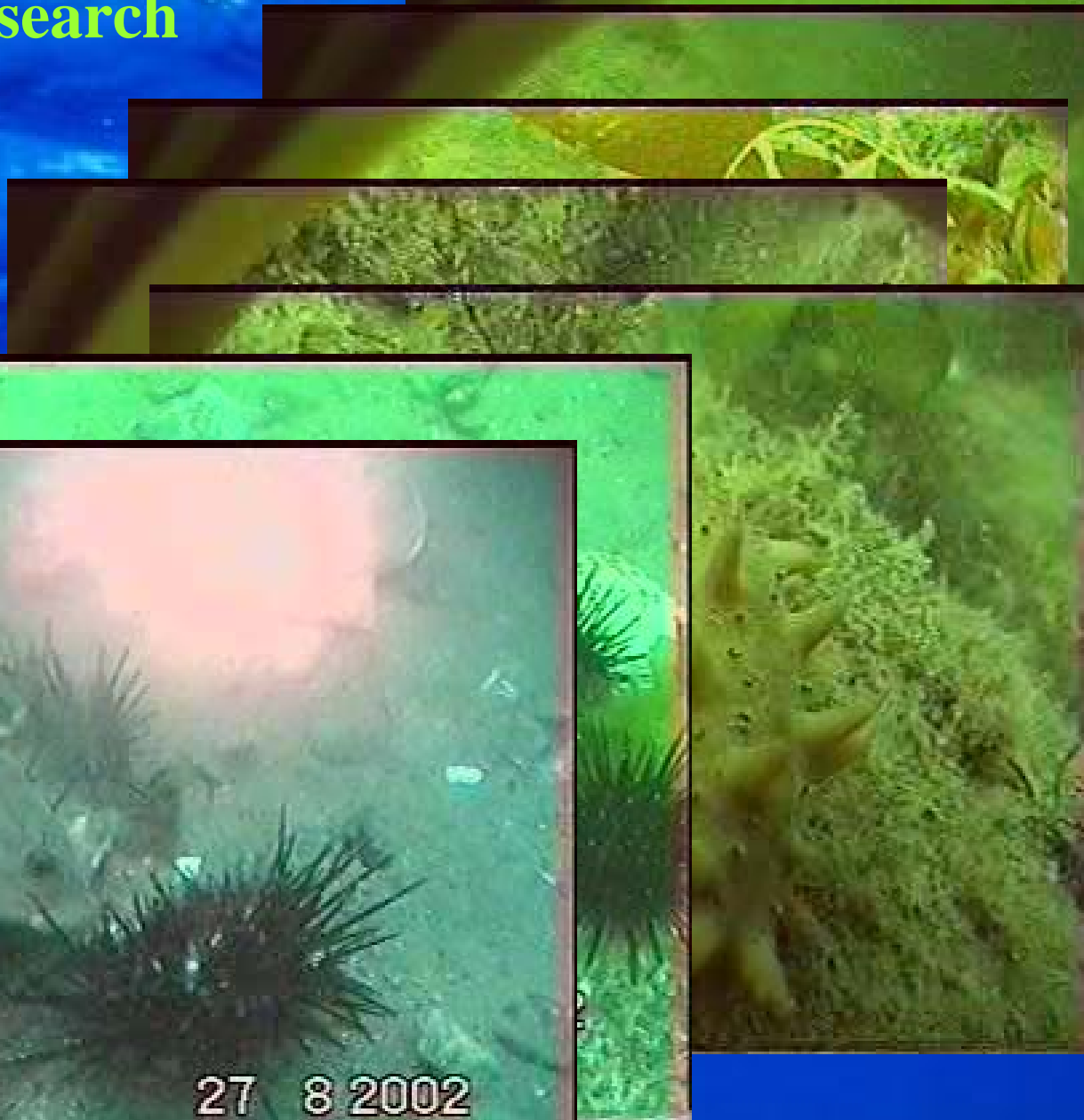
Folder The 3-year biomonitoring by measurement of EROD activities in fish

↪ Seasonal variation of EROD activity induction



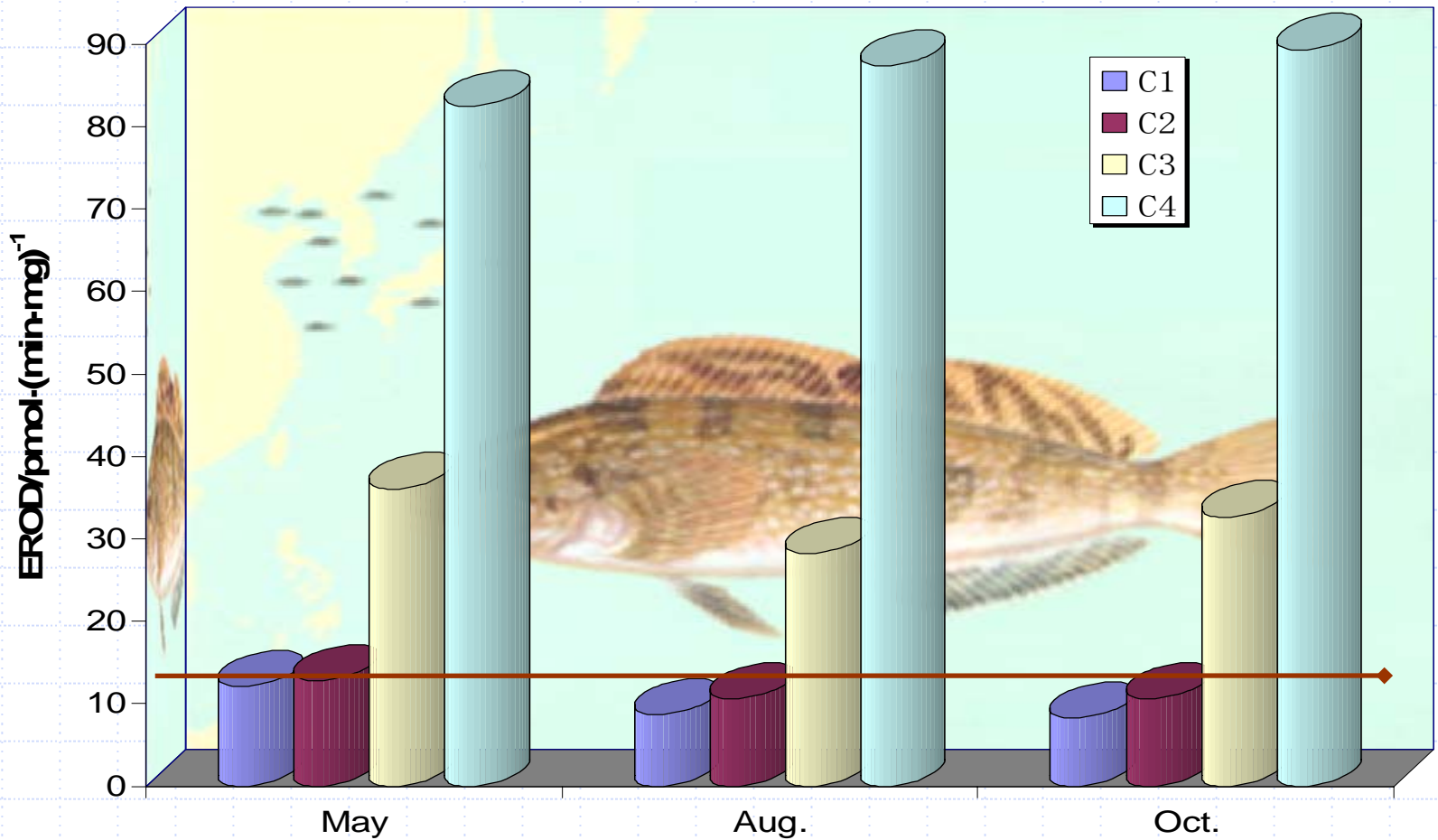


Habitat research
for the
tested fish



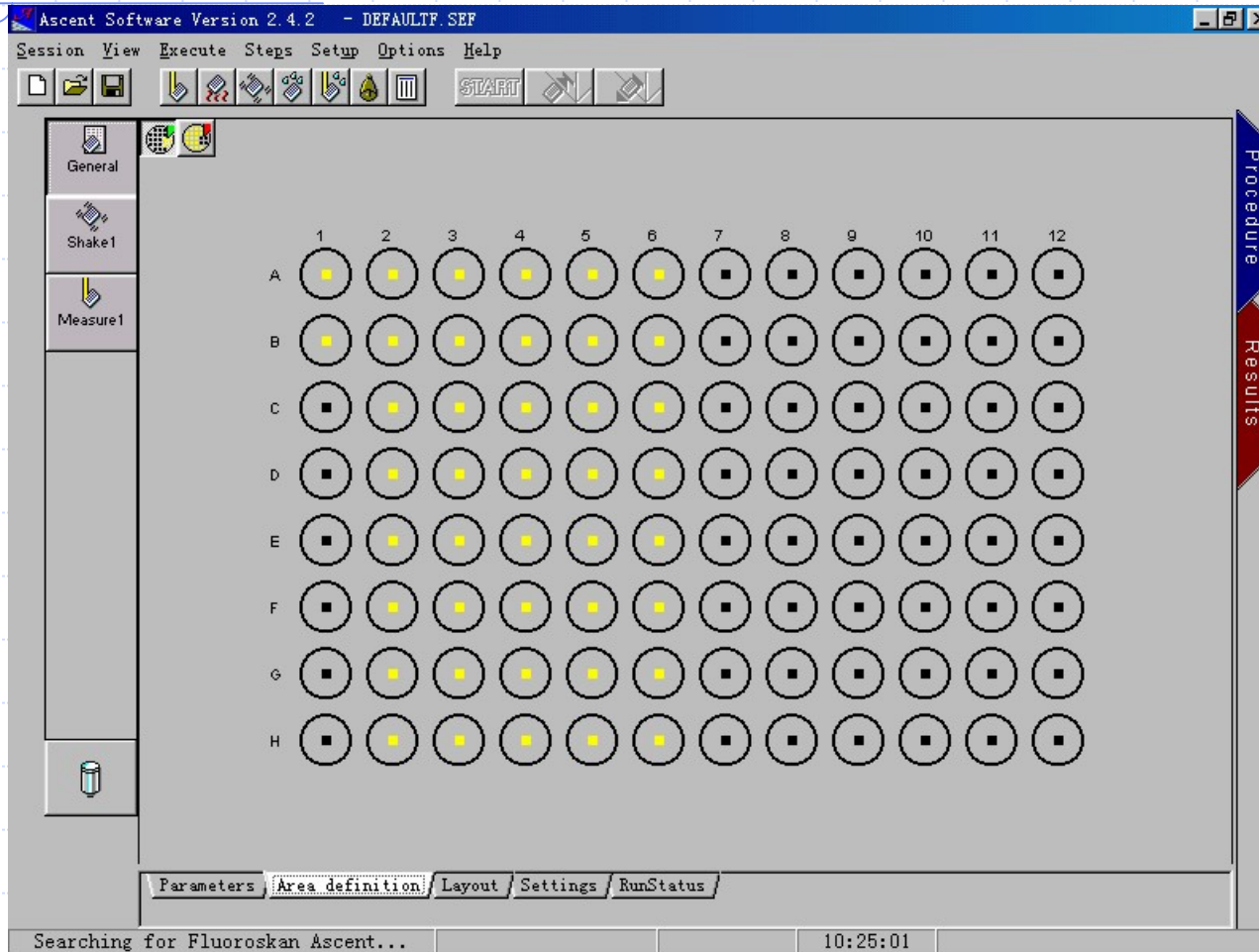


Seasonal variation of EROD activity induction





The hepatic EROD activities analysis by using a fluorescence plate-reader



The test species, test organ, conditions including sampling principle, sampling time and measuring method for analysis of EROD activity were the same as before.

Illustration of Micro-plate with 96 wells



The hepatic EROD activities analysis of fish by using a fluorescence plate-reader

Material and method

Plate-reader

Exposure to CB-28

Preparation of extracts

*Vivisection
Tip of liver*

*Homogenating
Centrifugating*

Supernatant
as enzymatic solution

Proteins contents

*Spectro-
photometer*

595nm

Protein analysis
By Bradford

Multiskan Ascent

EROD activity [pmol prod./ (min·mg) Pro]

Regressed

(slope)

Liner regression $\rightarrow k$

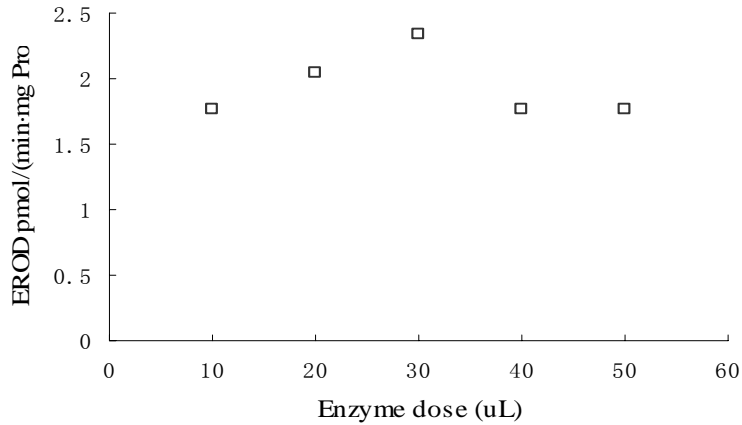
*Fluoro-
photometer*

E_X 530nm
 E_M 590nm

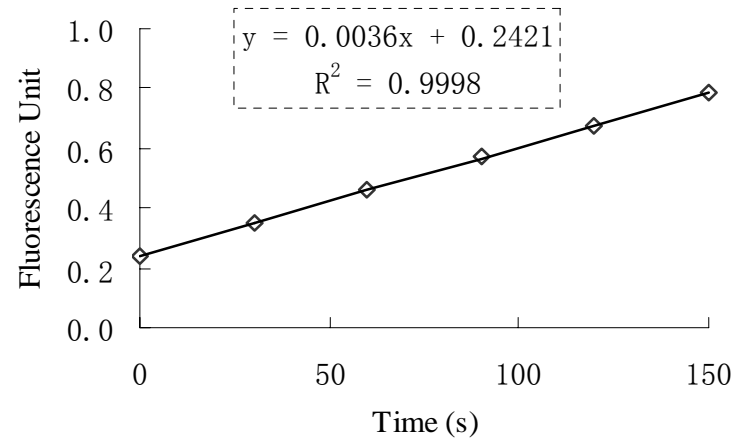
Analysis of EROD Activity
by kinetics fluorophotometry

Fluoroskan Ascent

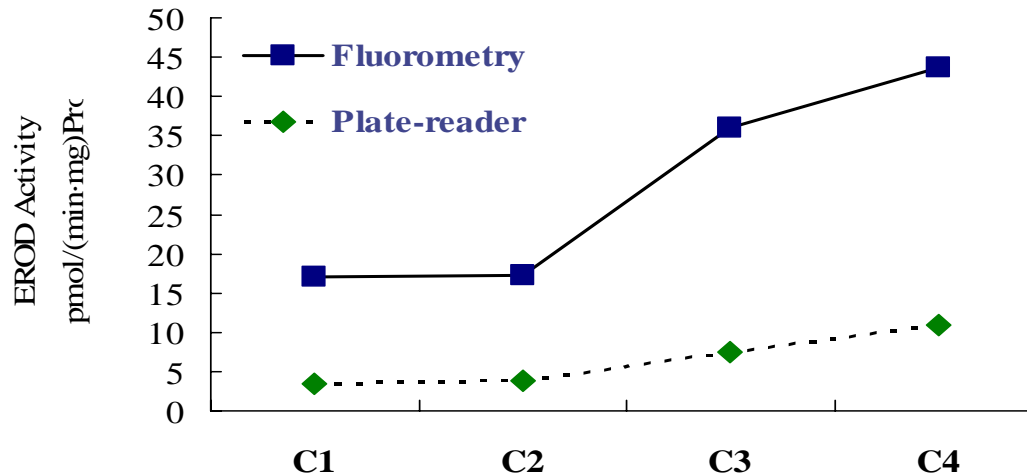
Influence of enzymatic solution dose on EROD activity



Calculation of slop with kinetic measurement data



Comparison of the EROD activity measured with the plate reader method and fluorescence spectrophotometry





Summary

- EROD induction was a potential biomarker and feasible indicator of screening contaminants in seawater such as PCB;
- The results showed the feasibility of using such an indicator in the field either with Kinetics Fluorescence Spectrophotometry or with fluorescence plate-reader;
- This approach can be a highly informative & cost-effective;
- The assessment would be more precise using biomarker combining with chemical analysis;
- It has to be noted that the factors affecting EROD activities in organisms are various;
- The capability of monitoring contamination by using single biomarker is limited, and a biomarker system should be more persuadable.



3 Study on other Biomarkers



Benzo[a]pyrene hydroxylase (BPH)

The study on benzo(a)pyrene hydroxylase (BPH) activity in the marine mussel, as a potential biomarker of contamination by PAHs-type compounds. (SOA youth grant No. 2002603)



Aryl hydrocarbon hydroxylase (AHH)

The study on Aryl hydrocarbon hydroxylase (BPH) activity for monitoring the marine benthic environment.(SOA youth grant No. 2003613)



4 Work in the future



Biomarker system

Biomarkers can provide a rapid and cost-effective screening tool, which can complement other testing techniques by **significantly reducing the number of samples** (not for specific evaluation); Thus, biomarker-based techniques have a major role to play in the **overall effort** of environmental monitoring and protection.



Methodology

Different levels: Protein level, Gene level, DNA level.



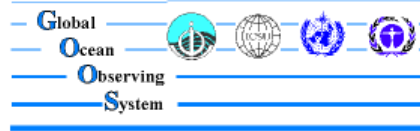
Scientific and Technical Advisory Panel to the Global
Environment Facility:
the use of bioindicators, biomarkers and analytical methods
for the analysis of POPs in developing countries

May 2004

Scientific and Technical Advisory Panel (STAP) to the Global Environment Facility (GEF)

May 2004

The use of bioindicators, biomarkers and analytical methods for the analysis of POPs in developing countries



Intergovernmental Oceanographic Commission

The Final Design Plan for the
HOTO Module of GOOS

GOOS Report No. 99
IOC/INF-1167

UNESCO 2001

The Final Design Plan for the HOTO (Health of the Oceans) Module of GOOS (Global Ocean Observing Systems)

5.2. BIOLOGICAL EFFECTS MEASUREMENTS

5.2.1 Molecular and Cellular Biomarkers.

5.2.2 Cellular Pathology

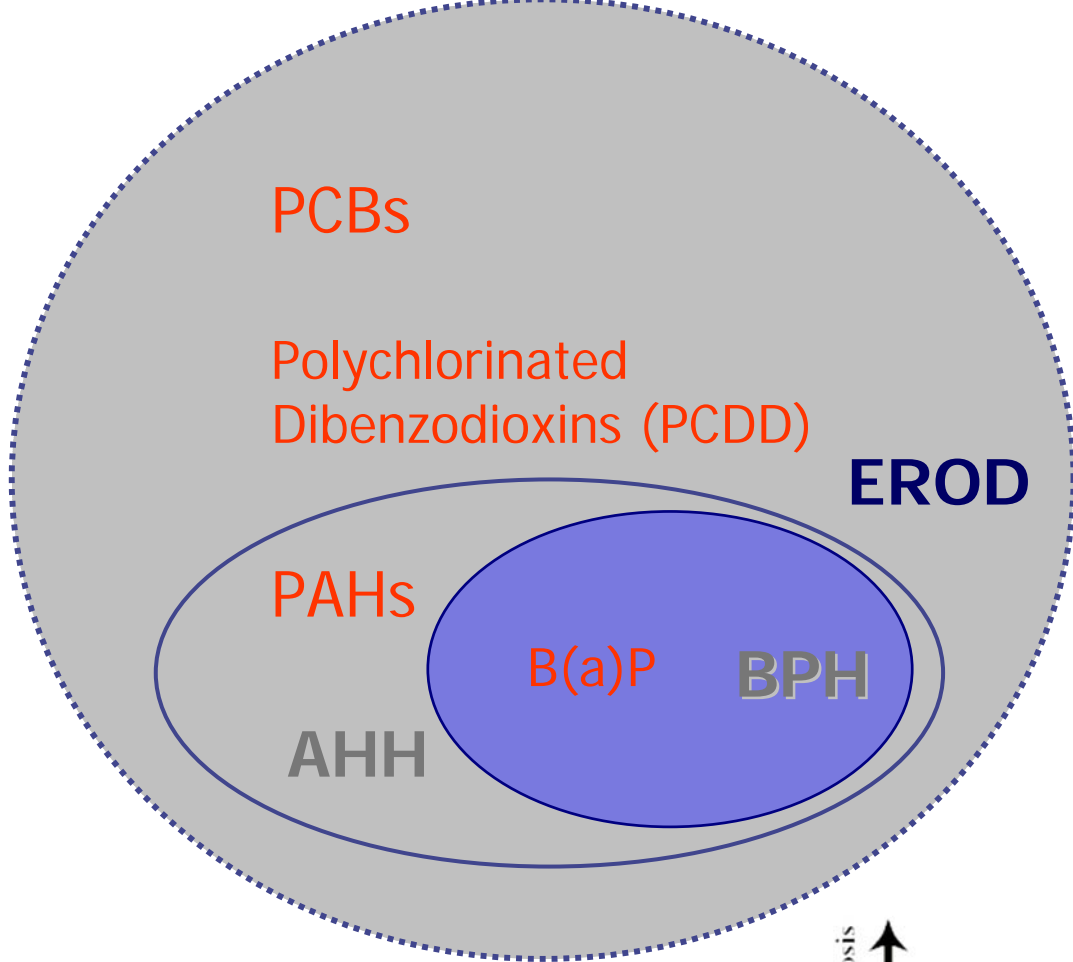
5.2.3 Physiological and Behavioural Responses


5.2.4 Population and Community Monitoring



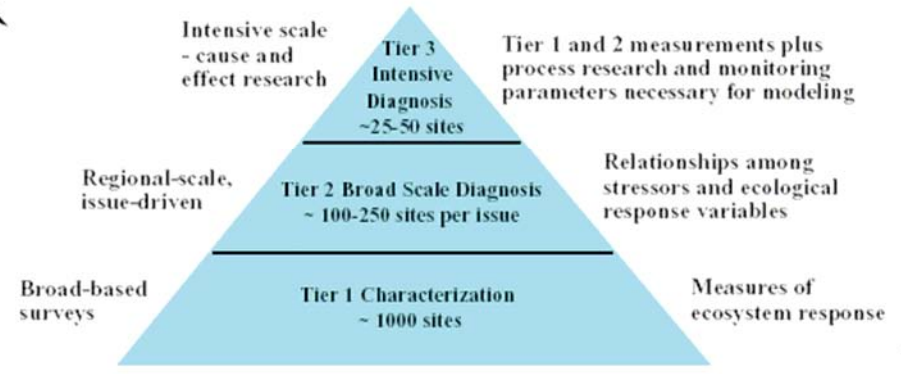
Biomarker system (focus on POPs)

Biomarkers	Issues addressed	Organism
EROD	Measures induction of enzymes which detoxify planar organic contaminants (e.g., PAHs, planar PCBs, dioxins)	Fish
AHH	Measures induction of enzymes which detoxify PAHs	Fish
BPH	Measures induction of enzymes which detoxify PAHs (BaP)	Bivalve molluscs
DNA adduct formation	PAHs, Other synthetic organics, e.g., nitro organics, amino triazine pesticides (triazines)	Fish Bivalve molluscs
AchE	Organophosphates (OP) and carbamates or similar molecules, Possibly algal toxins	Fish, crustacea, bivalve molluscs



 **Biomarker system**

↑ Increased site-specific diagnosis



↓ Increasing national coverage

Physical, chemical and biological parameters are measured in all tiers at different temporal and spatial intensity.

Source: Clean Water Action Plan, USA, 2000



Thank you!



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<http://www.nmemc.gov.cn>