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

HUO Chuan-Lin

National Marine Environmental Monitoring Center, SOA
Dalian, China

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Outline

1. Brief introduction to Biomarker
2. $\textit{EROD}$ as biomarker for monitoring marine contaminants along the Dalian coasts
3. Study on other biomarkers ($\textit{AHH}, \textit{BPH}$)
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
“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.
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)

Distress signals damage

Ecological significance

early

late

high
difficulty increasing for measurement
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

In general MFO

Enzyme Activity
- CYP1A protein
- mRNA

Induction can be measured at different levels

AHH EROD

Molecular effect
- Early warning
- Indicate
- Induce

High level Respond
- Substrates & inducer, (PCBs, PAHs & dioxin)

3 levels

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*

**CB28**

- **Exposure to CB-28**
  - Preparation of extracts
    - **Vivisection**
      - Tip of liver
    - Homogenating
    - Centrifugating
  - Supernatant as enzymatic solution

- **EROD activity [pmol prod.\(\text{min} \cdot \text{mg}\)^{-1} Pro]**
  - Proteins contents
    - Spectrophotometer
      - 595nm
  - Protein analysis
    - By Bradford
  - Liner regression \(\rightarrow k\)
    - Fluorophotometer
      - \(E_X\) 535nm
      - \(E_M\) 585nm
  - Analysis of EROD Activity
    - by kinetics fluorophotometry

**Fluorometry**
**Results**

**EROD activity [pmol prod.(min·mg)-1 Pro]**

<table>
<thead>
<tr>
<th>Organ</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>0.056</td>
</tr>
<tr>
<td>Intestines</td>
<td>0.814</td>
</tr>
<tr>
<td>Heart</td>
<td>0.842</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.185</td>
</tr>
<tr>
<td>Gill</td>
<td>0.170</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.056</td>
</tr>
</tbody>
</table>

65.78

**Selection of Ex & Em**

**Fluorescence Unit**

**Ex**

**Em**

**Test organ**

**Kirby et al. (1999)**
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*

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

*Eggen et al., 1992*
Relationship between CB-28 contents and response of EROD activity (dose-effect/dose-response)

\[ y = 67.07 \ln(x) + 87.3 \]

\[ R = 0.9636 \]
Investigation using EROD activity as biomarker and chemical analysis

Test species: *Hexagrammos otaki*
Sampling and pretreatment
Investigation area and Results
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

![Bar chart showing EROD activity (pmol·(min·mg)^{-1}) for May, Aug., and Oct. with categories C1, C2, C3, and C4. The graph illustrates higher EROD activity in October compared to May and August.]
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

Exposure to CB-28

Preparation of extracts

Vivisection
Tip of liver

Homogenating
Centrifugating

Supernatant
as enzymatic solution

Plate-reader

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

Proteins contents

Liner regression \( k \)

Regressed

Analysis of EROD Activity
by kinetics fluorophotometry

Analysis of EROD Activity
by kinetics fluorophotometry

Fluoroskan Ascent

Multiskan Ascent

Fluorophotometer

Spectrophotometer

Protein analysis
By Bradford

Protein analysis
By Bradford

By Bradford

595nm

590nm

EX 530nm

EM 590nm

EX 530nm

EM 590nm
Influence of enzymatic solution dose on EROD activity

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

Calculation of slope with kinetic measurement data

\[ y = 0.0036x + 0.2421 \]

\[ R^2 = 0.9998 \]
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.
The use of bioindicators, biomarkers and analytical methods for the analysis of POPs in developing countries
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)

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Issues addressed</th>
<th>Organism</th>
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<tbody>
<tr>
<td>EROD</td>
<td>Measures induction of enzymes which detoxify planar organic contaminants (e.g., PAHs, planar PCBs, dioxins)</td>
<td>Fish</td>
</tr>
<tr>
<td>AHH</td>
<td>Measures induction of enzymes which detoxify PAHs</td>
<td>Fish</td>
</tr>
<tr>
<td>BPH</td>
<td>Measures induction of enzymes which detoxify PAHs (BaP)</td>
<td>Bivalve molluscs</td>
</tr>
<tr>
<td>DNA adduct formation</td>
<td>PAHs, Other synthetic organics, e.g., nitro organics, amino triazine pesticides (triazines)</td>
<td>Fish, Bivalve molluscs</td>
</tr>
<tr>
<td>AchE</td>
<td>Organophosphates (OP) and carbamates or similar molecules, Possibly algal toxins</td>
<td>Fish, crustacea, bivalve molluscs</td>
</tr>
</tbody>
</table>
Source: Clean Water Action Plan, USA, 2000

PCBs
Polychlorinated Dibenzodioxins (PCDD)
PAHs

EROD

B(a)P
BPH

Biomarker system

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

Email: clhuo@nmemc.gov.cn
http://www.nmemc.gov.cn