국내 초청강연 - 2

다단계 바이오마커를 이용한 생태독성 모니터링

Ecotoxicity mionitoring using multilevel biomarkers and its application in invertebrates

최진희

서울시립대학교 환경공학과

Abstract

Monitoring toxicity levels in specific biological compartments is necessary to evaluate the ecotoxicological risk associated with environmental pollution. Biomarkers are increasingly used as rapid early warning systems in environmental monitoring and ecological risk assessment procedures. Despite this increasing use, biochemical endpoints alone are not sufficient to diagnose environmental quality. Changes in biomarkers should be investigated in connection with effects at higher levels of biological organization, to ensure that they can really be considered as an "early warning" signal. Numerous studies demonstrate that the simultaneous use of several biologicalparameters can provide complementary information about the effects of chemical exposure.
Keywords. biomarker, environmental monitoring, ecological risk assessment, early warning system, multilevel biomarker, invertebrate

1. Introduction

In many developed countries, the enforcement of specific regulations had a significant positive effect on the level of environmental pollution in the last decades, especially through a reduction in point source pollution (e.g. building of

sewage treatment plants) and the ban of some persistent chemicals (e.g. DDT, toxaphene). However point source pollution is still a matter of concern in numerous countries and non-point source pollution by organic (e.g. pesticides, dioxins) and inorganic (e.g. heavy metals) compounds is still a matter of concern worldwide.

The assessment of environmental quality implies that the biological effects of pollutants could be monitored using adapted tools. Ecotoxicology is a multidisciplinary science which focus on the adverse effects of toxicants at various levels of biological organization and which may provide such tools. Ecotoxicological researches have first been devoted to the study the effects of environmental contaminants at the population, community or ecosystem levels (Forbes & Forbes, 1994). However, these traditional approaches are sometimes inefficient, especially to adequately assess the effects of chronic exposure of organisms to low levels of xenobiotics and to detect early biological responses. Therefore, therehas been a shift in emphasis towards understanding the sublethal effects of long-term exposure to contaminants at the individual level where exposure can be adequately described and assessed (Newman & Jagoe, 1996). It has been necessary to perform studies on individuals at the biochemical and molecular levels where toxicant-induced responses are initiated.

The effects of toxicants usually begin through an interaction between toxicants and biomolecules (e.g. enzymes, receptors, DNA). Effects then cascade through the molecular, biochemical, subcellular, cellular, tissue, organ, individual, population, community and ecosystem levels of organization. Therefore, the understanding of the effects of toxicants at the molecular or biochemical levels may provide some insights into the cause of effects identified at higher levels (Newman & Unger, 2003). The biomarker approach can be an extremelyuseful tool for this kind of investigation and it has been increasingly used for environmental hazard assessment during the last ten years (Delpedge & Fossi, 1994; Fossi et al, 2000).

2. Biomarker-based environmental monitoring

The historical development of the biomarker approach is closely linked to advancesin medicine and vertebrate biology (NRC, 1987). Biomarker measurements are now equally feasible in many plants and animal species (Livingstone, 1991; Depledge & Fossi, 1994 Fossi et al., 2000 Lagadic *et al.*, 2000). Biomarkers were originally defined as xenobiotically-induced variations in cellular or biochemical components or processes, structures or functions that are measurable in a biological system or sample (NRC, 1987). They were first classified as markers of exposure to a toxicants, markers of effects of exposure and markers of susceptibility to the effects of exposure (NAS/NRC, 1989). This definition has been challenged by several authors (Adams, 1990; Engel & Vaughan, 1996; McCarty & Munkittrick, 1996) and the term biomarker is now more commonly used in a more restrictive sense, namely sublethal biochemical changes resulting from individual exposure to xenobiotics (Hyne & Maher, 2003).

The biomarker approach has receivedconsiderable attention in ecotoxicology as a new and potentially powerful and informative tool for detecting and documenting exposure to, and effects of, environmental contamination (Newman & Jagoe, 1996). The primary use of biomarker in environmental monitoring is to assess the health of organismsin order to detect and identify potential problems so that unacceptable and irreversible effects at higher levels of biological organization can be avoided. It is important, however, to keep in mind that our current understanding of biomarker responses in wild species is limited. To achieve the full potential of this tool for the protection of the environment, a great deal of research is still needed to develop, validate andinterpret biomarker based monitoring.

3. Potentials and limitations of biomarker in environmental monitoring

Chemical pollution is often caused by a complex mixture of compounds, which makes the exhaustive analysis of the contaminants present in polluted environment impossible (Risso-de-Faverney et al., 2001 Meregalli et al., 2002).

Moreover, the mere presence of a pollutant does not indicate an impact on organisms, as its bioavailability may be influenced by many factors (see e.g. Landrum & Robbins, 1990). The use of biomarkers to assess the biological and ecological significance of environmental contaminants is a complementary approach to chemical analysis and is becoming an important component of many environmental monitoring programs. Organisms can provide more complete information on the impacts of the toxicants than chemical analysis alone because some of them can integrate the exposure to contaminants and respond in some measurable and predictable ways (Vermeulen, 1995). Responses can be observed at several levels of biological organization from the biomolecules level, where pollutants can cause damage to critical cellular targets and elicit cellular mechanisms of defensesuch as detoxication (e.g. cytochrome P450 associated enzymatic activities, glutathione S-transferases) and repair process (e.g. DNA repair enzymes), to the organismal level, where severe disturbances such as impairment in growth, reproduction, developmental abnormalities, or decreased survivalmay be observed (Newman & Jagoe, 1996).Biomarkers can provide not only evidence of exposure to a broad spectrum of anthropogenic chemicals, but also a temporally integrated measure of bioavailable contaminants. A suite of biomarkers should preferably be used to determine the magnitude of the problem at the individual level and evaluate possible consequences at the population or community levels (Cormier & Daniel, 1994).

Recently, the growing awareness of the possibility of using wildlife animals as sentinels for human environmentally-induced diseaseshas created a demand for biomarkers that are nonlethal and correlate with adverse effects in humans (Kendall et al., 2001). Links between wildlife and human health can serve as a premise for extrapolation in risk assessment. Indeed, humansshare many cellular and subcellular mechanisms with wildlife species. Humans and wildlife also overlap in their environments and may therefore be exposed to thesame contaminants. There is evidence to suggest that when highly conserved systems are targeted by environmental toxicants, both ecosystem and human health suffer

(Kendall et al., 2001).

As biochemical changes are usually detectable before adverse effects may be seen at higher level of biological organization, the biochemical marker approach is often considered as an early warning or proactive tool. This is a great advantage because responses at higher levels are usually measurable only after asignificant or permanent damage has occurred. The early detection of sublethal effects may also be used to identify the need for remedial action at a contaminated location and to monitor the recovery period after cleanup of the site (Peakall & Shugart, 1993 Depledge & Fossi, 1994; Lagadic et al., 2000). Regardless of their proactive or retroactive utility, the ecological realism of biomarkers is lower than for indicators based on higher-level of biological organization such as species richness or reproductive failure (Newman & Unger, 2003).

The choice of the appropriate biomarker requires an accurateknowledge of a variety of factors (Mayer et al., 1992; Peakall & Shugart, 1993). Thus, it is critical to use well-defined biological material, for which the changesin biochemical activity with development, age and tissue is known, in order to predict toxicity from changes in biochemical biomarker response following the exposure to a chemical (Hyne & Maher, 2003). The selection of biomarkers applicable in many species is frequently limited by a lack of knowledge on their intrinsic characteristics (e.g. basal level, feedback control, role of repair mechanisms). The reliability of use of biomarkers depends on knowledge of the mechanism involved in the particular response. Once suitable biomarkers are selected, it is important to conduct field studies to establish how environmental and biotic factors will modify the biomarker responses to toxicants relative to those seen in laboratory conditions where those factors are controlled (Hyne & Maher, 2003).

4. Multilevel biomarker based approach

As mentioned above, biomarker responses could be used as an early warning system for environmental monitoring (Peakall & Shugart, 1993 Depledge & Fossi,

1994; Lagadic et al., 2000). Nevertheless, biochemical endpoints alone do not seem to be sufficient to assessenvironmental quality. Pollutant-induced biochemical effects may potentially have consequences at higher levels of biological organization, such as changes in population dynamics or in biological diversity at both the intra- and interspecific levels (Depledge et al., 1993 Caquet & Lagadic, 2000). Such changes may have adverse ecological consequences (Caquet & Lagadic, 2000). Therefore, multilevel biomarker approach, evaluating different biological responses ranging from molecular to physiological level, would be more conservative for useful environmental monitoring (Depledge & Fossi, 1994; Lagadic et al., 1994, 2000 Dickerson et al., 1994; Choi et al., 2002).

The multilevel biomarker concept is originally based on the fact that biological responses of an organism in natural environment progresses through homeostasis, compensatory and repair phases, as the exposure level or duration increases (Depledge, 1994). While an organism is exposed to contaminants, physiological compensatory mechanisms become active and changes in physiological processes or functions occur, which indicate that exposure has occurred. If the exposure persists or the level of exposure increases, these compensatory mechanisms become overwhelmed, damages occur, and physiological repair mechanismsbecome active. Under natural environmental conditions, as an organism progresses through these phases, the energy allocated for natural maintenance is reduced as more energy is needed for compensatory response and repair. The organism weakens and may be quickly eliminated from the population.Therefore, *in situ* survey of populations may not allow to detect diseased organisms even though exposure and effects have occurred (Newman & Jagoe, 1996). In the context of the multiple-response paradigm, the objective is not to quantitatively measure the amounts of different toxicants, but to determine wherean organism is located on the continuumbetween homeostasis and disease. Responses indicate whether the organism is challenged but readily coping with toxicant stress (compensatory phase) or is deeply stressed and needs to use its energy resources to repair damages. This approach is essential to determine the

general health status of the organism; moreover, it makes possible to extrapolate the relationship between responses at different levels of biological organization (Fossi et al., 2000).

Some biochemical biomarkers do not appear to have a direct relationship to a defined mechanism of toxicity. In this case, the use of such biomarker will not give a reliable prediction of toxic effects and is, therefore, only ever likely to indicate exposure to chemicals. These biomarkers of exposure cannot be used to predict effects at the population level from biomarker changes measured in a sample of individuals (Hyne and Maher, 2003). To relate the effects measured at the individual level to higher levels of biological organization, the biomarker response should be related to an impairment of growth, reproduction, or metabolic function which directly affects the survival of the organism and which can be attributed to exposure to a known amount of specific contaminants (Delpedge & Fossi, 1994).

5. Ecotoxicological significance of invertebrate biomarkers

To link the measurement of a biomarker in individuals to changes at the population level, it is necessary to understand the mechanisms, which link the effects at the subcellular level to the response of individuals. Quantitative dose-response relationships for the biomarker may then link the molecular effect of the toxicant to the toxic response of the individual organism. Linkage of whole organism responses to changes in populations can then be obtained by statistical or numerical inferences (Hyne & Maher, 2003). Invertebrates are good biological models for such studies.They are major components of all animal communities and they represent 95% of all animal species on Earth (Barnes, 1968). Their populations are often abundantand their life cycles are frequently short, so samples can be taken for analysiswithout significantly affecting population dynamics and population level effects can be examined concomitantly with the response of biomarkers. Increasing knowledge of the biochemistry of invertebrates (James, 1989; Livingstone, 1991), now permits reasonable

interpretation of biomarker responses in terms of ecological risk assessment (Depledge, 1994; Depledge & Fossi, 1994). Numerous biomarkers are extensively studied in various invertebrates species to evaluate their potential for predicting population level changes. This is for example the case of DNA damage (Deplege, 1998; Wilson et al., 1998; Atienzar et al., 1999; Fossi et al., 2000; Guecheva et al., 2001), heat shock proteinsinduction (Snyder & Mulder, 2001; Wheelock et al., 2002; Guecheva et al., in press), energy reserves (Baturo & Lagadic, 1996) or of the alteration of the activity of various enzymes (Abele-Oeschger, 1996; Baturo & Lagadic, 1996; Fossi et al., 2000; Hyne & Maher, 2003).

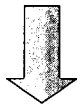
ECOTOXICITY MIONITORING USING MULTILEVEL BIOMARKERS : APPLICATION IN INVERTEBRATES

최 진 희

서울시립대학교
환경공학부

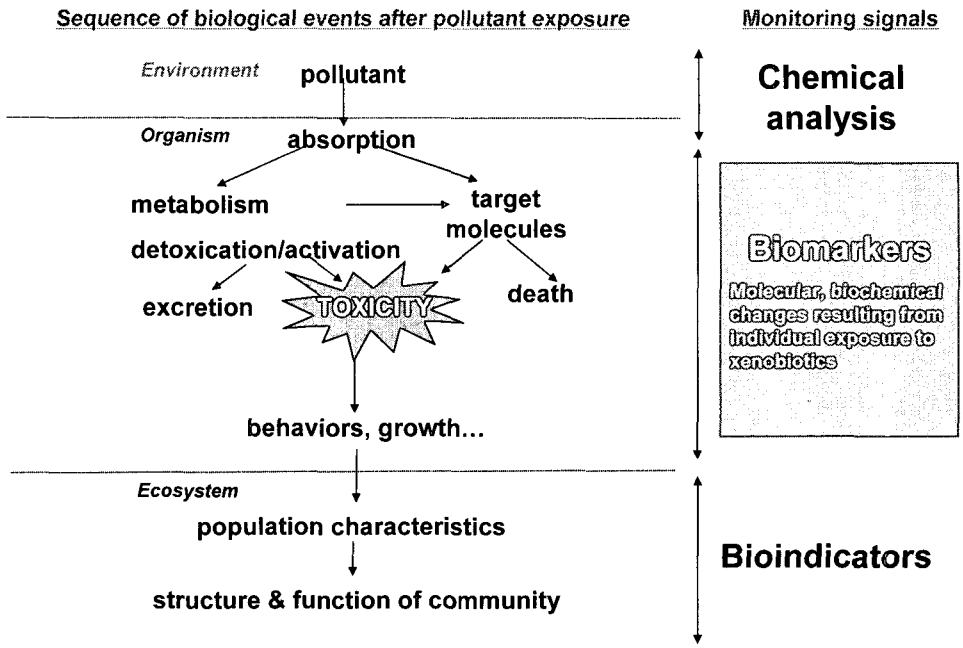
Ecotoxicology

- **study the effects of environmental contaminants at the population, community or ecosystem levels**

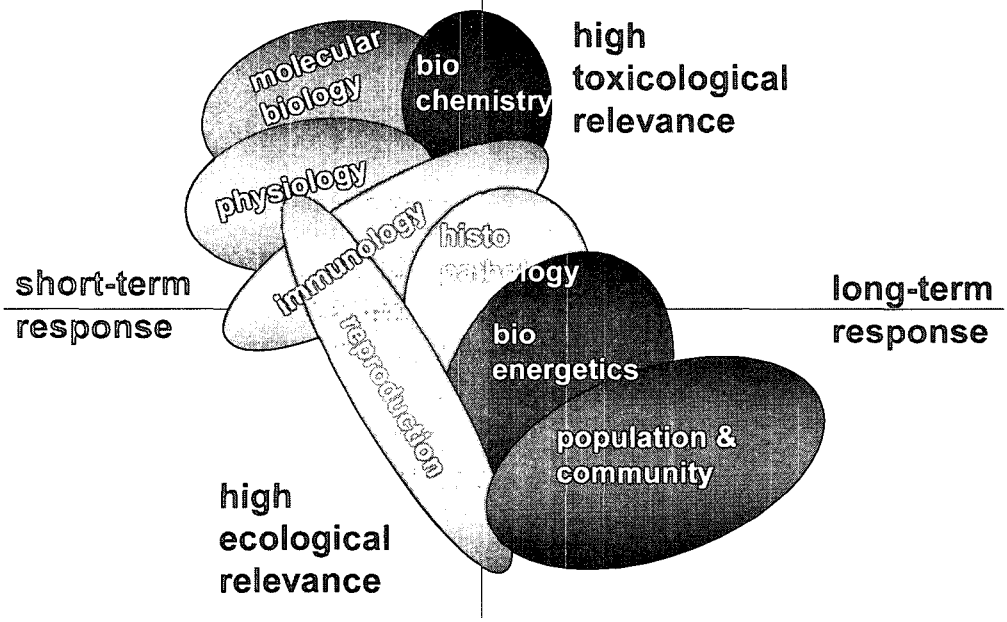


- **study on individuals at the biochemical and molecular levels where toxicant-induced responses are initiated**

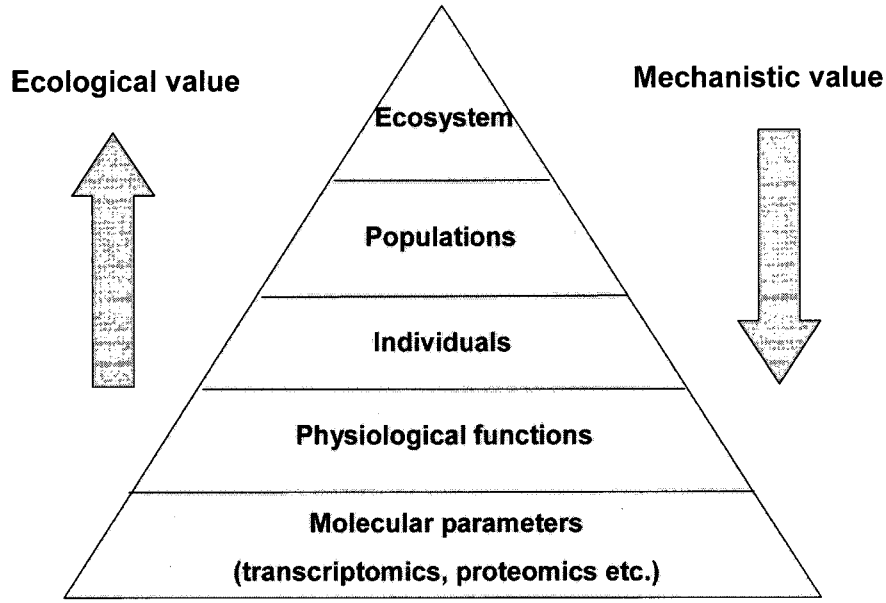
Biomarkers in Ecotoxicology



Relationships between responses at levels of biological organization and the relevance and time scales of responses



Conceptual framework for Ecotoxicology



. Ecotoxicological significance of invertebrate biomarkers

Invertebrates are good biological models for such studies.

They are major components of all animal communities and they represent 95% of all animal species on Earth (Barnes, 1968).

Their populations are often abundant and their life cycles are frequently short, so samples can be taken for analysis without significantly affecting population dynamics and population level effects can be examined concomitantly with the response of biomarkers.

Increasing knowledge of the biochemistry of invertebrates (James 1989; Livingstone, 1991), now permits reasonable interpretation of biomarker responses in terms of ecological risk assessment (Depledge, 1994; Depledge & Fossi, 1994).

Numerous biomarkers are extensively studied in various invertebrates species to evaluate their potential for predicting population level changes.

This is for example the case of **DNA damage** (Deplege, 1998; Wilson et al., 1998; Atienzar et al., 1999; Fossi et al., 2000; Guecheva et al., 2001), **heat shock proteins induction** (Snyder & Mulder, 2001; Wheelock et al., 2002; Guecheva et al., in press), **energy reserves** (Baturo & Lagadic, 1996) or of the **alteration of the activity of various enzymes** (Abele-Oeschger, 1996; Baturo & Lagadic, 1996; Fossi et al., 2000; Hyne & Maher, 2003; Guecheva et al., in press).

Invertebrates

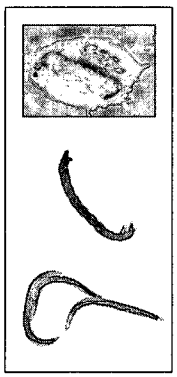
Aquatic

Water quality monitoring : freshwater crustacean (*Daphnia magna*),

Sediment toxicity monitoring : larva of aquatic midge (*Chironomus riparius*)

Teresteral

Soil toxicity monitoring : soil nematode (*Caenorhabiditis elegans*)



Daphnia magna

Chironomus riparius

Caenorhabiditis elegans

Invertebrates

Daphnia magna* / *Chironomus riparius* / *Caenorhabiditis elegans

- **widely used in environmental monitoring /laboratory toxicity testing**
- **ubiquitously distributed**
- **sensitive to many pollutants**
- **easy to culture**
- **short life cycle**
- **suitable for ecotoxicological monitoring**

- ***Daphnia magna***
- plays a pivotal role in aquatic food webs
- international Daphnia Genomics Consortium - to develop Daphnia as a model system for ecological genomics.

- ***Chironomus riparius***
- *Chironomus* Hbs : high degree of polymorphism/ high affinity for oxygen / extracellular localization

- ***Caenorhabiditis elegans***
- Since its genome has been completely sequenced, the functional relations of gene expression and phenotypic response have been investigated to a considerable extent.

Research Schemes

Species

Daphnia magna / Chironomus riparius / Caenorhabiditis elegans

Environmental Contaminants

benzo[a]pyrene, carbon tetrachloride, cadmium chloride, lead(II)nitrate, potassium dichromate chloropyriphos, fenitrothion, endosulfan, paraquat dichloride, nonylphenol, Bisphenol A, 17α-ethynyl estradiol, bis (2-ethylhexyl) phthalate, Octachlorostyrenes

Environmental samples (unknown contaminants)

Toxic Effects

1. **Acute toxicity**
   : **24h LC50 / EC50**
2. Biomarker ***(1/10, 1/100 and 1/1000 of L(E)C50)***
   : **pollutant metabolism & oxidative stress... molecular/biochemical/**
3. **physiological/individual/population levels effects**

Correlation study

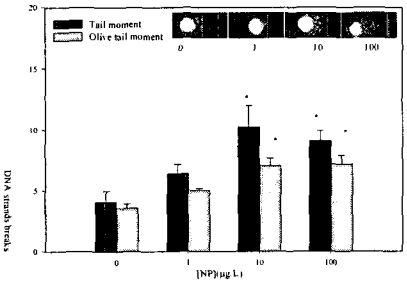
Biomarkers

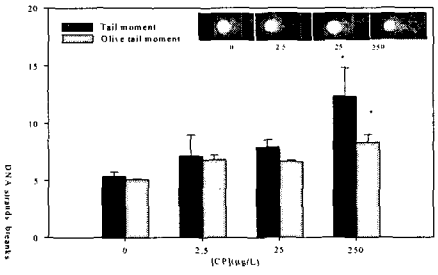
Invertebrates	media	Biomarkers							Eco-physiological markers
		Gene expression profiling	Stress protein	DNA damage	Oxidative stress	Detoxication	Neurotoxicity	Hb	
D.magna	Water			Strand break (SCGE)	Antioxidant enzyme activity/ MDA	GST (enzyme activity)	AChE (enzyme activity)	Hb (gene expression)	Reproduction
C.riparius	sediment	DEG	HSP70 HSC70	Strand break (SCGE)	Antioxidant enzyme activity/ MDA	GST (enzyme activity)	AChE (enzyme activity)	Hb Gene /protein expression /function)	Growth Development (Male/female sensitivity)
C.elegans	soil	DEG DNA chip	HSPs MTs ...		SOD,CAT (enzyme activity/ gene expression)	CYP/GST (gene expression)			Growth Development Reproduction

Acute Toxicity

chemicals	EC50 (㎍/L) *Daphnia magna* (95% confidence interval)	LC50 (mg/L) *Chironomus riparius* (95% confidence interval)	LC50 (mg/L) *C. elegans* (95% confidence interval)
nonylphenol	303.45 (229.56~397.10)	0.6880 (0.5380~0.8020)	0.1000 (0.0300~0.6000)
bisphenol A	352.49 (187.09~498.27)	6.0300 (3.7880~7.4100)	80.000 (2.0000~250.00)
ethynyl estradiol	7194.2 (6075.7~8232.7)	9.1360 (7.3410~24.965)	-
bis(2-ethylhexyl)phthalate	710.93 (539.46~2777.3)	258.36 (123.94~3600.5)	20.000 (4.0000~57.000)
endosulfan	887.74 (726.71~1527.7)	0.4760 (0.0270~ 2.0900)	1.5000 (0.4160~ 2.7480)
paraquate dichloride	1126.3 (135.52 ~1834.2)	1325.8 (1008.4~2121.9)	200.00 (58.000~264.00)
chloro pyriphos	0.9530 (0.762~4.360)	2.7740 (0.8610~5.1880)	1.0000 (0.5700~1.5000)
fenitrothion	1.8860 (1.377~2.763)	8.7210 (6.8760~19.160)	0.5000 (0.0700~1.4000)
cadmium chloride	866.33 (749.57~1009.5)	212.23 (174.15~27750)	850.00 (636.00~1064.0)
lead(Ⅱ)nitrate	18153 (1080.4~40171)	6693.5 (3559.6~39879	40.000 (23.000~45.000)
potassium dichromate	1456.4 (941.40~1919.9)	51.309 (23.304~62.540)	120.00 (84.000~151.00)
benzo[a]pyrene	29.304 (13.321~242.19)	31.592 (23.304~62.546)	-
Carbon tetrachloride	28607 (16554~64031)	26.105 (18.867~28.943)	-

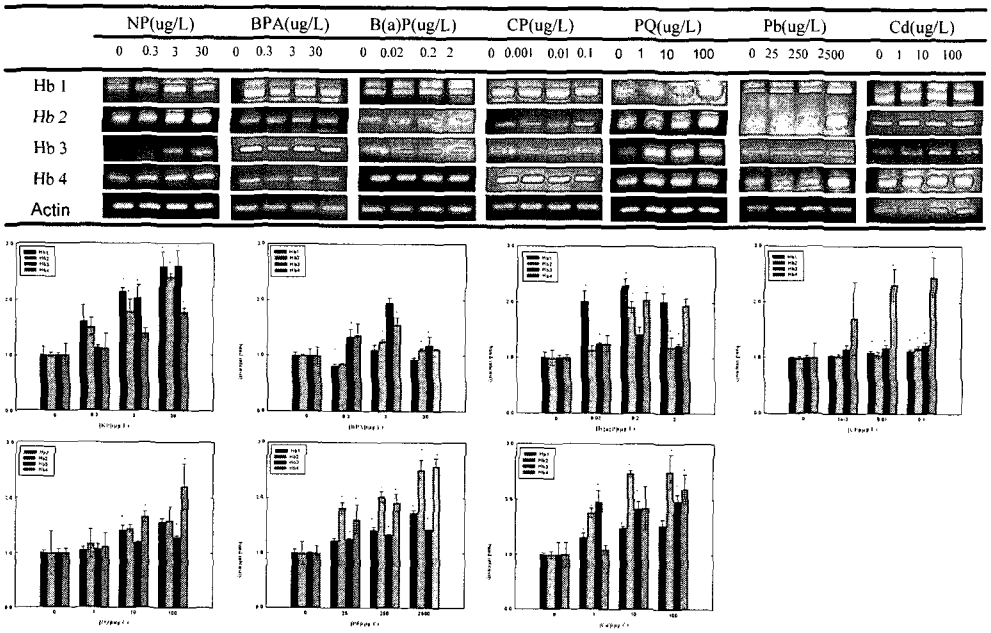
DNA damage
D.magna





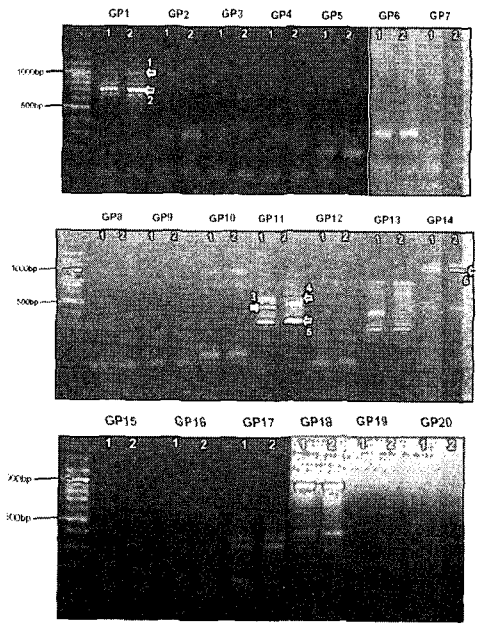
Hb gene expression

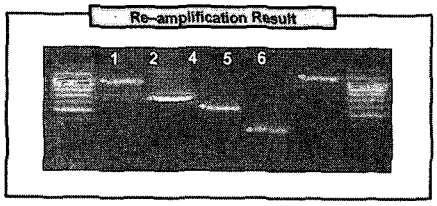
D. magna



Gene expression profiling

C. riparius

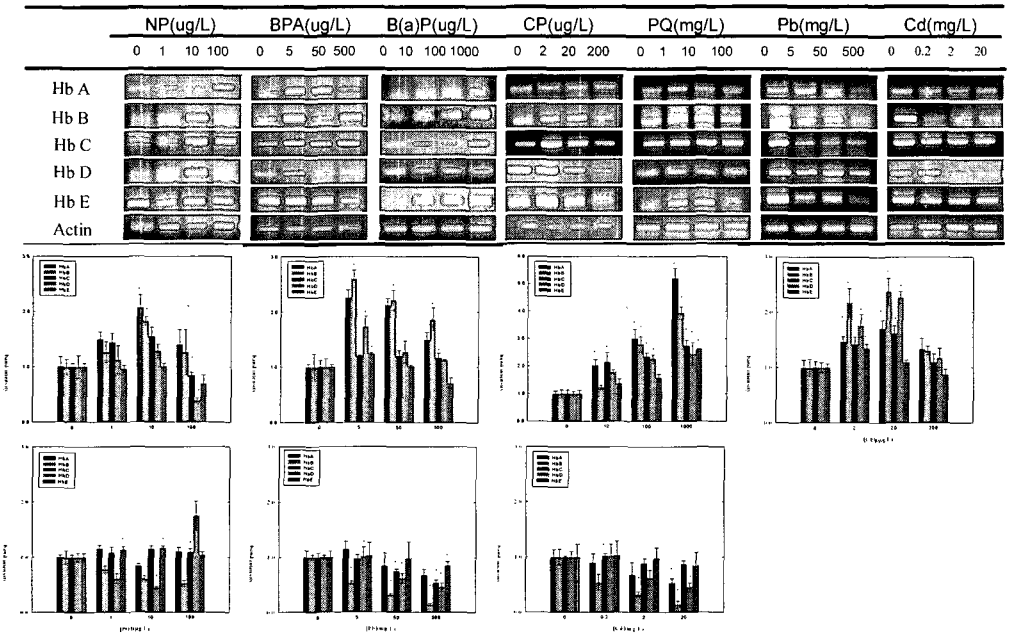




1: control
2: OCS (5mg/L)

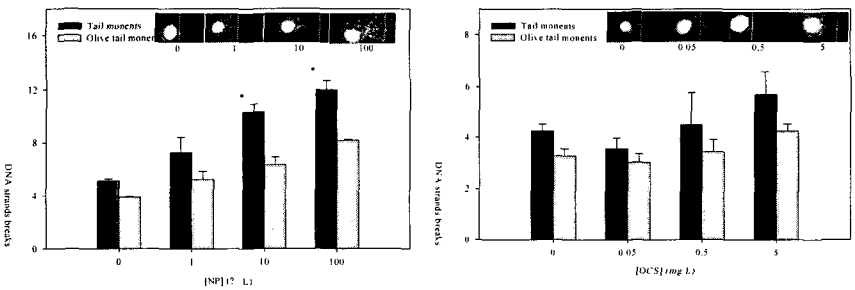
Hb gene expression

C. riparius

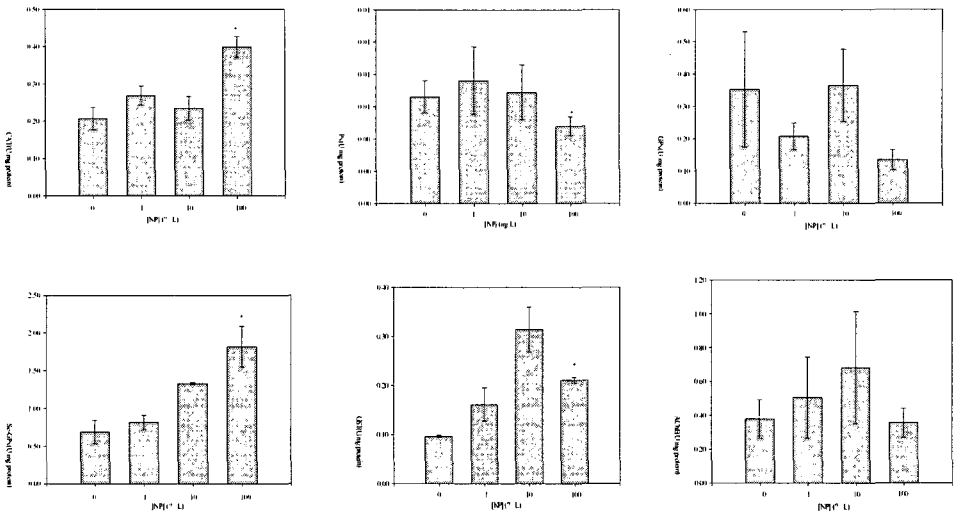


DNA damage

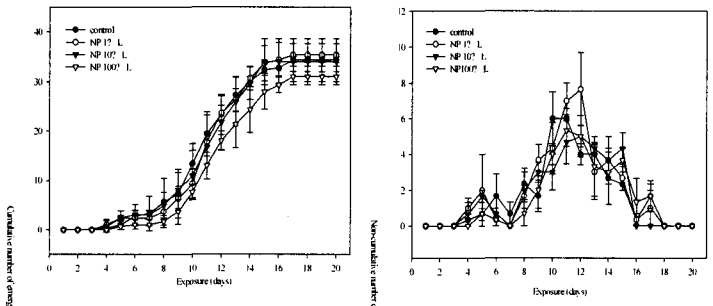
C.riparius



Enzyme activities
(Oxidative stress /Detoxication / neurotoxicity)
C.riparius



Development
c.riparius



NP (µg/L)	Pupation failure (%)	Emergence failure (%)	
	larva - //- > pupa	pupa - //- > adult	larva - //- > adult
0 (control)	26 ± 1.15	5.33 ± 1.76	31.4 ± 0.67
1	24.67 ± 0.67	11.33 ± 3.33	36 ± 4.0
10	20.67 ± 4.81	11.33 ± 0.67*	32 ± 5.29
100	20 ± 2.0	18 ± 2.31*	38 ± 1.15*

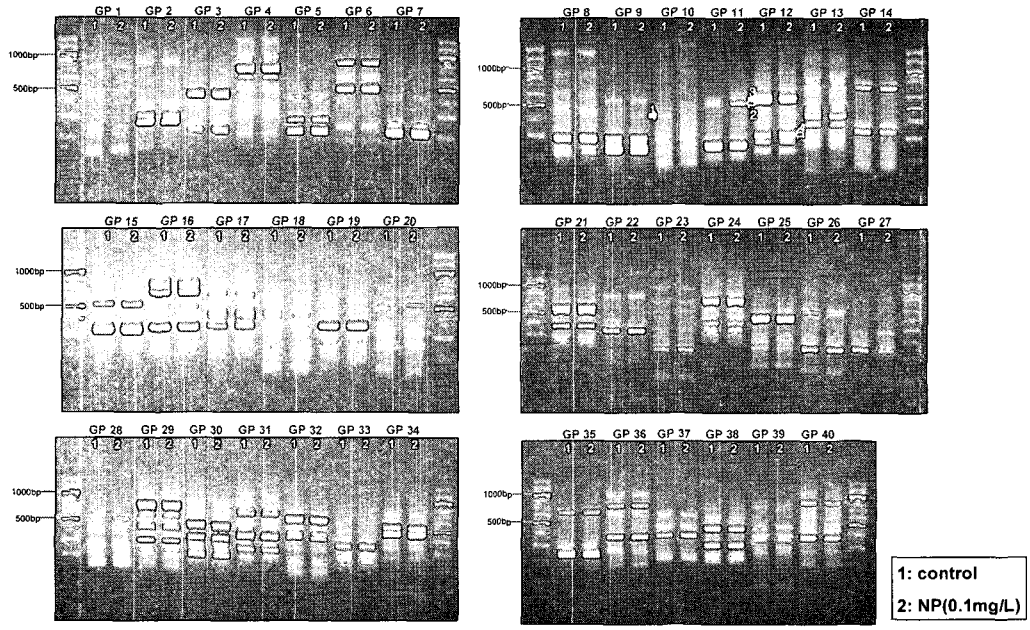
Correlation study

c.riparius

	Molecular -		Biochemical -					Physiological -			Ecophysiological levels				
	tail moments [2]	HSP70 [3]	CAT [4]	PX [5]	GPX [6]	Se-GPX [7]	GST [8]	AChE [9]	Water [10]	BDW [11]	Pupation [12]	Emergence [13]	Time [14]	Male [15]	Female [16]
[NP] [1]	470 (530)	- 113 (887)	.951* (.049)	-.979* (021)	- 741 (259)	864 (136)	198 (802)	- 443 (557)	232 (768)	380 (620)	- 708 (292)	744 (256)	634 (366)	- 740 (260)	- 680 (920)
tail moments [2]		786 (214)	457 (543)	- 459 (541)	- 178 (822)	850 (150)	.954* (046)	118 (882)	712 (288)	470 (530)	-.950* (050)	284 (716)	- 484 (596)	211 (789)	- 127 (873)
HSP70 [3]			- 186 (814)	054 (946)	453 (547)	380 (620)	932 (068)	167 (833)	424 (576)	023 (977)	- 613 (387)	- 346 (654)	- 762 (238)	621 (379)	198 (802)
CAT [4]				- 869 (131)	- 901 (099)	829 (171)	171 (829)	- 166 (834)	442 (558)	631 (369)	- 652 (348)	912 (088)	515 (485)	- 627 (373)	- 383 (617)
PX [5]					595 (405)	- 545 (455)	- 212 (788)	601 (399)	- 686 (914)	- 196 (804)	712 (288)	- 594 (406)	- 626 (374)	772 (228)	- 124 (876)
GPX [6]						- 545 (455)	102 (898)	- 124 (876)	- 485 (515)	- 723 (227)	328 (672)	-.993** (007)	- 495 (505)	526 (474)	677 (323)
Se-GPX [7]							662 (338)	- 195 (805)	546 (454)	497 (503)	-.964* (036)	668 (332)	129 (871)	- 320 (680)	- 123 (877)
GST [8]								197 (803)	648 (352)	318 (682)	- 832 (168)	013 (987)	- 626 (374)	450 (550)	- 023 (977)
AChE [9]									715 (285)	639 (361)	133 (867)	184 (816)	- 691 (309)	748 (252)	- 812 (188)
Water [10]										912 (088)	- 576 (424)	581 (419)	- 507 (493)	418 (582)	- 775 (225)
BDW [11]											- 418 (582)	831 (169)	- 171 (829)	123 (876)	- 921 (079)
pupation [12]												- 412 (588)	102 (898)	103 (897)	033 (967)
emergence [13]													398 (602)	- 448 (552)	- 710 (290)
time [14]														-.978* (022)	195 (805)
male [15]															- 230 (770)

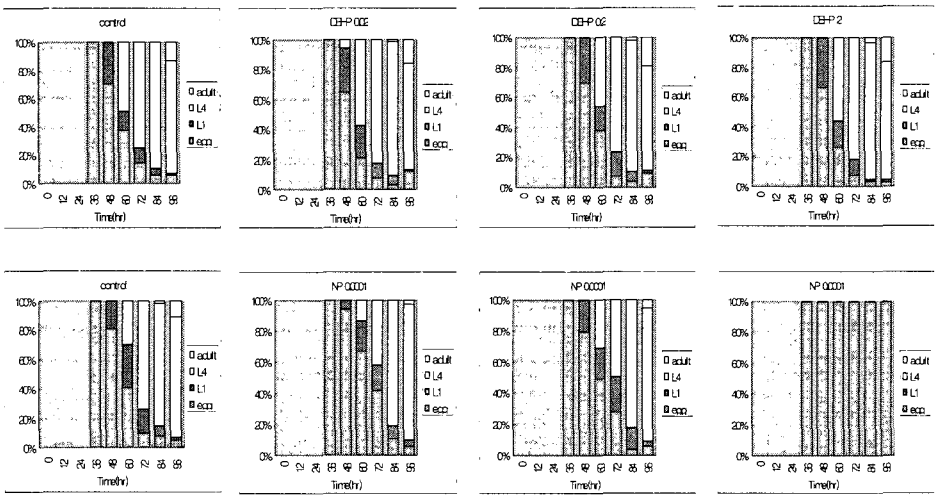
Gene expression profiling

C.elegans



Development

C.elegans



CONCLUSIONS