

넙치 혈액중의 TBT축적과 환경모니터링의 응용  
**Accumulation of Tributyltin in Blood of Olive Flounder, *Paralichthys olivaceous*, and its application for Monitoring in Marine Environment**

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TBT contamination has been monitored mainly with bivalves, gastropods, and fish. Most of studies have analyzed muscle and liver (Kannan *et al.*, 1995a; Krone *et al.*, 1996), without focusing on the accumulation of TBT in blood in different from other hydrophobic compounds. In analytical aspects, blood has the advantages that specimen can be repetitively collected without destruction of organisms and a time consuming clean-up step is unnecessary. Although TBT could be detected in fish blood, very little is known about the accumulation pattern of TBT in blood. The applicability of TBT in fish blood has rarely been verified with field samples either.

Tributyltin (TBT) was highly accumulated in the serum of olive flounder, *Paralichthys olivaceous*, with an in vivo 30-day exposure experiment. Tributyltin was more quickly accumulated in serum than in the other tissues (liver, muscle, and gill). The accumulated TBT concentrations were in order of serum > gill > liver > muscle on dry weight basis. Tributyltin was also detected in the serum of feral fine-spotted flounder, *Pleuronichthys cornutus*, collected from the coastal area moderately polluted with TBT (Shim *et al.*, 1999). The mean TBT concentration in blood serum (247 ng Sn/g wet weight) of the fine-spotted flounder was about four times higher than that in liver (60 ng Sn/g dry weight) and twenty times higher than that in muscle (27 ng Sn/g dry weight). The TBT concentrations in serum and sediment showed strong correlation ( $r^2 = 0.97$ ). The percent TBT composition to total butyltin was much higher in the serum (71%) than in the other tissues and sediment (< 47%). These results suggest that the analysis of fish blood serum could be a tool for monitoring exposure to TBT in the marine environment.

#### References

Shim, W.J., J.R. Oh, S.H. Kahng, J.H. Shim and S.H. Lee. (1999) Horizontal distribution of butyltins in surface sediments from an enclosed bay system, Korea. *Environ. Pollut.* 106: 351-357.

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홍합-굴 감시 프로그램: 우리나라 연안환경의 유기주석화합물 오염 평가  
**Mussel and Oyster Watch : Assessment of Organotin  
 Contamination in the Marine Environment of Korea**

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Organotin compounds are one of the most widely used organometallic chemicals. They are actively applied to antifouling paints as biocidal additives to prevent adherence of sedentary organisms to ship hull and other structural surfaces immersed in water. Since their deleterious effects on non-target organisms were revealed in the early 1980s, many industrialized countries have placed regulations of ban on use of organotin compounds, especially tributyltin (TBT) and Triphenyltin (TPT) compounds, in ships.

Spatial distribution and quantification of the target pollutant is an essential corner stone to understand the exposure level of ecosystem, which can be used as important parameters to assess the risk of ecosystem. Contamination of organotin compounds has been monitored in many countries since the early 1980s (Shim *et al.*, 1998). Organotin concentrations in biota are thousands of times higher than those in ambient water and also reflect the integrated information of exposure more than several months long. Bivalve organisms such as mussels and oysters have been used as monitoring organisms due to their high accumulation of organotins, sessile characteristics and wide distribution

Tributyltin, Triphenyltin and their degradation products in several marine organism were quantitatively determined by gas chromatograph equipped with flame photometric detector (GC-FPD) to assess the extent of contamination of these compounds around the coast of Korea. The biota was taken from 12 regions (total 67 sites) near harbor and/or shipyard. All the biota samples contained detectable amount of butyl- and phenyltins. Tributyltin concentrations in bivalves reached up to 1,630 ng Sn/g on dry weight basis and triphenyltin up to 328 ng Sn/g. The distribution patterns of TBT and TPT in biota were closely related to boating and dry-docking activities.

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Shim, W.J., J.R. Oh, S.H. Kahng, J.H. Shim and S.H. Lee. (1998) Accumulation of tributyltin compounds in Pacific oyster, *Crassostrea giags*, from the Chinhae Bay System, Korea. Arch. Environ. Contam. Toxicol. 35: 41-47.

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**우리나라 연안환경에서 TBT의 생태위해성평가**  
**Probabilistic Ecological Risk Assessment of Tributyltin compound**  
**in the Marine Environment of Korea**

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Tributyltin is considered one of endocrine disrupting chemicals, as it is known to cause imposex (imposition of male sexual organ on female) in marine gastropod well below ppb level (Shim *et al.*, 200). Furthermore, TBT cause adverse effects on from microorganism to mammals. Nevertheless of high toxicity of TBT, its ecological effects on marine ecosystem has not been quantified yet. In this study, exposure, effect and risk characterization was done with TBT contamination in Korea and toxicity database. The ecological risk imposed by TBT on marine community was quantified with probabilistic risk assessment model.

Ecological effects of tributyltin (TBT) has been assessed in the coastal area of Korea with TBT exposure and effect database. Approximately 30% of TBT water concentration exceeded provisional US EPA water quality criterion (10 ng TBT/L) and 85% exceeded UK water quality criterion (2 ng TBT/L). In addition, 35% of bivalve tissue residue levels were over allowable daily intake (converted to tissue burden as 1.8 g/g TBT dry wt.). Approximately 20% of TBT sediment concentrations were above screening level (250 ng TBT/g dw). However, The effects of TBT on marine organisms are confined near TBT source areas such as harbors and shipyards in Korea.

To assess the quantitative risk of marine organisms imposed by TBT in the marine environment of Korea, logistic regression risk assessment model (95% species protection) was run with expected TBT concentrations in coastal waters and literature surveyed toxicological data of TBT. Based on the acute and chronic toxicity data and the mean water TBT concentration (20.2 ng TBT/L) in the coastal area of Korea, the current TBT water concentration would be protective of more than 93% of marine species. However, approximately 25% of species would be affected by the current TBT water contamination in chronic threshold toxicity levels.

#### References

Shim, W.J., S.H. Kahng, S.H. Hong, N.S. Kim, S.K. Kim and J.H. Shim (2000) Imposex in the rock shell, *Thais clavigera*, as evidence of organotin contamination in the marine environment of Korea. *Mar. Environ. Res.* 49: 435-451.

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**Freezing filtration와 고상 추출 전처리 후 GC/MS-SIM 방법을 이용한  
생물시료 중 Bisphenol A, Alkylphenols와 Chlorophenols의 분석  
Analysis of Bisphenol A, Alkylphenols and Chlorophenols in Biota Samples  
by Freezing Filtration, Solid Phase Extraction and GC/MS-SIM**

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최근 환경오염 및 내분비계장애물질로 연구대상이 되고 있는 생리활성이 큰 페놀류들 중 농약, 목제 방부제, 살균제등의 생산에 중간체로 쓰이는 클로로페놀류, 계면 활성제의 원료인 알킬페놀 에톡시레이트 (alkylphenol ethoxylate, APE)의 생산 원료인 알킬페놀류 (대표적인 nonylphenol과 이 보다 내분비 장애 효과가 30 배 이상 높은 것으로 알려진 *tert.*-butylphenol) 및 에폭시 수지 및 방염제의 중간체, 코팅제와 포장제로 사용되는 비스페놀 A들은 환경 중 농축에 의한 인체에 유입을 통해 내분비계장애물질로 작용하여 암 발생 및 발생기 과정에서 성결정 (fetal sex determination)에 영향을 주고, 야생 동물에 여러가지 문제를 야기할 뿐만 아니라 인간에게도 커다란 영향을 주리라 예상되고 있기 때문에, 환경시료 중에서 극미량 ppt (parts per trillion) 수준의 이들 오염물질을 검출할 수 있는 분석법의 개발은 국민 보건상, 환경 보호 차원에서 매우 중요하다.

내분비계장애물질로 알려진 8종 알킬페놀류, 2종 클로로페놀류와 비스페놀 A를 포함하는 11종 페놀류를 생물시료 중 ppt 단위로 정량할 수 있는 방법을 개발하였다. 생물시료를 아세트니트릴로 ultrasonication 추출후, freezing filtration하여 과량의 지질을 제거하였다.

추출물을 XAD-4를 이용하여 고상추출 후 isobutylchloroformate를 이용하여 isobutoxycarbonyl (isoBOC) 유도체화하여 기체크로마토그래피/질량분석기-선택이온 모니터링 방법으로 분석하였다. 회수율은 92.3~150.6% 이었으며, US-EPA에서 규정한 분석방법 검출한계는 비스페놀 A의 경우 0.062  $\mu\text{g}/\ell$  이었다.

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## Bisphenol A-metabolites induces Oxidative DNA damage and reduced cell proliferation

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Bisphenol A, a monomer of polycarbonate and epoxy resins, has been detected in canned food and human saliva. BPA stimulate cell proliferation and induces expression of estrogen-response genes in vitro. This report considers the hypothesis that BPA is converted in vivo to hydroxylated metabolites with enhanced estrogenicity and cytotoxicity. The purpose of the this study was to evaluate the cytotoxicity and cell proliferation of bisphenol A in the presence of a rat liver S9 mix containing cytochrome P 450 enzymes and Cu(II). In the present study, we found that BPA in combination with Cu(II) exhibited a enhancement in cytotoxicity, which was inhibited by reactive oxygen species scavenger. For cell proliferation assay MCF-7 cells were seeded on a 96-well multi-well-plate at  $1.5 \times 10^3$  cells per well. After 24hr cultivation, the S9 mix and Cu(II) was added to the wells as an S9 mix group (+S9), and medium was added to the other wells as a none-S9 mix group (-S9), then 5 different concentrations of various BPA were added to each well. After 5 days, a sulforhodamine B (SRB) assay was conducted to measure cell proliferation. +S9 mix group enhanced the proliferation of MCF-7 cells at much lower concentrations than -S9 mix group which was inhibited by the ROS scavenger. These results suggest that reactive oxygen species reacts with Cu(I) leading oxidative stress. Also the formation of reactive oxygen species induced by BPA was dose-dependently by inhibited by tamoxifen, which suggests that the effect of BPA was estrogenic action via estrogen receptors.

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## Oxidative damage by bisphenol A induced lipid peroxidation and apoptosis

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It is very important to understand the ROS generation of estrogen-like chemicals. Of such chemicals, we were especially interested in bisphenol A for its wide spreadness in environmental and structural similarity with aneuploidogenic stilbene estrogen diethylstilbestrol. The purpose of this study was to evaluate the lipid peroxidation and DNA fragmentation by bisphenol A in the presence of a rat liver S9 mix containing cytochrome P 450 enzymes and Cu(II) in HaCaT cell lines. The specific content of malondialdehyde, an end product of lipid peroxidation, was also found to increase with concentration. The fragmentation of intact DNA, a parameter of apoptotic cell death, was evaluated qualitatively by agarose gel electrophoresis analysis and quantitatively by diphenylamine reaction method. BPA induced apoptotic cell death in a dose-dependent manner. When HaCaT cells were exposed to 50uM BPA for 48h, the DNA fragmentation was significantly increased to 54%. The effect of radical scavenger on the apoptotic cell death induced by BPA was investigated. The DNA fragmentation induced by BPA was significantly inhibited by addition of ROS scavenger to the culture medium. Also we examined the enzyme activities of Cu,Zn-SOD, Mn-SOD, catalase, and GPx in the cells. The activities of Cu,Zn-SOD, glutathione peroxidase, Catalase were found to decrease with concentration. However, the activity of Mn-SOD were unchanged. This indicated that elevated oxidative stress caused by an imbalance between the production and removal of ROS and free radicals occurred in cells.

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과량의 아연에 의해 유도된 사이토카인 생산에 미치는 인도메타신의 영향  
**Effects of indomethacin on the production of cytokines  
 following exposure to excessive zinc**

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Zinc plays an important role in immunobiological responses, while excessive zinc attenuates immune functions in a dose-dependent manner. Zinc excess increased levels of plasma prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), which is known to inhibit production of Th (helper T) 1-associated cytokines from Th cells and to induce inflammatory responses. Thus, this study was investigated the effect of indomethacin, a potent inhibitor of PGE<sub>2</sub> synthesis, on the excessive zinc-induced production of proinflammatory cytokines and lymphokines in ICR mice. Indomethacin at doses of 5 mg/kg was administered *i.p.* 30 minutes before zinc chloride (Zn) 30 mg/kg orally daily for 10 days. Zn remarkably increased tumor necrosis factor (TNF)- $\alpha$  and interleukin (IL)-1 $\beta$  levels in both serum and splenic supernatants compared with those in controls, while indomethacin significantly reduced the Zn-induced levels of IL-1 $\beta$ . Zn significantly decreased the levels of serum IL-2 and interferon (IFN)- $\gamma$  compared with those in controls, whereas indomethacin significantly enhanced the Zn-decreased levels of serum IFN- $\gamma$  but did not affect the Zn-decreased levels of serum IL-2. All of Zn, indomethacin, and combination of Zn and indomethacin significantly enhanced IL-2 levels in supernatants compared with those in controls, but indomethacin didn't affect the Zn-induced production of IL-2. These data, therefore, suggest that indomethacin attenuated the IL-1 $\beta$  production induced by excessive zinc and restored the excessive zinc-suppressed production of serum IFN- $\gamma$  but not IL-2.

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암유발 생쥐에서 리포폴리사카라이드에 의해 유도된 사이토카인 생산에  
미치는 인도메타신의 영향

Effect of Indomethacin on the Lipopolysaccharide-induced Production of  
Cytokines in Tumor-bearing Mice

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Indomethacin is well known as a prostaglandin (PG) E<sub>2</sub> synthetase inhibitor, which has antipyretic and anti-inflammatory effects and reduces the risk of cancer. Growing tumors greatly induce hypersensitive responses to lipopolysaccharide (LPS). Thus, this study was investigated the effect of indomethacin on the LPS-induced production of cytokines in sarcoma-bearing ICR mice. Indomethacin at doses of 5 mg/kg was administered orally 30 minutes before *i.p.* injection of LPS (8 mg/kg) 5 times for 7 days. LPS remarkably increased tumor necrosis factor (TNF)- $\alpha$  and interleukin (IL)-1 $\beta$  levels in both serum and splenic supernatants compared with those in controls, while indomethacin significantly reduced the LPS-increased levels of IL-1 $\beta$  in both serum and supernatants. LPS significantly enhanced IL-2 levels in serum and interferon (IFN)- $\gamma$  levels in supernatants, whereas indomethacin did not affect the LPS-increased levels of IL-2 and IFN- $\gamma$ . These data, therefore, indicate that indomethacin may attenuate the pathogenesis of IL-1 $\beta$  induced by LPS and maintain the tumoricidal cellular immune effects by LPS-increased production of IL-2 and IFN- $\gamma$  in tumor-bearing state.

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## Biological Responses of *Macoma balthica* Exposed to Ag and Cu Contaminated Sediments: long-term microcosm experiment

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If diet is a significant route of contaminant exposure from sediments, then bioassay protocols must be developed to evaluate effects of less than extreme sediment contamination. A sediment microcosm experiment was conducted to study the sub-acute effects of such exposures in the clam *Macoma balthica*. Clams were collected at late February, when they were developing gonads for spring reproduction. Animals were exposed to 4 levels of Ag and Cu-spiked sediments (maximum 0.9 mmol Ag/g + 7.8 mmol Cu /g), vertical redox gradients, and systematically varied AVS-SEM among experiments. Exposures were for 3 months with a salinity of 20 ppt in the cold room (15 oC). All 16 experimental containers (6L, 25 clams) were placed in two aquaria (150 L) with recirculation system (1000 L/hr). Spiked diatom *Phaeodactylum tricornutum* was provided daily, as supplemental food, and other phytoplankton species were added once a week to meet the nutritional quality. At 30 d, 60 d and 90 d, clams were removed and burrowing activity, clearance rate, glycogen content, condition index, gametogenesis and tissue metal content were analyzed. Within the first 60 d, metal exposure affected clearance rates and perhaps burrowing rates; both declined with increasing Ag+Cu concentrations. Small clams showed higher clearance rate than large clams on a dry weight basis, but Ag + Cu affected both sizes. Glycogen content was significantly less than the control in the highest metal exposure at 60 d, though the difference was small. Mean condition index (mg/cm<sup>3</sup>) showed no effect among the treatments; but closer inspection of data showed that large clams declined in condition compared to smaller animals, especially at the highest metal exposure. So the relationship between shell length and dry weight was poor as metal exposures increased. The complexities of dietary exposure require greater diligence to study that existing standard protocols, but offer the potential to better understand the type of metal exposures that occur in most estuarine environments affected by human activities.

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## 퇴적물내 아연의 분배와 독성에 대한 연구

## A Study on the Partitioning and Toxicity of Zn in Contaminated Sediments

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The influences of spiked Zn concentrations (1~40 mmol/g) and equilibration time (~100 d) on the partitioning of Zn between porewater (PW) and sediment were evaluated with estuarine sediments containing two levels (5 and 15 mmol/g) of acid volatile sulfides (AVS). Their influence on bioavailability was also evaluated by a parallel 10-d amphipod *Leptocheirus plumulosus* mortality test at 5, 20, and 85 d. During the equilibration, [AVS] increased (up to 2X) while weak acid extracted [Zn] (Zn SEM) remained relatively constant. Concentrations of Zn in PW decreased rapidly during the initial 30 d and decreased by 11~23X over the whole equilibration time. The apparent partitioning coefficient (KPW, ratio of [Zn] in SEM to PW) increased 10~20X with time. The decrease of [PW Zn] could be explained by a combination of changes in [AVS] and redistribution of Zn into more insoluble phases as the sediment aged. Toxicity to amphipods decreased significantly with the equilibration time and was correlated with decreases in [PW Zn]. The LC50 value (33 mM) in the second bioassay (20 d) was 2X the LC50 in the initial bioassay (5 d). Bioassay protocols employing a short equilibration time and high spiked metal concentrations could accentuate partitioning of metals to PW and shift the pathway for metal exposure toward PW.

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한국 전체연안에서 채취된 이매패류에서의 유기염소계 화합물의  
생물농축과 공간적 분포

**Geographical Distribution and Accumulation Features of Organochlorine  
Residues in Bivalves from Coastal Areas of South Korea**

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The contamination levels and accumulation features of polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) were assessed in 1999 from 82 bivalve samples collected from 66 locations along the entire coast of Korea. Investigated bivalves were oyster (*Crassostrea gigas*), mussel (*Mytilus edulis* and *Mytilus coruscus*), and clam (*Cyclina sinensis* and *Ruditapes philippinarum*). The dry weight based ranges of T-PCBs and T-OCPs were 1.80–421.99 (geometric mean = 35.75) and 5.34–131.37 (32.29) ng/g, respectively. 28 % of the samples, all of which were from areas near four large industrial complexes, contained elevated levels of T-PCBs (over 100 ng/g). Aroclor 1254 was the dominant PCB product found. The spatial distribution of T-OCP levels was correlated with that of T-PCBs ( $r^2 = 0.5$ ), which was attributable to the distribution patterns of DDT and CHL that were elevated in industrial areas. DDT, which constituted over 50 % of total T-OCP levels, was detected in all the samples as a dominant pesticide in Korea coastal. HCH and CHL constituted 20 % and 11 %, respectively, of total T-OCP levels. Isomer ratios of DDT, HCH, and CHL indicated that these pesticides may no longer be in use. Highly elevated organochlorine residues in bivalves from some areas raised a need of regulatory limits or guidelines to protect the marine system. Species specificity of bivalve PCB and OCP accumulation was investigated for oyster and mussel pairs from twelve different locations. There was no statistical difference in the accumulation level between the two species.

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인천북항에서 유기염소계 농약의 공간적 분포와 영양단계에 따른 생물전이  
**Distribution and trophic transfer of organochlorine pesticides  
 in Incheon North Harbor, Korea.**

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The present study was designed i) to investigate the high tidal range effect on the distribution of organochlorine pesticides (OCs) in abiotic media, and ii) to characterize the bioaccumulation of OCs in marine organisms (zooplankton, oyster, crab, and goby) on different trophic level. The high tidal range (~10m), multiple input sources and paths of varying strength, and proximity to the input sources could make the interpretation of the pesticides fates complicated. Due to the effect of the high tidal range, the levels of some pesticides (Schlordanes, SDDTs, and pentachlorobenzene) were higher at the intertidal zone than at the sub-tidal zone. Concentration gradients were established for chlordane and hexachlorobenzene along the direction of tidal currents. Multiple DDT sources appeared to exist, prohibiting such a gradient. A reversed gradient was observed for HCH, suggesting that direct terrestrial input was negligible. The compositional patterns of the pesticides were heterogeneous in the harbor basin. DDTs and chlordanes were most abundant in both sediment and the organisms, consistent with their high lipophilicity. Among DDTs, DDD and DDE were dominant in the sediment and the organisms, respectively. Trans-nonachlor, compared to other pesticides, highly accumulated with trophic level. The contamination levels of DDTs and chlordanes were high enough to warrant more detailed investigation and regulatory actions.

Key Words: organochlorine pesticides, tidal effect, trophic transfer, congener pattern

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## Prediction of Time-Dependent PAH Toxicity in *Hyalella azteca* Using a Damage Assessment Model

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A 'Damage Assessment Model (DAM)' was developed to describe and predict the toxicity time course for PAH in *H. azteca*. The DAM assumes that death occurs when the cumulative damage reaches a critical point and that the cumulative damage can be described by a combination of first-order toxicokinetic and toxicodynamic models. In aqueous exposures, body residues increase in proportion to the water concentration. Damage is assessed to accumulate in proportion to the accumulated residue and damage recovery in proportion to the cumulative damage when damage is reversible. As a result, the toxicity time course, LC50(t), is determined by both damage recovery rate and an elimination rate. The constant critical body residue (CBR) and the critical area under the curve (CAUC) models can be derived as two extreme cases from the DAM and all three models were re-analyzed using a hazard modeling approach. As a result, the critical cumulative damage ( $D_L$ ) is the determinant of the concentration-time-response relationship and not simply the CBR or the CAUC. Finally, from the DAM, two parameters, a damage recovery rate constant  $k_r$  and the killing rate  $k_+$  were estimated and found to be relatively constant for selected PAH. Therefore, to predict the toxicity time course of a compound, two experiment types, using the same treatment levels, are required; 1) toxicity experiments for gathering time-to-death data 2) and toxicokinetic experiment to estimate kinetic parameters ( $k_u$  and  $k_e$ ).

Keywords: damage assessment model, *Hyalella azteca*, polycyclic aromatic hydrocarbons, critical body residue, time course toxicity

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## Application of a sum of PAH Model and a Logistic Regression Model to Sediment Toxicity Data Based on a Species-Specific Water-Only LC50 Toxic Unit for *Hyaella azteca*

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Two models, a sum of PAH model based on equilibrium partitioning theory and a logistic regression model, were developed and evaluated to predict sediment-associated PAH toxicity to *Hyaella azteca*. A sum of PAH model similar to that of Swartz et al. (1995) developed for marine sediments was applied to freshwater sediments. This study is the first attempt to use a sum of PAH model based on water-only LC50 toxic unit (TU) values for sediment-associated PAH mixtures and its application to freshwater sediments. To predict the toxicity of contaminated sediments to *Hyaella azteca*, a calculated interstitial water toxic unit, the ambient interstitial water concentration divided by the water-only LC50 where the interstitial water concentrations were predicted by equilibrium partitioning theory, was used. Assuming additive toxicity for PAH, the sum of toxic units was calculated to predict the total toxicity of PAH mixtures in sediments. The sum of PAH model was developed from 10-d and 14-d *Hyaella azteca* water-only LC50 values. To obtain estimates of LC50 values for a wide range of PAH, a quantitative structure-activity relationship (QSAR) model ( $\log \text{LC50} - \log K_{ow}$ ) with a constant slope was derived using the time-variable LC50 values for four PAH congeners. The logistic regression model was derived to assess the concentration-response relationship for field sediments, which showed that 1.3 (0.6 - 3.9) TU were required for a 50% probability that a sediment was toxic. The logistic regression model reflects both the effects of co-occurring contaminants (non-measured PAH and unknown pollutants) and the over-estimation of exposure to sediment-associated PAH. An apparent site-specific bioavailability limitation of sediment-associated PAH was found for a site contaminated by creosote. At this site, there were no toxic samples below 3.9 TU. Finally, the predictability of the sum of PAH model can be affected by species-specific responses (*Hyaella* vs. *Rhepoxynius*), chemical specific (PAH vs. DDT in *H. azteca*) biases, which are not incorporated in Eq-P model, and the uncertainty from site-specific effects (creosote vs. other PAH contamination sources) on bioavailability of sediment-associated PAH mixtures.

Key words: Sum of PAH model, Toxic unit, Equilibrium partitioning model, Logistic regression model, *Hyaella azteca*

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**개체군 모델링 기법을 이용한 환경위해성 예측**  
**Prediction of population-level risk from *Daphnia magna* toxicity test data**  
**using population model techniques**

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Life Cycle toxicity statistics are used to evaluate the toxicity and risk of chemical stressors to aquatic invertebrate, *Daphnia magna*, which is used as one of the most sensitive test species for deriving freshwater national water quality criteria. Because these statistics are generated from responses of individual organisms, the relationships of these statistics to significant effects at higher levels of ecological organization are unknown.

In This study, population model estimates have been applied laboratory-derived toxicity data. These include the fecundity values from 21-day life cycle toxicity test. This study was conducted to evaluate the quantitative relationships between toxicity test data and a concentration-based exposure-response models relating projected population growth rate to stressor concentration. This statistic describes the concentration to decline in abundance as determined using population-level modeling techniques. Population risk analysis of responses of *D. magna* was conducted for 4 chemicals. These include tetradifon, 2, 3, 7, 8 TCDD, fenitrothion, and cadmium. The population model we used in this study is basic in which it does not consider density dependence, migration, predation, competition, and other factors that affect population growth.

In population model study, it has been discussed the inherent uncertainties that exist when making predictions of toxicity by extrapolating laboratory results to ecosystem levels. These uncertainties can be lessened by using long-term studies of contaminated ecosystems and processes at many levels of biological organization. Our Daphnid population model is just one of the steps in identifying the biological processes that control toxicity at a population level. This model is an attempt to reduce one of the many uncertainties and simultaneously enhance the ecological realism of a risk-based approach to environmental regulation.

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## **Distribution of Phenolic Chemicals in Water and Sediment from Coast & Creeks Around Industrial Area**

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Simple but effective extraction technique for simultaneous GC/MS determination of Bisphenol-A(BPA), Alkylphenols (APs), and Chlorophenols( CPs) from sediment samples was studied in this paper. The sediment samples were acid-digested then followed by liquid extraction with few milliliters of dichloromethane resulting quantitative recoveries higher than 90% within 30min. It does not require any expensive special equipment and heating process. Acid digestion and extraction condition are optimized in terms of time and acidity of digestion, extraction pH and solvent. The analytical characteristics of this extraction technique was compared to those of Soxhlet extraction and accelerated solvent extraction(ASE) techniques. This technique was applied to real marine sediment samples and obtained satisfactory results with high recovery and sensitivity.

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## Distribution and Characteristics of Polycyclic Aromatic Hydrocarbons in Seawaters of Coastal Korea

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PAHs (polycyclic aromatic hydrocarbons), one of persistent organic pollutants, are ubiquitous in marine environment. National status of PAHs contamination in seawater was investigated as a part of the 2000 Korean Mussel Watch Program. 31 sites along the West, South and East Coast were designated as representative of each coast, and 60 stations were selected in 5 major industrialized bays, i.e. Kyunggi, Kwangyang, Pusan, Ulsan and Young-il Bay.

A total of 91 samples of unfiltered seawater were collected during the period February - April 2000 and analyzed for 23 individual PAHs. In most of the stations, all 23 compounds were detected except acenaphthylene which serves as a minor component. As a total, coastal Korean seawaters were contaminated by PAHs from 39.9 ng/l to 10,200 ng/l with mean value of 551 ng/l. PAHs levels in three coasts were not different statistically ( $p > 0.1$ ), but average concentration of the East Coast was much lower than other two coasts. Contamination loads by riverine inputs, waste discharge by industrial complex, ship's activity and atmospheric deposition of pyrogenic PAHs were supposed to be major sources.

The highest concentration was found in the Ulsan Bay where refineries and other petrochemical complexes are located. Kyunggi Bay appeared to be highly contaminated by PAHs and the average concentration was highest among 5 bays. Young-il Bay and Kwangyang Bay showed similar concentration level and source. Away from hot spots, concentration level was similar to that of representative sites of each coast except Kyunggi Bay.

Benzo[a]pyrene which show carcinogenic activity, concentration was compared to guidelines of Canada and OSPAR. 2 stations of Kwangyang Bay exceeds this level and others in Kyunggi, Young-il Bay approximate.

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## Identification of PAHs Sources in the Intertidal Bivalves and Subtidal Sediments following the Sea Prince Oil Spill in Korea

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In 23 July 1995, oil tanker Sea Prince wrecked around Sori Island near Yosu and spilled 5,035 tons of crude oil into the marine environment. The effects of Sea Prince oil spill to the Sori Island and nearby areas have been investigated from 1996, and this study is the results of the 3<sup>rd</sup> assessment. Analysis of PAHs in 30 subtidal sediments, seasonal variation of PAHs loads to intertidal bivalves, and source identification was carried out during 1999 - 2000.

Spilled oil comprised of three types of crude oil and bunker C fuel oil. PAHs composition varied depend on the source oil, but low molecular weight, alkylated PAHs were dominant in all oil samples. Arabian light crude oil showed highest PAHs concentration above 0.2%. Weathering effects on spilled oil were monitored in one intertidal sediments where oil stranded and penetrated into rocky shore. Temporal changes of PAHs content were not shown but alkyl homologue distribution pattern and PAHs composition indicate that weathering was ongoing. Subtidal stations were divided into three groups, Sori Island (S), Kumo Island (K), and Yosu Bay (Y). Total PAHs concentrations ranged 50.8 - 923 ng/g dry wt., and average concentration in S, K, Y was 129, 196, 199 ng/g dw respectively. One station in K sites showed the highest concentration and nearby areas also showed elevated levels, which suggest relocation of spilled oil into subtidal or offshore sediments. Intertidal bivalves were analyzed seasonally at six sites including control. Average concentration of total PAHs was 183 - 465 ng/g dw, and mussels in Kumo Island showed the highest concentration. There was no significant difference between study area and control sites. Atmospheric deposition, major source of PAHs input into marine environment, was compared between downtown in Yosu and spilled area. Two most important PAHs sources in spilled area were remains of spilled oil and atmospheric deposition of PAHs produced by nearby Yosu, Yochon Industrial Complex.

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## Status of PCBs and Organochlorine Pesticides Contamination in bivalves from Coastal Korea

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PCBs and Organochlorine contamination in bivalves from intertidal area of three coast and 5 major bays in Korea were investigated as a part of Korean Mussel Watch Program.

Total PCBs (sum of PCB8, 28, 29, 44, 52, 66, 87, 101, 118, 128, 138, 153, 170, 180, 187, 195, 200, 206, 209) were ranged from 8.0 to 534 ng/g dw. Station 6 in Younil Bay showed the highest PCBs concentration and PCB153 and PCB180 were the major congeners. Profile of PCBs in coastal areas showed positive correlation with Aroclor 1260 ( $r^2 > 0.5$ ,  $p < 0.01$ ). DDT compounds were the major organochlorine pesticides and their concentrations were order of Pusan > Ulsan > Kyunggi > Yongil > Kwangyang Bay. The ratio of [DDT]/[DDE+DDD+DDT] were relatively higher at the East Coast than other coasts. Hexachlorobenzene and Aldrin were in lower level than DDTs. HCHs concentrations were in the range of nd - 41.2ng/g dw. At Pusan Bay, Ulsan Bay, Samchuck, Chinhae, and Kunsan sites,  $\gamma$ -HCH was the major congener among HCH isomers. But in the other areas  $\beta$ -isomer was highest. Concentrations of chlordane compounds were in the range of 1.34-7.12ng/g dw.  $\alpha$ -chlordane was the major compounds and ratio of trans-nonachlor/ $\gamma$ -chlordane were 0.73-21.8 and this means that recent input of technical chlordane into the environment are small.

DDE concentrations exceeded the guidelines of EAC (Ecotoxicological Assessment Criteria) suggested by OSPAR. But the concentration of aldrin, dieldrin, endrin, heptachlor, and DDTs were lower than the action level of US FDA and NSSP.

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## Distribution of Organochlorine Pesticides and PCBs in Surface Sediments from Coastal Korea

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As a part of Mussel Watch Program, distribution of organochlorine pesticides and PCBs in sediments of three coasts and 5 major bays were investigated.

PCB concentrations were in the range of 0.04-199 ng/g dw. Average PCB concentrations were highest in Pusan Bay. At most of study areas, concentrations of PCBs exceeded the guidelines of EAC (ecotoxicological assessment criteria) suggested by OSPAR. But at only one station in Pusan, PCB concentrations were higher than ERM level(189 ng/g). At Kwangyang, Ulsan, Kyunggi Bay and the other coastal areas, contamination levels were lower than ERL. Concentrations of DDT and its metabolites, DDD, DDE ranged from nd to 135 ng/g dw and the highest concentration was detected in the Pusan Bay. DDT composition (DDT/[DDD+DDE]) were highest in Ulsan Bay, which means recent usage of DDT. HCH compounds including  $\alpha$ -,  $\beta$ -,  $\gamma$ -isomer were also detected in the range of nd - 7.15 ng/g dw. At the West Coast,  $\beta$ -HCH was higher than the other isomers, but  $\gamma$ -HCH was the major isomer at the East Coast. Concentrations of chlordane compounds were nd - 3.15 ng/g dw. Oxychlordane and heptachlor epoxide, metabolites of chlordane compounds were also detected. DDT concentrations at two stations in Pusan Bay exceed ERM level (51.7 ppb) but the contamination level of other sites were lower than the guidelines. Lindane concentrations were lower than ERM (0.99 ng/g) except four stations of the Pusan and Ulsan Bay.

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**벤젠과 톨루엔에 대한 노출의 생체지표로서 소변 중  
대사산물인 muconic acid와 hippuric acid에 대한 평가**  
**Evaluation of Urinary Metabolites, Muconic Acid and Hippuric Acid,  
as Biomarkers of Exposure to Benzene and Toluene**

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대기 중에 흔히 존재하는 방향족 탄화수소 중 benzene과 toluene은 인간에게 유해한 영향을 끼치는 것으로 알려져 있다. 벤젠은 급성 독성이 낮지만, 인간에게 암을 일으키는 것으로 알려져 있다. 반면에, 톨루엔은 인간에 대한 발암성의 증거는 아직 없지만, 이 화합물에 대한 노출은 중추신경계에 영향을 주는 것으로 알려져 있다. 이 두 화합물은 석유 중 수%를 차지하고 있으며, 벤젠은 담배연기 중에서도 검출되고, 톨루엔은 여러 가지 용도의 용매로서 널리 사용되고 있다. 이러한 물질에 대한 연구는 대부분 작업장에서 노출과 관련되어 이루어졌지만, 일반 환경에서의 낮은 수준에서의 노출에 대한 연구는 비교적 적은 편이다. 점차 낮은 농도에서의 만성적인 노출과 질병과의 연관성에 대한 관심이 증가되면서, 이 화합물들에 대한 노출의 직접적인 생체지표에 대한 연구가 활발히 수행되고 있다. 벤젠과 톨루엔은 체내에 들어가 대부분 *t,t*-muconic acid(MA) 및 hippuric acid(HA)로 대사되기 때문에, 오래 전부터 이 화합물들을 benzene과 toluene에 대한 노출의 생체지표로 사용하는 것이 가능한지에 대하여 연구하였다. 이 연구에서는 GC/PDD(HID mode)를 이용하여 소변 중 MA 및 HA를 분석하는 방법을 확립하고, 환경에서의 노출시 이 화합물들이 각각 생체지표로 사용될 수 있는지를 평가하고자 하였다.

총 48명의 사람을 대상으로 대기 중 benzene과 toluene에 대한 노출을 측정하기 위해 각각 12시간 동안 총 24시간에 걸쳐 badge(SKC 575-001)를 착용하게 하였으며, 시료 채취 종료 후 아침의 첫 소변을 채취하였다. Badge 시료는 CS<sub>2</sub>로 추출한 뒤 GC/FID로 분석되었으며, 소변 시료는 황산으로 산성화한 후에 ethyl acetate로 추출하여 농축하고, MA와 HA는 10% H<sub>2</sub>SO<sub>4</sub> in CH<sub>3</sub>OH로 methyl ester 형태로 전환되었다. MA와 HA에 대하여 추정한 방법검출한계(MDL)는 각각 0.08 및 3.8mg/L이었다. 세 농도 수준에서의 재현성과 회수율을 실험한 결과 재현성은 모두 10% 이내로 나타났고, 회수율은 각 분석물질에 대하여 90~95% 및 70~101%이었다. 개인 시료 중 benzene 및 toluene에 대한 노출과 MA 및 HA와의 직선의 상관성을 조사한 결과 두 변수간에는 5%의 유의수준에서 상관관계가 없는 것으로 나타났다. 따라서, 일반적으로 작업장에서와 같이 고농도에서의 노출에 있어서 생체지표로 흔히 사용되는 MA와 HA는 환경에서의 낮은 수준의 benzene과 toluene에 대한 노출의 생체지표로 사용될 수 없음을 알 수 있었다.

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## **Evaluation of predictive models of ready biodegradability in the MITI-I test with the experimental data in Korea**

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Biodegradation is one of the most important degradation process of organic chemicals. Predictive models are important because the number of new and existing chemicals highly exceeds the number of chemicals tested and we usually face the difficulties in risk assessment due to lack of data. Two existing predictive models for assessing the biodegradability of a chemicals, BIOWIN© program and recently developed MITI models by Tunkel et al. (2000), were evaluated with the experimental data of 114 discrete chemicals in Korea as another validation set. The data were accumulated through the testing results using MITI-I method under GLP.

Whereas BIOWIN© poorly predicted the biodegradability of MITI-I test results (about 50% correct), MITI models, both linear and non-linear model, ameliorated the predictability at 80 % and 77 %. The verification results in this study strongly supports the model of Tunkel et al.

Two group of the subject chemicals which caused the discrepancy between model predictions and experimental results were suggested: chemicals whose sub-structures are not fully characterized among 42 fragments in Tunkel et al. and chemicals having low water solubility.

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## SFE를 이용한 잔류농약분석에 있어서 flow rate의 효과

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농약 등의 분석에 있어서 신속한 분석방법들이 크로마토그래피 등의 정량기와 더불어 SPE, ASE, SPME 및 SFE 등과 같은 추출장치들이 개발되었다. 초임계유체의 고유특성을 이용하여 추출하는 supercritical fluid extraction(SFE)는 1990년대 초부터 자동화와 진보된 장치로 광범위하게 적용되고 있다.

특히, 농산물 중, 잔류농약분석에 있어서 CO<sub>2</sub> 밀도(압력, 온도), CO<sub>2</sub>량, modifier 및 trap 등의 기기적인 요소들과 과일, 야채 등과 같이 많은 수분이 함유된 시료에 있어서 회수율이 낮기 때문에 수분조절제를 이용한 시료의 전처리 등에 관한 연구들이 진행되고 있으나, 낮은 회수율이 해결되지 않고 있는 실정이다. 따라서, 본 연구에서는 회수율에 영향을 미칠 것으로 예상되는 몇 가지 요인 중, CO<sub>2</sub> flow rate의 변화에 따른 회수율의 변화를 알아보려고 하였다.

추출조건은 수분조절제인 celite에 4 개의 농약(aldrin, dicofol, dieldrin, methoxychlor)을 처리하고, 360atm, 60°C에서 flow rate 1 ml/min(60 min), 3 ml/min(20 min) 및 5 ml/min(12 min)로 하여 에틸아세테이트로 포집하였다.

시험결과는 1, 3 및 5 ml/min에서 aldrin 40~50%, dicofol 58~78%, dieldrin 44~59%, methoxychlor 58~77%로 회수율이 다소 증가되었고, 5 ml/min(12 min)의 조건에서 전체적인 회수율은 각각의 농약에서 추출율이 50~78%, 잔류 20~24%였으며, 총 회수율은 71~99%가 회수되었다.

이러한 결과로 볼 때, SF-CO<sub>2</sub>를 이용하여 추출하는 경우, CO<sub>2</sub> flow rate가 높아짐에 따라 회수율의 증가와 시간의 단축효과가 있을 것으로 판단되었고, eldrine과 dieldrine과 같이 회수율이 낮은 농약에 대해서는 최적 조건에 대한 연구가 필요한 것으로 사료된다.

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**Antiestrogenic potentials of PCBs which detected  
in Korean adipose tissue**

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Polychlorinated biphenyls(PCBs) are members of the halogenated aromatic group of environmental pollutants. Because of their unique physical and chemical properties, notably their stability and widespread use, PCBs are sidely distributed and transported throughout the global environment. In fact, residues of PCBs have been identified in air, water, aquatic and marine sediments, and human tissue samples.

Although the mechanism of the effects of these PCBs on estrogenic function are still not entirely understood, the toxicities of the PCBs have been studied intensively. Some PCBs exert dioxin-like activities mediated through the aryl hydrocarbon receptor and some congeners are hypothesized to possess endocrine disruptive potential and to induce CYP1A(EROD)

Here we determined PCBs in Korean adipose tissues and their endocrine disrupting effects. Estrogenic activity was determined using E-Screen assay and whole cell competitive binding assay. CYP1A was determined using ethoxyresorufin o deethylase bioassay in H4IIE cell line. Aromatase enzyme activity was determined using tritiated water release assay in JEG-3 cell line.

As a result 5 PCB congeners(52, 118, 138, 153, 180) were detected, they have antiestrogenic potential. These studies demonstrate that selected PCB congeners and their mixture exhibit antiestrogenic activities and affect estrogen biosynthesis.

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## **Evaluation of Toxicity in water samples using cell culture system**

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So far, investigation of environmental pollution has been achieved in field study. This remains the most exhaustive approach, current dimensions of environmental researches and their inherent complexity require that relatively inexpensive and simple laboratory procedures are developed to make possible the screening of large numbers of sites and samples. At this point, microbioassay has been highlighted. The purpose of this study is to evaluate the water pollution using EROD-microbioassay. The methods were optimized and validated for the sensitive and quantitative determination of total toxic effects of the river water samples. The EROD-microbioassay was executed in rat hepatoma cell line, H4IIE and focused to detect PAHs, PCBs and dioxinlike components in the water. Gab stream and Mankyung river in last year were selected for this study. 50L of river water was absorbed using XAD-2 resin column. Pollutants adsorbed to the XAD-2 resin were extracted by elution with methanol. Total toxic effects of extracts were determined by cytotoxicity and EROD- microbioassay. The objectives of this study are : (1) to optimize and validate the cell culture bioassays for the sensitive and quantitative determination of total toxic effects of the environmental samples. (2) to quantitatively assess the toxicological effects of river water samples using toxicity equivalency of 3-MC. (3) to evaluate the water pollution by CYP 1A inducers, especially PAHs.

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**Modulating effects of bisphenol A on the various immunological parameters after subacute oral administration to mice.**

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Bisphenol A (BPA) is a monomer used in the manufacture of a multitude of chemical products including polycarbonates and epoxy resins and has been reported to induce estrogenic activity. We previously reported that BPA showed the modulating effects on the various immunological parameters after acute oral administration. In the present study, we examined the various immunological parameters after subacute oral administration of BPA (100, 500, 1000 mg/kg/day for 30 days) to mice. The weights of liver and kidney were slightly changed, however, hematological parameters, body-, thymus- and spleen-weight were not changed when compared with control group. Splenic IgM plaque forming cell (PFC) to SRBC and splenocytes proliferation to mitogen (Con A, LPS) were not much altered by BPA-treatment. However, in LPS-stimulated peritoneal macrophages obtained from mice exposed to BPA, nitric oxide production was showed a decrease tendency and that of the group given 500 mg/kg/day of BPA was significantly decreased to 34.3% of control, TNF- $\alpha$  production was slightly decreased, and expression intensities of surface marker, B7-1 and B7-2 on macrophages, were also decreased. These results indicate that BPA might show immunotoxic effects, and further investigation on immunomodulating mechanisms of BPA is required.

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## 발광성 미생물 Bioassay 기법에 의한 광양만 퇴적물 환경 평가

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*Photobacterium phosphoreum*은 polycyclic aromatic hydrocarbons (PAHs) 등의 유기 화합물질류에 민감하게 반응하기 때문에 유해 화학물질로 오염된 해양 퇴적물의 생물학적 독성 평가에 많이 이용되고 있는 발광성 미생물이다. 광양만은 반 폐쇄성 만으로 만 주변에 많은 유해 화학물질을 배출하는 오염원들(point sources)이 위치하고 있다. 특히 인근 제철소와 석유화학공업단지에서 발생하는 중금속류와 석유류 성분의 일종인 PAHs가 만 내 퇴적층에 축적되어 있을 가능성이 매우 높은 해역이다. 본 연구에서는 *P. phosphoreum*의 발광량 저해도를 측정하는 기법인 Microtox toxicity test system을 사용하여 광양만과 인접 수로의 퇴적물에 대한 상대적 오염 수준을 평가하였다.

광양만과 인접 수로의 30개 정점으로부터 퇴적물을 채집하였고, 유기용매로 추출한 후, 발광 저해도를 관측하였다. 그 결과, 여천공단과 광양 제철소 부근에서 높은 독성을 나타내었으며, 독성 오염물질이 여수 수로를 통해 외부로 확산되면서 오염원과의 거리가 멀어짐에 따라 독성이 감소하는 경향을 확인할 수 있었다. 이와 병행하여 여수 수로 일부 정점의 시기별 및 시료 채취 깊이별 독성 차이를 분석하였다. 시료 채취 시기별로 큰 차이가 보이지 않았으며, 이같은 결과는 퇴적물 내 존재하는 오염 물질의 자연적 감소가 퇴적물의 총체적 독성 변화에 큰 영향을 주지 못함을 시사하고 있다. 또한, 동일 정점 내 퇴적물 깊이별로 독성 수준이 비슷함을 보인 점은 퇴적물의 교란 현상에 기인한다고 사료된다.

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## FETAX와 Microtox 기법에 의한 Diazinon 및 diazinon 전구물질의 환경독성 비교

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Diazinon(O, O-diethyl O-[6-methyl-2(1-methylethyl)-4-pyrimidinyl]과 diazinon 전구물질인 2-isopropyl-6-methyl-4-pyrimidinol(pyrimidinol), 4(3H)pyrimidone 그리고 pyrimidine에 대한 환경독성을 FETAX와 Microtox 기법으로 평가하고 상호 비교하였다. FETAX(Frog Embryo Teratogenesis Assay-Xenopus)란 *Xenopus laevis* embryo를 시험물질에 노출시켜, 96시간 경과된 후 발생하는 기형율을 판정하는 시험기법이다. FETAX 시험에 의한 Teratogenic Index(기형성 지수, 96-hr LC50/96-hr EC50)가 1.5보다 높을 때, 해당 시험물질이 최기형성을 갖고 있다고 판단하게 된다. 또한 Microtox는 미생물 배양액에 독성물질을 첨가시킨 후 감소되는 미생물 발광량을 정량하여 해당물질의 상대적 독성을 평가하는 기법이다. Diazinon과 pyrimidinol에 대한 *Xenopus* embryo의 LC50는 각각 17.8 및 1503.249ppm이었으며, 총체적 기형발생 지표인 EC50는 각각 0.48ppm, 723.064ppm으로 측정되었다. Diazinon(TI=37.1)이 pyrimidinol보다 18.5배 높은 TI값을 나타내었다. 또한 Diazinon의 다른 전구물질인 4(3H)pyrimidone과 pyrimidine은 pyrimidinol과 비교하여 비슷한 치사율을 보였으나, 기형율은 작았다. 이와 병행하여 수행한 Microtox 독성시험의 Diazinon EC50@30min은 26.90ppm으로 일반적 농약의 독성수준을 나타내었고 Diazinon의 전구물질인 pyrimidinol, 4(3H)pyrimidone, 및 pyrimidine EC50@30min은 각각 1262.76, 13059.75 그리고 6275.85ppm으로 나타났다. 이러한 결과들은 전구물질에 비해 Diazinon이 독성도 클 뿐만 아니라 강력한 최기형성 물질임을 의미하고 있다. 이와 함께 Diazinon은 발광성 미생물보다 Frog Embryo에 대해 예민하게 반응하는 것으로 판단된다.

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## A comparative induction study using biomarker genes for screening of estrogenic effects in medaka.

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The aim of this study was to investigate and to compare the induction levels of possible biomarker genes, choriogenin (Chg), vitellogenin (VTG), and estrogen receptor (ER) to assess their utility as biomarkers for exposure to estrogenic compounds. Choriogenin and vitellogenin expressions are two estrogen-regulated processes and reproductive responses that are integral aspects of fish oogenesis.

17 $\alpha$ -ethinylestradiol was treated to matured male and juvenile medaka (3 weeks after hatching) by exposure in concentrations of 5, 10, 20, 50, 100, and 200 $\mu$ g/L for 6 days, respectively, and reverse transcription(RT)-PCR was used to measure the transcriptional expression levels of these genes. The synthetic oligo-primer sequences used for RT-PCR of the medaka Chg and ER were designed according to published their cDNA sequences, while the primers for the VTG RT-PCR assay were designed based on the analyzed nucleotide sequences in our previous study. Partial sequences of the induced mRNA products by 17 $\beta$ -estradiol using degenerate primers from the VTG conserved regions across several fish species were cloned, and sequence analysis of these clones confirmed vitellogenin gene transcript with homology to mummichog and rainbow trout vitellogenin cDNA sequences.

Treatment of matured males and juveniles with EE2 resulted in pronounced elevation of the liver Chg and VTG(VTGI and VTGII) mRNA levels, and the dose-dependent expression of these genes in treated fish was observed. ER mRNA was also induced by EE2, however, the potency of the induction level was not strong in comparison with choriogenin and vitellogenin. When we compared the sensitivity of these different genes mentioned above at the same concentration of 100 $\mu$ g/L by performing RT-PCR for 25cycles and 30cycles, respectively, Chg L was shown to be more responsive than Chg H, VTG, ER, and HSP70. We conclude that choriogenin L provides a sensitive means of detecting exposure to environmental estrogens.

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## Studies on the Endocrine Disruption in Wildlife Fish

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Endocrine disruption effects in crucian carp (*Carassius auratus*) living in the branch of Han River were examined. Vitellogenin level in plasma was measured using ELISA system and aromatase mRNA level in brain was observed using RT-PCR technique. In all female fish, vitellogenin levels were in the range of 20~40 $\mu$ g/ml and aromatase mRNA expression could be detected on the agarose gel after RT-PCR. However, in case of males, vitellogenin level was elevated in only one fish, while vitellogenin was hardly detected in others. Aromatase was expressed in all males although the levels were relatively lower than the level in female fish. Testis-ova and any other histological changes of reproductive organ were not shown in both sexes.

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OECD SIDS 사업(대량생산 화학물질 위해성평가)  
 -Disodium disulphite의 초기환경 위해성 평가 (환경, 생태분야)-

박광식, 박혜연, 권민정, 최윤호, 송상환, 구현주, 전성환, 나진균

인천광역시 서구 경서동 국립환경연구원

OECD SIDS (Screening Information Data Set)사업이란 각 국에서 연간 1000톤 이상 생산되는 화학물질을 대량생산화학물질 (High Production Volume Chemicals)로 규정하고 물질의 물리화학적 특성, 환경 중 거동, 생태독성, 인체독성에 대한 독성 및 노출자료 등 기본자료를 확보하여 해당 화학물질의 위해성(Risk)을 평가함으로써 우리나라가 OECD가입당시 회원국으로서 사업수행을 약속한 사항이다. 현재 전 세계적으로 초기 위해성 평가 대상물질 4600여종에 대한 효과적 업무 수행을 위해 대부분의 OECD 회원국이 업무를 분담, 동 사업을 수행 중에 있다. 우리나라는 현재 아세트아닐라이드 등 7개 물질에 대한 평가를 수행중에 있으며 이중 Disodium disulphite에 대한 초기 위해성 평가를 완료하였고 그 평가 결과는 다음과 같다.

Disodium disulphite은 우리나라에서 1998년 기준으로 대량생산화학물질(High Production Volume Chemicals)로서 1998년 환경부 유통량 조사 결과 연간 3200톤을 생산 및 수입하고 있으며, 음식첨가제 또는 탄닌제로 널리 사용되고 있는 화학물질이다.

본 물질은 무기화학물질로 가열시 이산화황 가스 형태로 분해되며(150℃), 높은 수용해도(470 g/l at 20℃)를 가지며, 수중에서 안정한 물질로 평가되었다. 또한 무기물질로서 환경 중 대부분 물에 분포하는 것으로 보이며, 토양환경에서의 이동성은 매우 높게 나타났다(토양흡착계수  $K_{oc}$ : 2.447). 이밖에 화학물질 특성상 생분해 실험은 적합하지 않으며, 생물농축성 또한 일어나지 않을 것으로 평가되었다.

생태독성을 평가하기 위한 기본 실험 가운데 송사리(Medaka, *Orizias latipes*)를 이용한 어류독성실험의 경우는 96시간 반수치사농도(LC<sub>50</sub>)가 >100mg/l 였으며, 물벼룩(Water flea, *Daphnia magna*)을 이용한 실험에서는 48시간 반수영향농도(EC<sub>50</sub>)가 88.76mg/l 였으며 조류(Algae, *Scenedesmus subspicatus*)실험에서는 72시간 반수영향농도(EC<sub>50</sub>)가 48.1mg/l로 관찰되었다. 그 외에 물벼룩(Water flea, *Daphnia magna*)을 이용한 21일-만성독성의 결과는 21일-무영향농도(NOEC) >10 mg/l로 나타났다. 따라서 생태독성 실험값 중 가장 민감한 물벼룩 만성독성 NOEC 값 >10mg/l을 가지고 평가지수 (assessment factor) 100을 적용하여 가장 엄격한 (conservative) 예측무영향 농도 (PNEC, predicted no effect concentration)값 0.1 mg/l을 구하였다. 이상 Disodium disulphite의 생태 및 환경에 대한 초기 위해성 평가 결과, 본 물질은 Post-SIDS 연구사업 수행시 우선순위가 낮은 것으로 권고되었다.

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**OECD SIDS 사업(대량생산화학물질 위해성평가)**  
**- Disodium disulphite의 초기 인체 위해성 평가(인체 건강분야) -**

박광식, 최윤호, 송상환, 박혜연, 권민정, 구현주, 전성환, 나진균

인천광역시 서구 경서동 국립환경연구원

OECD SIDS(Screening Information Data Set) 프로그램은 1000톤 이상 생산, 사용되고 있는 대량생산화학물질을 대상으로 위해성평가를 수행하는 사업으로서 OECD 회원국들간의 공동작업으로 진행되고 있으며, 초기 유해성 평가와 합의과정을 거쳐 후속 평가사업(further work)의 필요수준을 도출해 내는데 그 주요 목적이 있다.

그간 우리나라는 1999년부터 SIDS 사업참여의사를 공식적으로 표명한 이래, 7종의 대량생산화학물질에 대한 위해성 평가를 수행해 왔으며, 최근 11월 스위스 베른에서 개최된 제 13차 위해성평가 전문가 회의(SIAM13)에서 Disodium disulphite와 Acetanilide의 위해성 평가 보고서를 제출하여 그 심의를 통과 하였다. 그 중 본 발표는 SIAM13에 보고된 Disodium disulphite의 인체위해성 평가 결과를 소개하고자 한다.

Disodium disulphite은 세계적으로는 330,000 톤/년, 국내에서 3200 톤/년 가량 생산되는 대량생산화학물질로서, 탄닌제, 수처리제, 표백제, 사진현상제 혹은 식품제조 과정의 첨가제로 사용되고 있다. 국내 작업장 노출허용기준은 5 mg/m<sup>3</sup>(8hr TWA)로 규제하고 있으며 산업체에서나 최종소비자에서의 노출사례는 아직 국내에서 보고된 바 없다. Disodium disulphite의 급성독성 수준은 랫드 경구 투여시 반치사량(LD50)이 1,540 mg/kg bw로 다소 낮으며 피부 자극시험과 감작성 시험에서 각각 무자극성, 비감작성 물질로 보고되었다. 랫드를 이용한 약 2년간 반복투여 독성시험에서 전신독성(system toxicity)은 관찰되지 않았으나 위(stomach)에서의 염증이 관찰되어 국소독성(local toxicity)을 유발할수 있으며, NOAEL값은 217 mg/kgbw/day로 평가되었다. 또한 3세대에 걸친 연구에서 생식능에 유의한 이상이 관찰되지 않아 생식독성을 유발시키지 않을 것으로 판단된다. 시험관내 유전독성 연구에서는 시험환경에 따라 양성 혹은 음성 결과가 혼재하였으나 생체 유전독성 연구에서는 확실한 양성 결과를 보여 인체노출시 돌연변이원이 아님을 보여주었고 또한 다세대 반복투여에 의하여 유의한 암 발생은 관찰되지 않았다.

Disodium disulphite에 노출된 사람에게 천식 등의 호흡기 장애가 발생된 사례가 보고된 바 있으나, 직접적인 감작성 물질이라고 판단하기는 어려우며, 민감한 사람에게는 천식 등의 감작 반응을 증진시킬 수 있는 것으로 보인다.

국내 노출 현황 및 인체 독성자료를 바탕으로 Disodium disulphite는 향후 post-SIDS 연구사업 대상 선정에 있어 낮은 우선순위인 물질로 권고되었다.

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## 자주달개비 분석법을 이용한 환경 중 돌연변이유발물질의 감시

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산업화로 인한 다양한 화학물질의 개발 및 사용량 증가는 생활환경중의 환경오염물질의 증가를 초래하고 있을 뿐 아니라 이에 따른 노출기회의 증가로 인간의 건강을 크게 위협하고 있다. 따라서 환경오염물질의 염색체변이유발과 세포유전독성을 신속하게 확인할 수 있는 방법이 필요하게 되었다. 이러한 요구조건에 적합하게 활용할 수 있는 방법이 자주달개비 (*Tradescantia*)를 이용한 분석법이다.

자주달개비는 분류학적으로 달개비과 (Commelinaceae)에 속하는 현화식물로 자주달개비 屬 내에 수많은 종이 속해 있다. 한 화서는 8~12개의 꽃봉우리로 구성되며, 각각의 수술대에는 20~35개의 단세포로 이뤄진 수술털이 40~150개 있다.

자주달개비 수술털 분석법 (Trad-SHM)은 수술털의 선단세포는 분열능력이 있어서 발생 또는 분열과정 중 돌연변이원에 노출되면 쉽사리 체세포 돌연변이를 일으킨다. 즉 화서의 발생 또는 수술털 세포의 분열과정 중에 돌연변이원에 노출된 경우 우성형질 (표현형)이 자주색이지만 염색체가 손상을 받아 절단이 일어나면 상동염색체상에 존재하는 열성형질이 발현되어 꽃잎 또는 수술털의 전체 또는 일부분이 분홍색을 띠게 된다. 이러한 색깔변화는 관찰이 용이하기 때문에 돌연변이원의 영향을 역추정할 수 있는 분석법이다.

자주달개비 미세핵분석법 (Trad-MCN)은 자주달개비의 화분모세포 (PMC: pollen mother cell)는 감수분열을 통하여 4개의 꽃가루를 형성하는 응성 생식세포로서 돌연변이원에 매우 민감하다. 특히, 감수분열중인 화분모세포의 염색체는 동일개체의 분열중인 체세포 염색체보다 민감하다고 알려져 있다. 환경 중 돌연변이유발물질에 노출되었을 경우 무동원체 염색체 조각 (acentric fragment)이나 점착성 염색체 복합부위 (sticky chromosome complex)가 감수분열의 4분자염색체 (tetrad)시기에 미세핵으로 남게 된다. 많은 수의 4분자염색체중에 미세핵의 빈도는 염색체 손상의 지표로 신뢰할 수 있다. 이러한 실험은 1970년대 초부터 포유동물 감수분열 세포에서도 미세핵의 생성은 염색체 손상을 의미하는 유용한 지표로서 이용되기 시작하면서부터 동물세포는 물론 식물세포에 있어서도 미세핵은 염색체 손상을 나타내는 명백한 지표로서 이용되기에 이르렀다. 자주달개비 미세핵분석법은 신속하고, 간단하며, 환경유해물질의 염색체변이영향을 결정하는 생물학적 분석방법이다..

자주달개비를 이용한 분석방법은 방사선에 대한 안전성평가, 대기오염, 수질오염, 폐기물침출수, 토양오염 및 액체상 또는 기체상의 화학물질에 대한 유전독성평가 등 생물학적 영향에 대한 감시수단으로서 활발히 이용되고 있다.

앞으로 본 자주달개비 분석법을 이용하여 다양한 환경중 돌연변이유발물질에 대한 생물학적 영향평가를 위한 감시모델로 유용하게 활용할 수 있을 것으로 판단된다.

<책임연구자>

성 명:

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## Oligonucleotide Microarray-based Enteric Virus Detection

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**Background:** A novel system for the rapid and accurate detection of three major groups of enteric viruses (pan-enterovirus, adenovirus and rotavirus) in clinical samples (stool and cerebrospinal fluid) as well as in environmental samples (water, sludge, and soil) was developed. The system employs a DNA chip with microarrayed oligonucleotide probes that are specific to the target sequences of given enteric viruses.

**Methods:** Triplex (RT)-PCR was performed targeting the 5' non-coding region for pan-enterovirus, the hexon gene region for adenovirus, and the VP7 gene for rotavirus. The reaction product of the (RT)-PCR was labeled with cyanine-5 and then hybridized to the immobilized probes followed by fluorescence scanning for the specific virus group identification.

**Results:** The enteric virus detection system was validated by testing corresponding references of the three enteric virus groups and also successfully applied to clinical samples after optimization of experimental details.

Differentiating the enteric adenoviruses (types 31, 40 and 41) from non-enteric adenoviruses at the same time would be an additional attraction of the system. The DNA chip detection system turned out to be more sensitive than cell culture method statistically. Complete virus detection procedure of the system took only 5-6 h while it often takes days and weeks in cell culture method.

**Conclusion:** The first DNA chip system for enteric virus detection was developed and proved to be highly specific, sensitive, and faster than ever. The system, targeting the three major enteric virus groups, will obviously upgrade the enteric virus detection methodology for clinical specimens and environmental samples as well.

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