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Phenanthrene-induced changes in blood organic components, transaminase and ALP activities of *Paralichthys olivaceus*

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Phenanthrene-induced changes in blood organic components, transaminase and ALP activities of *Paralichthys olivaceus* were investigated. The aim of this study was to obtain a holistic view of the toxic responses, and compensations of fish exposed to waterborne phenanthrene. Fish were exposed to 0, 0.5, 1, 2 μM of phenanthrene for 4 weeks. The most noticeable changes were concentration-dependent increase in levels of blood GOT and GPT activities. At concentrations 1.0 and 2.0 μM of phenanthrene GOT, GPT and ALP enzyme activities in plasma were increased. Plasma albumin concentration was stable in phenanthrene-exposed fish, however, plasma total protein level was reduced significantly at the 4-week sampling points (2.0μM). Plasma glucose levels were found to be increased significantly over the control throughout duration of the experiment with phenanthrene. Overall the results suggest that phenanthrene has the potential to alter some physiological functions in olive flounder.

Keyword : *Paralichthys olivaceus*, Phenanthrene, Plasma, Haemochemical enzyme, Organic compounds

Introduction

Polycyclic aromatic compounds are widespread in the environment. Because of their carcinogenic and mutagenic properties (Lehr and Jerina, 1977), these compounds have been intensively studied in the various compartments of the environment (McElroy *et al.*, 1989). Approximately 230,000 tons of total PAHs enter aquatic environments yearly, worldwide (Eisler, 1987). A high incidence of liver tumors has been reported in several species of bottom-feeding fish collected from areas contaminateds with these chemicals (Baumann *et al.*, 1982). Phenanthrene is a polycyclic aromatic hydrocarbon (PAH), ubiquitous in the environment as a product of incomplete combustion of fossil fuels and wood. It has been identified in ambient air, surface and drinking water, and in food (U.S. EPA, 1988). Phenanthrene (CAS Reg. No. 85-01-8), with three aromatic rings, accounts for 0.3-3% of the total composition of crude oil (NASA, 1985). Although a large body of literature exists on the toxicity of PAHs, primarily benzo[*a*]pyrene, toxicity data for phenanthrene are very limited.

Measurement of biochemical and physiological parameters is a commonly used diagnostic tool in aquatic toxicology and biomonitorings (McDonald and Milligan, 1992; Folmar, 1993; Soimasuo *et al.*, 1995). In toxicological studies of acute exposure, changes in concentrations and enzyme activities often directly reflect cell damage in liver and kidney (Casillas *et al.*, 1983). Livers of vertebrates are the main organs of biotransformation, therefore, they are the organs of choice for biomonitoring effects of aquatic pollutants of coastal environment (Von Landwust *et al.*, 1996; Broeg *et al.*, 1999). Determination of transaminase enzymes (glutamic-acid-oxylacetic-acid-transaminase - GOT, glutamic-acid-

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pyruvic-acid-transaminase - GPT) is a routine method in human diagnostics. Under normal conditions these enzymes can be found within cell membranes, cytoplasm and mitochondria. If cells are damaged and disintegrated (cell necrosis) large amounts of those enzymes are released into the blood circulation, resulting in a dramatic increase in blood serum activities (Jeney, 1992). GOT and GPT transaminases are enzymes frequently used in the diagnosis of damage caused by pollutants in various tissues such as liver, muscle and gills of fish, also (de la Torre *et al.*, 1999).

Total protein (TP) is used as an indicator of liver impairment. Decreased concentrations can be caused by structural liver alterations (e.g., cirrhosis) reducing aminotransferase activity with a concurrent reduction in the deamination capacity. Hematological parameters reflecting alterations on the carbohydrate matabolism, such as plasma glucose and lactate concentrations, have the potential as general stress indicators in fish (Adams, 1990). Flounder seems to be suitable for monitoring of sediment pollution exposure and effects in coastal area as it is a bottom-dwelling species. European flounder (*Platichthys flesus* L.) are thus often strongly inflicted by liver cancer because of their close contact to sediments loaded with hepatotoxic compounds (Malins *et al.*, 1984; Malins et al., 1985; Myers *et al.*, 1990). Flounder is a stationary, benthic organism and one of the most important fish in Korean aquaculture.

In the present experiment, the effects of chronic exposure to phenanthrene on plasma organic components and transaminase activities in flounder were investigated. The present study is part of a larger scale study, focusing on environmental diseases in relation to exposure levels of various environmental pollutants via coastal toxicants.

Material and Methods

Animal

Olive flounder (*Paralichthys olivaceus*), 51 g mean body weight, were obtained from a commercial farm in Pohang, Kyongbook, Korea and transferred to a maintenance room where temperature was kept at 20±1℃. Fish were acclimated to the laboratory condition for two weeks and the feed was withdrawn one day prior to experimentation. Test chamber (glass aquaria, 50 ℓ capacity) were filled with 40L of sea water. Water characteristics, measured by the method described in APHA(1985), were as follows: pH, 8.03±0.4; temperature, 20±1℃; salinity, 31.8±0.7‰; and dissolved oxygen 7.5-7.8 mg/ℓ.

Exposure

Fifteen minutes prior to introduction of fish to 50-L aquaria, solutions of phenanthrene (96% purity, Sigma Chemical, St. Louis, MO) were mixed with water to attain a nominal concentration of 0.5, 1, 2 μM. In each test chamber, a group of nine fish was exposed for a period of 2, 4 weeks.

Sampling

At the end of each period (at 2, 4 weeks) fish were anesthetized in buffered 3-aminobenzoic acid ethyl ester methanesulfonate (Sigma). They were quickly transported to the processing table where they were measured, weighed, and bled from the caudal vessel using heparinized syringes. Blood were centrifuged (6,000 rpm for 5 min, MIKRO 22R, Hettich, Germany), and the plasma collected was frozen (-80℃) until required for analysis. All procedures were performed within 3 h of blood collection.

Assay of blood organic components, transaminase and ALP activities

The activities of GOT, GPT and alkaline phosphatase (ALP) and levels of plasma albumin, glucose and total protein were determined spectrophotometrically using a spectrophotometer (DR 4000, HACH). GOT, GPT and ALP assays were performed using specific kit and reagent (Asan Pharm. Co. Ltd.). Blood albumin(BCG method), glucose (glucose oxidase/peroxidase method) and total protein (colorimetric method) were determined on plasma using Sigma diagnostic kit.

Statistical analysis

Statistical analyses were performed with the aid of the SPSS/PC+ statistical package. Significant differences between means were determined using one-way ANOVA and the Duncan's test for multiple comparisons, or the Student's *t*-test for two groups (Duncan, 1955). Significance level was established at P<0.05.

Results

Blood organic compounds

There was a decline (P=0.056) in albumin concentration at 4 weeks in the phenenthrene-treated fish (1.0μM) compared to controls (Fig. 1). However, there were no significant changes in all exposure group compared to controls (P>0.05). Plasma glucose levels were found to be increased significantly over the control throughout duration of the experiment with phenanthrene (Fig. 2). Compared with the values found in the control group, the values were about two times higher for plasma glucose concentration in fish exposed to 2.0 μM phenanthrene at 4 weeks (P<0.01). Plasma total protein levels showed insignificant changes after 2 weeks, but thereafter significant reduction was observed in the group with the highest concentration (P<0.05).

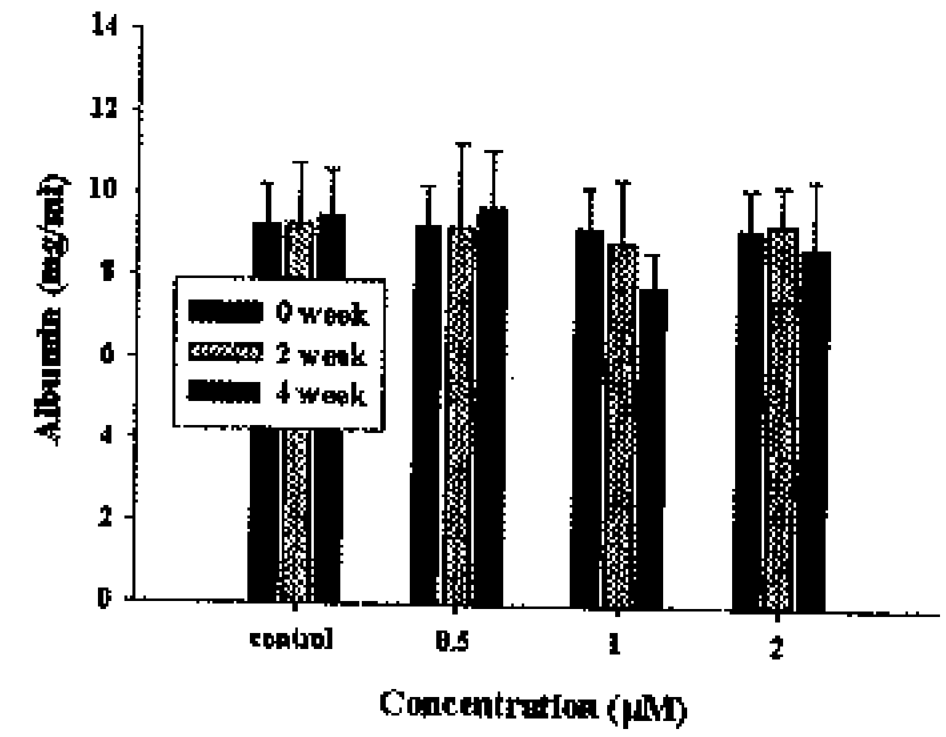

Fig. 1. Changes of blood albumin in olive flounder, *Paralichthys olivaceus* exposed to different concentrations of phenanthrene. Vertical bars indicate standard deviation.

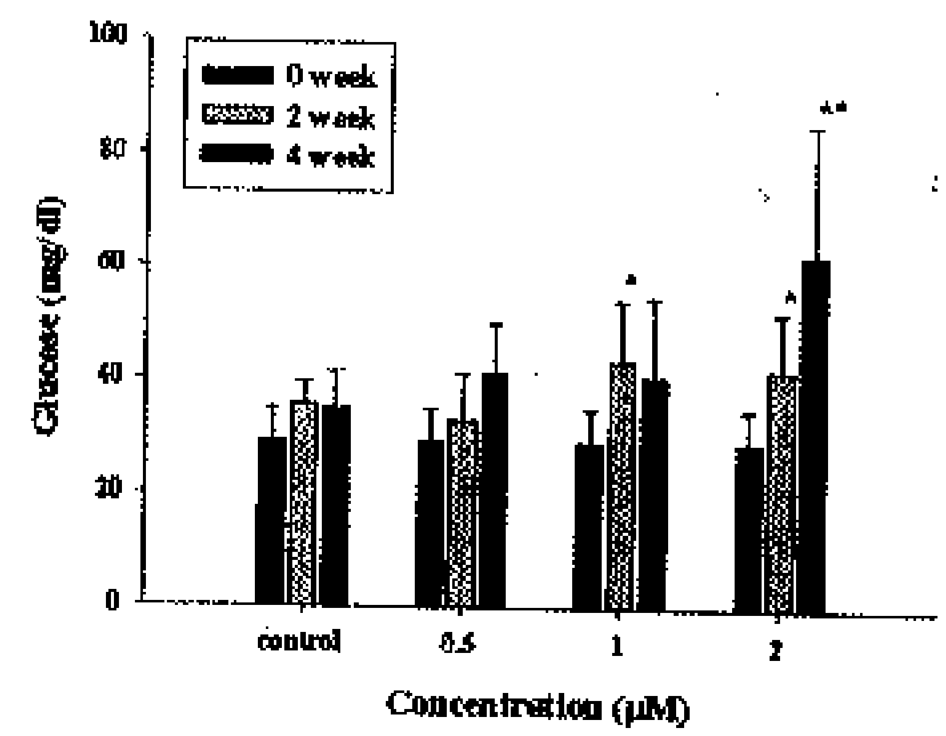

Fig. 2. Changes of blood glucose in olive flounder, *Paralichthys olivaceus* exposed to different concentrations of phenanthrene. Vertical bars indicate standard deviation.
* significant different between control and phenanthrene exposure group ; P<0.05

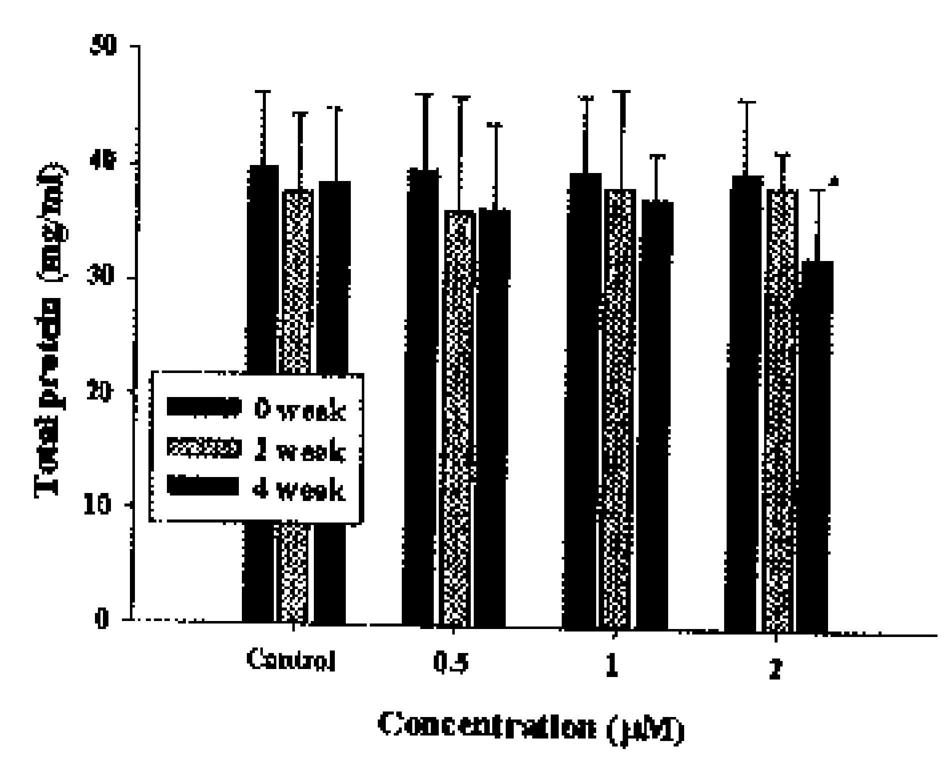

Fig. 3. Changes of blood total protein in olive flounder, *Paralichthys olivaceus* exposed to different concentrations of phenanthrene. Vertical bars indicate standard deviation.
* significant different between control and phenanthrene exposure group ; P<0.05

Plasma transaminase and ALP

GOT activity in the plasma of *P. olivaceus* showed a significant increase in exposure to higher concentrations of phenanthrene (>1.0 μM) throughout the exposure period (Table 1). GPT activity increased at the concentration of 1.0 and 2.0 μM at 4 weeks ($P<0.05$ and $P<0.01$, respectively). Plasma alkaline phosphatase level showed insignificant changes at 2 weeks, thereafter significant elevation was observed. The elevation was found to be more pronounced with 1.0 μM phenanthrene. Plasma ALP activities were increased (by 47.2 and 36.6%, respectively) in flounder exposed to 1.0 μM and 2.0 μM phenanthrene at 4 week($P<0.05$ and $P<0.01$, respectively), but there was no significant change at the other exposure period (Table 2).

Discussion

The measurement of enzyme activity to diagnose pollutant effects in fish may constitute a useful ecotoxicological tool. The evaluation of liver GPT activity may provide indications of metabolic and cytotoxic alterations (Pacheco and Santo, 2001). In the present study, exposure of flounder to phenanthrene, resulted in a progressive increase in blood GOT and GPT activity continuing over the 4 weeks exposure period, indicating significant cytotoxic damage. Transaminases are intracellular enzymes which exist at low levels in normal serum. Therefore, damage to the liver cell may result in leakage of the enzymes into the serum due to a large concentration gradient (Wroblewski and La Due, 1955). Generally elevated alkaline phosphatase is

Table 1. The variation of blood GOT and GPT activities in phenanthrene-treated *Paralichthys olivaceus* for 4 weeks.

Phenanthrene concentration		Control	0.5 μM	1 μM	2 μM
GOT	0 week	59.27±6.28(7)			
	2 week	53.65±8.07(9)	58.13±7.52(9)	64.67±16.59(9)	75.58±19.84(9)*
	4 week	57.22±10.20(8)	56.57±10.33(8)	76.75±12.80(8)*	74.92±15.99(8)*
GPT	0 week	18.53±3.39(7)			
	2 week	18.78±4.43(9)	18.17±3.03(9)	20.95±4.91(9)	21.42±4.82(9)
	4 week	18.44±2.40(8)	17.69±2.27(8)	24.16±5.04(8)*	26.68±6.28(8)**

GOT and GPT activities is expressed as Karmen Unit. The results represent the means of the number of experiments indicated in parentheses ±SD. * $P<0.05$, ** $P<0.01$.

Table 2. The variation of blood ALP activities in phenanthrene-treated *Paralichthys olivaceus* for 4 weeks.

Phenanthrene concentration		Control	0.5 μM	1 μM	2 μM
ALP	0 week	3.58±1.07(7)			
	2 week	3.90±1.17(9)	3.75±1.09(9)	4.33±0.90(9)	4.21±0.99(9)
	4 week	3.74±0.98(8)	4.76±1.05(8)	5.27±0.91(8)*	4.89±1.50(8)*

ALP activities is expressed as King-Armstrong units. The results represent the means of the number of experiments indicated in parentheses ±SD. * $P<0.05$.

observed as a result of intra- and extra-hepatic obstruction to biliary passage. Hence, estimation of serum alkaline phosphatase is used as a test for liver function. Gupta and Dhillon (1983) studied the effects of sublethal concentrations of xenobiotics in the plasma of *Claris batrachus* and *Cirrhina mrigala*, suggesting liver damage which might have induced production alkaline phosphatase, which are subsequently released into blood. The elevation in alkaline phosphatase suggests an increase in the cell necrosis due to xenobiotic toxicity. Reddy *et al.* (1992) studied the effects of the herbicide diuron on *Sarotherodon mossambicus*. They ascribed elevated ALP level to liver damage and alterations in liver function. Goel et al. (1984) observed elevated levels of ALP in *Claris batrachus* exposed to Alachlor and suggested tissue damage. Hence the increased activity of alkaline phosphatase in the present investigation, evident with 4 weeks exposure to 1.0 μM and 2.0 μM of phenanthrene ($P<0.05$), could be due to cell necrosis in liver.

Althought some studies indicate a decrease in albumin content during aquatic pollutant exposure, no significant changes were observed in the plasma of phenanthrene-treated flounder. The adverse effect of pollutants on the plasma total protein levels of fishes has been studied in many investigations (McKim *et al.*, 1970; Ramalingam and Ramalingam, 1982; Gluth and Hanke, 1984). They found that the decrease in total protein in fish exposed to toxic levels of toxicants could be attiributed to either a state of hydration and change in water equilibrium in the fish or a disturbance in liver protein synthesis, or both. In the present study, significantly reduced total protein levels were observed in the plasma of highest phenanthrene concentration ($P<0.05$).

Toxic substances such as pesticides might induce hyperglycemic condition caused by hypersecretion of adrenaline which in turn stimulates the breakdown of glycogen to glucose (Gupta, 1974). Depletion of glycogen content in several organ of fish during toxic stress by toxic materials was reported (Ghosh, 1987). Hyperglycaemia can be viewed as a physiological response of the fish to meet the critical energy needs under toxic stress. In the present study, exposure of phenanthrene to *P. olivaceus* induced significant elevation of blood glucose level. Compared with the values found in the control group, the values recorded were about two-fold higher for plasma glucose concentration in fish exposed to 2.0 μM phenanthrene at 4 weeks ($P<0.01$). Plasma total protein levels showed insignificant changes after 2 weeks, thereafter significant reduction was observed in the group with highest concentration ($P<0.05$). This generalized stress response typically includes effects on the carbohydrate metabolism, such as the plasma glucose increase (Hontela and Santos, 1996; Kennedy, 1995). The present study represents the first assessment of the chronic changes in plasma enzyme activities in fish species exposed to phenanthrene.

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References

Adams, S. M. : Biological indicators of stress in fish. Am. Fish. Soc. Symp., 1-8, 1990.

APHA (American Public Health Association), American water works association, and water pollution control federation : Standard methods for the examination of water and waste water. 16th edition. APHA, Washington, D. C. : 1985.

Baumann, P. C., Smith, W. D. and Ribbick, M. : In polynuclear Aromatic Hydrocarbons: Physical and Biological Chemistry. Battelle Press, Columbus, OH, 93-102, 1982.

Broeg, K., Zander, S., Diamant, A., Kruner, G., Paperna, I. and Westernhagen H. V. : The use of fish metabolic, pathological and parasitological indices in pollution monitoring. I. North Sea. Helgoland Marine Res., 53 : 171-194, 1999.

Casillas, E., Meyers, M., and Ames, W. E. : Relationship of serum chemistry values to liver and kidney histopathology in English sole (*Parophrys vetulus*) after acute exposure to carbon tetrachloride. Aquat. toxicol., 3 : 61-78, 1983.

de la Torre, F. R., Salibian, A., Ferrari, L. : Enzyme activities as biomarkers of freshwater pollution: responses of fish branchial (Na+K)-ATPase and liver transaminases. Environ. Toxicol., 14 : 313-319, 1999.

Duncan, D. B. : Multiple-range and multiple F tests. Biometrics, 11 : 1-42, 1955.

Eisler R. : Polycyclic aromatic hydrocarbons hazards to fish, wildlife, and invertebrates:a synoptic review. Fish. Wild. serv. Biol. Rep., (US) Washington, DC. 104C : 67-70, 1987.

Folmar, L. C. : Effects of chemical contaminants on blood chemistry of teleost fish: A bibliography and synopsis of selected effects. Environ. toxicol. Chem., 12 : 337-375, 1993.

Ghosh, T. K. : Impact of commercial grade hexagor and sumidon on behaviour and some aspects of carbohydrate metabolism in fish *Cyprinus carpio*. Uttar Pradesh Journal of Zoology., 7 : 48-62, 1987.

Gluth, G. and Hanke, W. : A comparison of physiological changes in carp *Cyprinus carpio*, induced by several pollutants of sublethal concentration. II. The dependency on the temperature. Comp. Biochem. Physiol., 79C : 39-45, 1984.

Goel, K. A., Kalpana, S. and Agarwal, V. P. : Alachlor toxicity to a freshwater fish *Clarias batrachus*. Curr. Sci., 53: 1051-1052, 1984.

Gupta, A. K. and Dhillon, S. S. : The effects of a few xenobiotics on certain phosphates in the plasma of *Clarias batrachus* and *Cirrhina mrigala*. Toxicol. Lett., 15 : 181-186, 1983.

Gupta, P. K. : Malathion induced biochemical changes in rats. Acta Pharmacologia et Toxicologia., 35 : 191-194, 1974.

Hontela, A., Daniel, C and Ricard, A. C. : Effects of acute and subacute exposures to cadmium on the interrenal and thyroid function in rainbow trout (*Oncorhynchus mykiss*). Aquat. Toxicol., 35 : 171-182, 1996.

Jeney, G., Nemcsok, J., Jeney, Zs. and Olah, J. : Acute effect of sublethal ammonia concentrations on common carp (*Cyprinus carpio* L.). 2. Effect of ammonia on blood plasma transminases GOT, GPT), GlDH enzyme activity, and ATP value. Aquaculture, 104 : 149-156, 1992.

Kennedy, C. J., Sweeting, R. M., Johansen, J. A., Farrell, A. P. and McKeown, B. A. : Acute effects of chlorinated resin exposure on juvenile rainbow trout, *Oncorhynchus mykiss*. Environ. Toxicol. Chem., 14 : 977-982, 1995.

Lehr, R. E. and Jerina, D. M. : Metabolic activation of polycyclic hydrocarbons. Arch. Toxicol., 39 : 1-6, 1977

Malins, D. C., McCain, B. B., Brown, W. D., Chan, S. L., Myers, M. S., Landahl, J. T., Prohaska, P. G., Friedman, A. J., Rhodes, L. D., Burrows, D. G., Gronlund, W. D. and Hodgins, H. O. : Chemical pollutants in sediments and diseases in bottom-dwelling fish in Pudged Sound, Washington. Environ. Sci. Technol., 18 : 705-713, 1984.

Malins, D. C., Krahn, M. M., Myers, M. S., Rhodes, L., Brown, W. D., Krone, C. A., McCain, B. B. and Chan, S. : Toxic chemical in sediments and biota from a creosote-polluted harbour: relationship with hepatic neoplasms and other hepatic lesions in English sole (*Parophrys vetulus*). Carcinogenesis, 6 : 1463-1469, 1985.

McDonald, D. G., and Milligan, C. L. : Chemical properties of the blood. In "Fish Physiology" (W. S. Hoar, D. J. Randall, and A. P. Farrell, Eds.), Academic Press, San Diego, 55-133, 1992.

McElroy, A. E., Farrington, J. W. and Teal, J. M. : Bioavailability of polycyclic aromatic hydrocarbons in the aquatic environment. In Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment (Varanasi, U., ed.). CRC press, Boca Raton, FL, 1-40, 1989.

McKim, J. M., Christensen, G. M. and Hunt, E. P. : Changes in the blood of brook trout *Salvelinus fontinalis* after short-term and long-term and long-term exposure to copper. J. Fish. Res. Bd. Can., 25 : 1883-1889. 1970.

Myers, M. S., Landahl, J. T., Krahn, M. M., Johnson, L. L. and McCain, B. B. : Overview of studies on liver carcinogenesis in English sole from Pudget Sound; evidence for a xenobiotic chemical etiology. Pathology and epiziitiology. Sci. Total. Environ., 94 : 33-50, 1990.

NASA (National Academy of Sciences) : Oil in the sea-inputs, fates and effects. National Academy Press, Washington, DC, 601, 1985.

Pacheco, M and Santo, M. A. : Tissue distribution and temperature-dependence of *Anguilla anguilla* L. EROD activity following exposure to model inducers and relationship with plasma cortisol, lactate and glucose levels. Environ. Int., 26 : 149-155. 2001.

Ramalingam, K. and Ramalingam K. : Effects of sublethal levels of DDT, malathion and mercury on tissue protein of *Sarotherodon mossambicus*(Peter). Proc. Ind. Acad. Sci. Anim. Sci., 91 : 501-505, 1982.

Reddy, D. C., Vijayakumari, P., Kalarani, V. and Ronald, W. Davis : Changes in erythropoietic activity of *Sarotherodon mossambicus* exposed to sublethal concentration of the herbicide diuron. Bull. Environ. Contam. Toxicol., 49 : 730-737, 1992.

Soimasuo, R., Ilmari, J., Kukkonen, J., Petanen, T., Ristola, T. and Oidari, A. : Biomarker responses along a pollution gradient: Effects of pulp and paper mill effluents on caged whitefish. Aquat. Toxicol., 31 : 329-345, 1995.

U.S. EPA : Drinking water criteria document for polucyclic aromatic hydrocarbon (PAHs). Prepared by the environmental criteria and assessment office, office of Health and environmental assessment, U.S. Environmental Protection Agency, Cincinnati, OH, for the Office of Drinking Water. ECAO-CIN-D010, 1988.

Von Landwust, C., Holst, S., Moller, H., Anders, K., Momme, M., von Neuhoff, N., Scharenberg, W., Weis, N., Lee, K., Kohler-Gunther, A., Cameron, P., Brauer, F., Pluta, H.J., Kruse, R., Ballin, U., Soffker, K. and Dethlefsen, V. : Fischkrankheiten in der Nordsee, Umweltbundesamt Nr. 106 03 900, UBA Texte 57/96, Umweltbundesamt Berlin, 1-557, 1996.

Wroblewski, F. and La Due, J. S. : Serum glutamic oxaloacetic transaminase activity as an index of liver cell injury. A preliminary report. Ann. Intern. Med., 43 : 345-360, 1955.