J. Fish. Sci. Tech., 7(3), 171-173, 2004

JOURNAL OF
FISHERIES SCIENCE
AND TECHNOLOGY

Notes

Effects of Diethyl Phthalate on Acetylcholinesterase Activity in Olive Flounder (*Paralichthys olivaceus*) Following Short-term Exposure

Jung-Hoon Jee[1], Yoo-Hwa Keum[2] and Ju-Chan Kang[2]*

[1]*Institute of Fisheries Sciences, Pukyong National University, Busan 612-021, Korea*
[2]*Department of Aquatic Life Medicine, Pukyong National University, Busan 608-737, Korea*

Activity of acetylcholinesterase (AChE) is well known as a biomarker of exposure to organophosphate compounds in aquatic organisms. However, the effect of diethyl phthalate (DEP), a widely used plasticizer, on the chance of AChE activity is not yet known. Olive flounder (*Paralichthys olivaceus*) were exposed to DEP 300 and 1,000 mg DEP/kg b.w. through three times of intraperitoneal injection and effects were assessed in AChE activity of brain, muscle, heart and eyes of the exposed fish. AChE activity in various tissues of flounderwas inhibited after exposure to DEP as a concentration-dependent manner, especially in brain, muscle and heart. Among tissues examined, heart is supposed to be a major part of body which is seriously damaged by DEP exposure. It indicates that DEP induces toxic effects in various organs (brain, muscle and heart), and changes of AChE activities. Such changed activities of AChE might be a useful biomarker to assess the impacts induced by phthalate esters including DEP.

Key words: Acetylcholinesterase, Biomarker, *Paralichthys olivaceus*, Diethyl phthalate

Diethyl phthalate (DEP) is a dialkyl ester of phthalic acid. DEP is an important industrial chemical with numerous applications, the largest being as plasticizer for cellulose ester plastic films and molded and extruded articles (U.S. EPA, 1991). Thus, it has been identified in many ecological systems. In a compilation of concentrations (1984-1997) of DEP in North American and western European surface waters (USA, Canada, United Kingdom, Germany, Netherlands, Sweden), geometric mean concentrations ranged from about 0.01 to 0.5 μg/L (Staples et al., 2000). Colon et al. (2000) studied the serum of Puerto Rican girls containing high levels of phthalate esters, which are suspected to be responsible for premature breast development. However, little is known on aquatic toxicity of DEP.

One of the biomarkers most frequently used in fish for the diagnosis of exposure to organophosphate and carbamate compounds is the measurement of the inhibition of enzyme cholinesterase (Sancho et al., 2000). Analysis of acetylcholinesterase (AChE; EC 3.1.1.7) activity, as a biomarker, in different tissues provides a method for diagnosing poisoning and detecting water contamination by anticholinesterase pesticides. Although AChE inhibition has been widely monitored in terrestrial and coastal aquatic environments as a biomarker of aquatic toxicants, including organophosphorus (Holland et al., 1967), the effect of DEP on AChE has not yet been known. In this study, we tried to determine the neurotoxic effects of DEP exposure on AChE activity in brain, muscle, heart and eyes in olive flounder (*Paralichthys olivaceus*).

All chemicals were of analytical grade. Diethyl phthalate (CAS registry number 84-66-2) was purchased from Sigma (St. Louis, MO, USA). Healthy, cultured olive flounder (*P. olivaceus*) were obtained from a local fish farm in Korea, transferred to a tank and kept at 20±1℃. Prior to exposure, fish were held 3 weeks for acclimatization and evaluation of over-all fish health under the laboratory condition in 12:12 h light/dark cycle for further studies. During

*Corresponding author: jckang@pknu.ac.kr

acclimation fish were fed with basic diet twice daily. After acclimatization, fish (mean length, 18.20±0.18 cm, body weight 52.50±2.17 g) were selected for the experiments of examination. The exposure took place in 80 L aquaria containing 7 fish each under flow-through conditions. Each tank received a flow of 9 L/h with continuous aeration. Water characteristics, measured by the method described in APHA (1995), were as follows: pH, 8.0±0.5; temperature, 20±1℃; salinity, 32.1±0.9 psu and dissolved oxygen 7.7-8.0 mg/L. Fish were administered two concentrations of DEP (300, 1000 mg DEP/kg body weight) by three times of intraperitoneal injection during 3 days. A 50-μL volume was injected into the peritoneal cavity of each fish at the ventral surface midline by using a 1-mL tuberculin syringe. To control for possible side effects of the solvent used, a control group of fish was injected with sunflower seed oil (Sigma Chemical, St. Louis, MO) as same volume of treatment group. Twenty-four hours after the last injection of DEP, fish were anesthetized with 3-aminobenzoic acid ethyl ester methanesulfonate (Sigma Chemical, St. Louis, MO, USA). AChE activity was determined in (1:50) brain and heart homogenate in 0.1 M phosphate buffer containing 0.1% Triton X100 (Sigma Chemical, St. Louis, MO, USA), pH 8. Muscle (1:10) and whole eyes homogenates (1:100) were also prepared in the same buffer. The crude homogenates were then decanted into Eppendorf tubes and centrifuged at 12,000 g for 20 min at 4℃. The supernatants were removed and used to test AChE activity, which was measured by an automated adaptation of the Ellman assay (Ellman et al., 1961) using a microplate spectrophotometer (Zenyth 200, Anthos Labtec Instruments GmbH, Austria). AChE activity was normalized to protein content and expressed as nmole min^{-1} mg $protein^{-1}$. Protein concentration was determined using Bradford's method (1976), with a bovine serum albumin (Sigma Chemical, St. Louis, MO, USA) as standard. Statistical analyses were performed using SPSS/PC+ statistical package. Significant differences between groups were determined using one-way ANOVA's and Duncan's test for multiple comparisons (Duncan, 1955). Significance level was established at $P<0.05$.

Effects of DEP on AChE activity in the brain, muscle, heart and eye of olive flounder are given in Fig. 1. AChE activity was inhibited by exposure to DEP in a concentration-dependent manner. DEP produced 29 and 38% decrease in brain AChE ac-

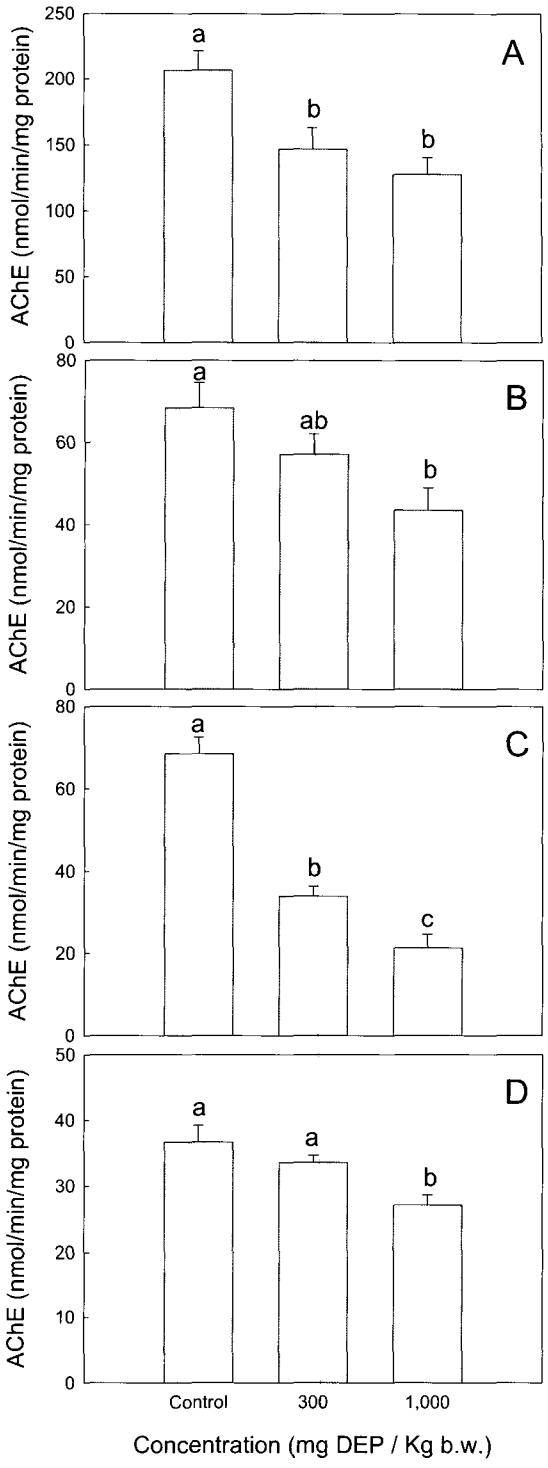

Fig. 1. Acetylcholinesterase activities in brain (A), muscle (B), heart (C) and eyes (D) of olive flounder (*Paralichthys olivaceus*) exposed to diethyl phthalate. Values are mean±SE (n=7). Values with different superscript are significantly different ($P<0.05$) from controls by ANOVA and Duncan's multiple comparison.

tivities of olive flounder when exposed to 300 and 1,000 mg/kg b.w., respectively. Muscle AChE activity in olive flounder were significantly reduced after thrice injection of DEP. 17% and 37% reductions were observed in both DEP 300 and DEP 1,000 group after exposure. Heart is supposed to be a major part of bodywhich is seriously damaged by DEP exposure since the percentage inhibition of AChE was significantly higher than heart and eyes when exposed to 300 mg DEP/Kg b.w. (50%) or 1,000 mg DEP/Kg b.w. (69%) of DEP. However, AChE activity in eye was not declined as much as in brain and muscle.

The high activity of AChE in brain and muscle lead to consider those organs useful for monitoring purpose, as is the case in other species (Bocquené et al., 1990; Jee and Kang, 2003). Commonly, AChE inhibition in either brain or muscle is accepted as an adverse effect because activity in these target tissue is known to participate in neurotransmission (Padilla, 1995). In this study, brain showed the highest AChE activity when compared with the other tissues in nonexposed olive flounder. Indeed, the percentage decrease of AChE activity in brain and muscle was apparent. Recently, Ghorapade et al. (2002) showed significant decrease in brain AChE activity in DEP treated *Cirrhina mrigala* by waterborne exposure. The most interesting finding of this study was the highest AChE activity inhibition of heart tissue. This study confirms that the measurement of AChE of olive flounder is a valuable tool that should be incorporated into a series of biomarkers to maximize the confidence with which ecotoxicologists assess the impacts of DEP pollution in the aquatic environment.

References

APHA. 1995. Standard Methods for the Examination of water and wastewater, 19th Ed. American Public Health Association, Washington, DC.

Bocquené, G., F. Galgani and P. Truquet. 1990. Characterization and assay conditions for use of AChE activity from several marine species in pollution monitoring. Mar. Environ. Res., 30, 75-89.

Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72, 248-254.

Colon, I., D. Caro, C.J. Bourdony and O. Rosario. 2000. Identification of phthalate esters in the serum of young Puerto Rican girls with premature breast development. Environ. Health Perspect., 108, 895-900.

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

Ellman, G.L., K.D. Courtney, V.J. Andreas and R.M. Featherstone. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol., 7, 88-95.

Ghorpade N., V. Mehta, M. Khare, P. Sinkar, S. Krishnan and C.V. Rao. 2002. Toxicity study of diethyl phthalate on freshwater fish *Cirrhina mrigala*. Ecotoxicol. Environ. Saf., 53(2), 255-258.

Holland, H.T., D.L. Coppage and P.A. Butler. 1967. Use of fish brain acetylcholinesterase to monitor pollution by organophosphorus pesticides. Bull. Environ. Contam. Toxicol., 2, 156-162.

Jee, J.H. and J.C. Kang. 2003. Effects of phenanthrene exposure on the acetylcholinesterase activity of olive flounder (*Paralichthys olivaceus*). J. Fish Sci. Technol., 6(4), 225-227.

Padilla, S. 1995. Regulatory and research issues related to cholinesterase inhibition. Toxicology, 102, 215-220.

Sancho, E., J.J. Cern and M.D. Ferrando. 2000. Cholinesterase activity and hematological parameters as biomarkers of sublethal molinate exposure in *Anguilla anguilla*. Ecotoxicol. Environ. Saf., 46, 81-86.

Staples, C.A., T.F. Parkerton and D.R. Peterson. 2000. A risk assessment of selected pthalate esters in North American and Western European surface waters. Chemosphere, 40, 885-891.

U.S. EPA (U.S. Environmental Protection Agency). 1991. Drinking Water Criteria Document for Phthalic Acid Esters (PAEs). Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH, for the Office of Water. ECAOCIN-D009.

(Received July 2004, Accepted September 2004)