Bull. Environ. Contam. Toxicol. (2004) 72:750–755

Bulletin of Environmental Contamination and Toxicology

Effects of Lead as an Environmental Pollutant on EROD Enzyme in *Gammarus pulex (L.) (Crustacea: Amphipoda)*

M. Kutlu, F. Susuz

Anadolu University, Department of Biology, Faculty of Science, Tepebaşı, 26470 Eskişehir, Turkey

Received: 31 August 2002/Accepted: 8 December 2003

There has been a constant dramatic increase in the number of chemicals synthesized and encountered in the environment. While concern has been expressed regarding the potential hazards of synthetic and natural chemicals in the environment, it is not generally realized that some of the best studied and most effective mutagens are synthesized in humans as the result of normal metabolic processes. These include nitrosamines and heavy metal ions (Singer et al; 1994).

It is well accepted that the aquatic environment is becoming threatened by an increasing number of chemicals, as a result of high technological and industrial development. The marine environment today is loaded with about 60.000 different chemicals. Chemical analyses doesn't always reveal the impact of chemical pollution on the aquatic environment (Arınç et al; 1999).

Utilization of biochemical factors to evaluate biological responses to pollutants, especially to carcinogenic compounds, such as PAHs (Polycyclic Aromatic Hydrocarbons) and PCBs (Poly Chlorinated Biphenyls), has increased considerably over the past 15 years.

Induction of cytochrome p4501A and of its monooxygenase activities, namely arylhydrocarbon hydroxylase (AHH) and 7-ethoxyresorufin o-deethylase (EROD) activities, in fish by these chemicals are probably the most widely used biochemical measurements in biomonitoring of environmental contaminants (Arınç et al; 1999).

Interest in aquatic toxicology results not only from the comparative point of view with possible benefits to the fishing profession but also from the human health standpoint, since aquatic organisms may be sensitive first line indicators of environmental contamination which could have important public health significance.

It has been the desire and need to establish baseline information to define the aquatic model systems as completely as possible (Hendricks, 1982). After exposure mammalian tissues or cells in culture to certain chemicals, the enzyme activity of the AHH system increases and different forms of cytochrome-p450 are synthesized. This induction process may provide an important mechanism for detoxification of the chemicals on one hand, or in some one cases it provides a pathway for formation of carcinogenic intermediates (Singer et al; 1994). Some recent studies have employed EROD as a potential biomarker for xenobiotic exposure in fish (Arınç et al; 1994).

Correspondence to: M. Kutlu

This study focuses on the effects of lead acetate on EROD activity and its characteristics in *Gammarus pulex (L.)*.

MATERIALS AND METHODS

G. pulex were collected from the Porsuk River at Eskişehir (Turkey). They were taken to the laboratory and transferred into aquarium. Animals were acclimated to laboratory conditions in a recirculating aquarium containing tap water for at least 7 days prior to use in exposure studies. The temperature was controlled and maintained at 10-12 °C. The water was aerated continuously. At the end of acclimation the animals that appeared healthy were used in the experiments. They were sorted according to their length and only animals with 5-8 mm length were used. Pb solutions were prepared by dissolving lead acetate in distilled water. All experiments were carried out in triplicate.

For enzymatic studies animals were homogenized on ice with a teflon pestle and Potter- Elvehjem glass homogenizer after addition of 0.1 M Tris-HCI buffer pH: 7.8. the homogenate was centrifuged 20,000 rpm for 15 min at 0 °C. The pellet was discarded and the supernatant was used for measuring EROD activity. EROD activity was determined by the spectrofluorometric method of Prough, Burke and Mayer (1976) with some modifications. EROD activity was determined by measuring the rate of formation of product, resorufin per miligram of protein.

The incubation mixture for the ethoxyresorufin dealkylation reaction consists 0.1 ml of microsomal protein (0.1-1.0 mg/ml) and 1.8 ml of 0.1M Tris-Chloride buffer pH 7.8. After adjusting the excitation and emission wavelengths to 530 and 585 nm, respectively, the rate of fluorescence change vs time is recorded prior to adding 10 µl of NADPH. In a final volume of 2.0 ml. The reaction was initiated by the addition of substrate and followed for 2 min in a Shimadzu (RF 5301 PC) spectrophotoflorometer.

The animals were exposed to a single toxicant concentration (EC_{50}) for various time periods (4, 8, 16, 32, 64 and 96 hours) (Kutlu et al; 1998). To assess the relationship between cytosolic EROD activity and the exposure time of Pb at EC_{50}, the activity was determined after 4, 8, 16, 32, 64 and 96 hours of exposure.

Control groups of animals were subjected to the same procedures but exposed only to clean, conditioned tap water. At the end of the exposure times, animals were homogenized as mentioned above. After centrifugation enzyme activity was measured.

The effect of pH on EROD activity was also investigated. For this purpose, 0.1 M Tris-HCl (pH: 3, 4, 5, 6, 7, 7.8, 8) were used as working buffers. Protein concentration was determined by the method of Lowry et al (1951).

RESULTS AND DISCUSSION

7-Ethoxyresorufin o-deethylase (EROD) plays a very important role in catalyzing

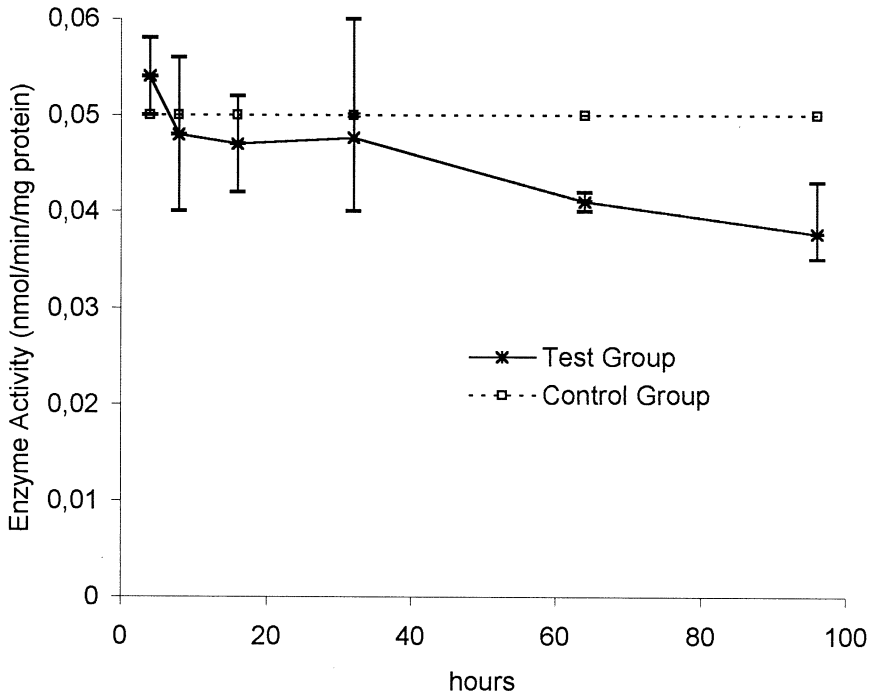

Figure 1. EROD activity for *Gammarus pulex* at different hours (EC_{50} concentrations). Data are the means of at least three seperate determinations.

the conjugation and detoxification of toxic molecules. In this study, we determined the effect of lead acetate on EROD enzyme in *Gammarus pulex*.

Gammarids are common and abundant in many regions and also easily collected and sensitive to a wide variety of chemicals (Arthur; 1980, Kuhn and Streith; 1994). *Gammarus* also one of the most sensitive genus to some heavy metals such as Cd, Cu and Pb according to the U.S EPA Sources (Diamond et al; 1994).

In a series of studies, we first observed that the treatment of lead acetate slightly inhibited the activity of EROD in *G. pulex*. Figure 1 shows the activity of EROD during the 96 hours of exposure at the toxicant concentration of EC_{50}.

As a result, the activity of EROD was inhibited by Pb acetate in *G. pulex*. In a period of 96 hours of exposure, the activity became slightly low. It is also reported that metals are known to inhibit cytochrome p-450 dependent monooxygenase activities (Arınç and İşcan 1983; Förlin et al; 1986).

Data regarding the effect of pH on EROD activity in *G. pulex* indicate that activity is maximal in the pH range 7.0-7.8 decreasing at higher values (Fig.2). Similar to our results, it has reported that the activity of EROD in rat intestine, liver and lung was maximal in the pH range of 7.8 and declined at higher values (Prough et al; 1976 , Burke et al; 1974).

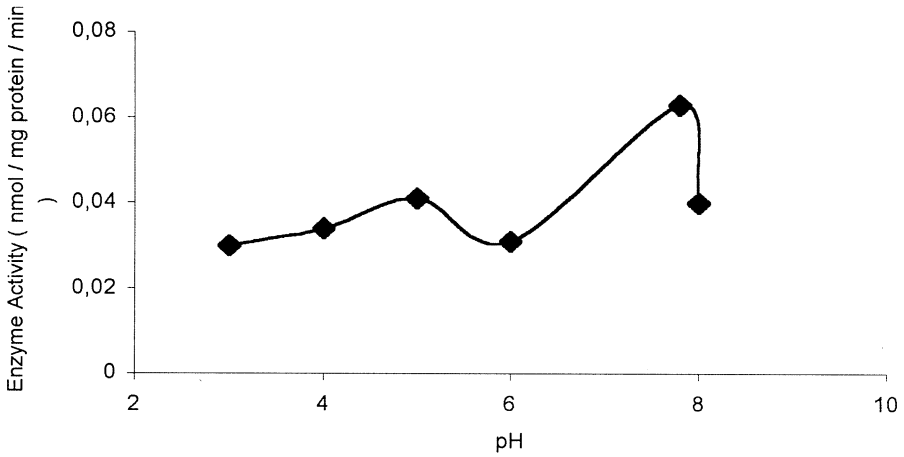

Figure 2. pH-activity of *Gammarus pulex* cytosolic EROD in the presence of different buffers.

In the attempt to define and measure the effects of pollutants on an ecosystem, biomarkers have attracted a great deal of interest. The principle behind the biomarker approach is the analysis of an organism's physiological or biochemical response to pollutant exposure. It is suggested that 7-EROD is a very useful indicator because of its high sensivity, specificity and practicability of the reaction (Burke et al; 1985).

Dicari showed that the induction of EROD in sunfish liver (forex by benzo(a) pyrene) can be inhibited by the simultaneous application of hepatotoxines such as carbon tetrachloride (Dikari et al; 1992). Urbanization industrial activies impact heavily on the water quality. There has been growing concern that inputs of organic contaminants.

In our study we investigated the effects of lead acetate, an important heavy metal pollutant on *G. pulex* under laboratory conditions, several field studies have supported this by establishing correlations between EROD activity and contaminant distribution in fish and their associated sediments (Bequalm newsletter 1999). In contrast to EROD induction detected in many studies (Arınç and Şen; 1999, Stephensen et al; 2000), we observed a slight inhibition of the enzyme. In this case, high consuming will inhibit the action of EROD.Thus, the EROD levels must be measured using another enzyme. In addition to the hepatic detoxification enzymes, EROD has been used as a biomarker for contaminant exposure in mammalian and aquatic invertebrates.

Our data demonstrate that overall toxicity cannot be predicted on the basis of the results because the effects observed can be very different. More detailed studies are needed to explain these phenomena.

Acknowledgments This work was supported by grants from the Research Fund of Anadolu University, Eskişehir,Turkey (Project 011044) and Presented at First International Conference on Pollution Eco-Chemistry and Ecological Processes, August 27-31, 2002, Shenyang, China.

REFERENCES

Arınç E, İşcan MY (1983) Comparative studies of sheep liver and lung microsomal aniline 4-hydroxylase. Comp Biochem Physiol 74 ; 151-158

Arınç E, Şen A (1994) Effects of benzo (a) pyrene treatment on liver microsomal mixed-function oxidase activities of gilthead seabream (Sparus aurata). Comp Biochem Physiol 107 ; 339-404

Arınç E, Şen A (1999) Hepatic cytocrome P4501A and 7-ethoxyresorufin o-deethylase induction in mullet and common sole as an indicator of toxic organic pollution in İzmir Bay, Turkey. Mar Environ Res 48; 147-160

Arthur JW (1980) Rewiew of freshwater bioassay procedures for selected amphipods. ASTM STP American Soc Test Mat 715: 98-100

Bequalm (1999) Hepatic mono-oxygenase (EROD) activity in fish. (Biological effects quality assurance in monitoring programmes) Newsletter Essex, UK

Burke MD, Mayer RT (1974) Ethoxyresorufin: Direct fluorometric assay of a microsomal o-dealkylation which is prefentially induced by 3-methylcholanthrene. Drug Metab Disp 2: 583-588.

Burke MD, Thompson S, Elcombe CR, Halpert J, Haaparanta T, Mayer RT (1985) Ethoxy-pentoxy and benzyloxphenoxazones and homologues: A series of substrates to distinguish between different induced cytochrome p-450. Biochem Pharmacol 34, 3337-3345.

Diamond JW, Hall JC, Pattie DM, Gruber D (1994) Use of an integrated monitoring approach to determine site spesific effluent metal limits. Water Environ Res 66 : 773-743

Dikari A, Jimenez B (1992) Effects of hepatotoxicants on the induction of microsomal monooxygenase activities in sunfish liver by beta naphthoflavone and benzo(a) pyrene. Ecotoxicol Environ Safety 23 : 89-102.

Förlin L, Haux C, Karlson-Norrgen L, Runn P, Larson A (1986) Biotransformation enzyme activities and histopathology in rainbow trout salma gairdneri, treated with cadmium. Aquat Toxicol 8: 51-64

Hendricks JD (1982) Aquatic Toxicology. Raven Press: New York, 149p

Kuhn K, Streit B (1994) Detecting sublethal effects of organophosphates by measuring acethylcholineesterase activity in *Gammarus*. Bull Environ Contam Toxicol 53: 398-404

Kutlu M, Sumer S (1998) Effects of Lead on the activity of δ-aminolevulinic acid dehydratase in *Gammarus pulex*. Bull Environ Contam Toxicol 60: 816-821

Lowry OH, Roenbrough NJ, Farr AL, Randal RJ (1951) Protein measurement with Folin phenol reagent. J Biol Chem 193: 265-275

Prough RA, Burke MD, Mayer RT (1976) Direct fluorometric methods for measuring mixed-function oxidase activity. Meth Enzymol 39: 372-377

Singer B, Gerunberger D (1983) Molecular biology of mutagens and carcinogenes. New York, Plenum pp 1-20

Stephensen E, Svavarsson J, Sturve J, Ericson G, Adolfsson-Erici M, Förlin L (2000) Biochemical indicators of pollution exposure in shorthorn sculpin

(*Myxocephalus scorpius*), caught in four harbours on the southwest coast of iceland. Aquat Toxicol 48 : 431-442