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Effects of Lead on the Activity of δ-Aminolevulinic Acid Dehydratase in *Gammarus pulex*

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Lead is one of the most important heavy metal pollutants in aquatic environments. Transport, industrial and domestic waste products are the main sources of this pollutant. It causes not only water pollution, but also air pollution due to the directly releasing into the atmosphere.

Selective inhibition of fish δ-amino levulinic acid dehydratase (ALAD; EC 4. 2. 1. 24) was introduced to characterize this enzyme as an important biomarker for determination of environmental lead pollution (Conner and Fowler 1994). ALAD catalyzes the formation of one molecule of porphobilinogen from two molecules of amino levulinic acid. This reaction involves an aldol condensation between two o-ALA molecules, formation of a C-N bond, and elimination of water molecules to produce a pyrrole, porphobilinogen which is the aromatic precursor for porphyrins and other tetrapyrrole-like compounds (Gurba et al. 1972).

In the present study, we chose the freshwater amphipod *Gammarus pulex* as a sensitive indicator organism for environmental pollution. The freshwater invertebrate has been used as test organism to aquatic toxicology for many years (Macek et al. 1976). The genus *Gammarus* has being particularly popular (Arthur 1980).

MATERIALS AND METHODS

Gammarus pulex were collected from the Porsuk River at Eskisehir (Turkey). They were taken to the laboratory and transferred into aquaria. 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 healty were used in the experiments. They were sorted according to their length, and only animals that were 5-8 mm were used.

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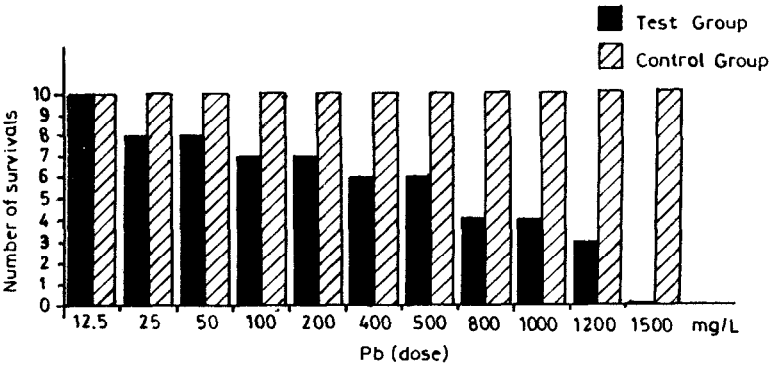

Figure 1. Survival data for *Gammarus pulex* exposed to several Pb concentrations for 96 h.

Pb solutions were prepared by dissolving lead acetate in distilled water. For the determination of LC_{50}, groups of 10 animals were exposed to eleven different lead acetate concentrations (12.5-1500 µg/L) for 96 hours. Control groups were maintained in conditioned tap water alone. Mortalities on each concentration were recorded. All experiments were carried out in triplicate.

The LC_{50} (96 hr) value and its confidence limits were calculated by EPA Probit Analysis Program (Horning and Weber, 1985). This value is based on mortalities after a 96 hr exposure to the toxicant and a further 24 hr in toxicant free water.

For enzymatic studies animals were homogenized on ice with a teflon pestle and Potter-Elvehjem glass homogenizer after addition of 4 volumes of KCI containing 0.1 M phosphate buffer (pH: 6.8). The homogenate was centrifuged at 8000xg for 10 minutes at 4°C. The supernatant was used for measuring ALAD activity. ALAD activity was determined by measuring the rate of formation of product, porphobilinogen, using the method of Coleman (1970). Protein concentrations were determined using the method described by Lowry et al. (1951). One unit of enzyme activity was defined as the amount of enzyme that produced 1.0 nanomole of porphobilinogen in 1 hr at 37°C. Specific activity was defined in terms of units per mg of protein.

The animals were exposed for various time periods (4, 8, 16, 32 and 64 hr) in a single toxicant concentration. 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, ALAD activity was measured to quantify enzyme inhibition.

Atomic-absorption studies were performed on the animals exposed to lead at LC_{50} for 4, 8, 16, 32 and 64 hr time periods and on the unexposed control. The lead analysis was carried out by graphite furnace atomic absorption spectrophotometry (Hitachi spectrophotometer, model 180-70) using the method of Kikbright (1980).

All results are expressed as mean ± S.D. Statistical variations among the experiments were evaluated by Student's t-test for two-sample comparisons. In each case, P values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

It is very important to get some information on the acute toxicity of substances to aquatic invertebrates in the assessment of their hazard in aquatic environments. Amphipods are one of the most sensitive groups of organisms to toxic substances in acute tests. Gammarids are common and abundant in many regions and also easily collected and maintained. Previous studies showed that freshwater gammarids are sensitive to a wide variety of chemicals (Arthur 1980, Kuhn and Streit 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 Figure 1, toxicity of lead as inorganic salt to *Gammarus pulex* in 96 hr is presented. The LC_{50} (96 hr) value for lead in *Gammarus pulex* was found to be 0.394 mg/L (0.226-0.746 mg/L with the 95% confidence limits.

In a previous study, 96 hr LC_{50} value was found to be 0.175 mg/L for lead in *Gammarus pulex* at pH: 7.2-7.6 (Bascombe et al. 1990). The variability in value may be explained by different water quality parameters such as pH. In our study, toxicity tests were conducted at pH: 7.8-8.0. Many studies confirm that lead is more toxic at lowered pH (Mackie 1989, Schubauer-Berigan et al. 1993).

We have plotted ALAD activity versus time for lead exposed and control groups (Fig. 2). The lead exposed group at LC_{50} concentration showed a significant ALAD inhibition, the degree depending on the exposure time. Activity was gradually decreased during the time period and showed the lowest activity at 64 hr of exposure. Lead content of *Gammarus pulex* was determined by atomic absorption spectrophotometry as mentioned above.

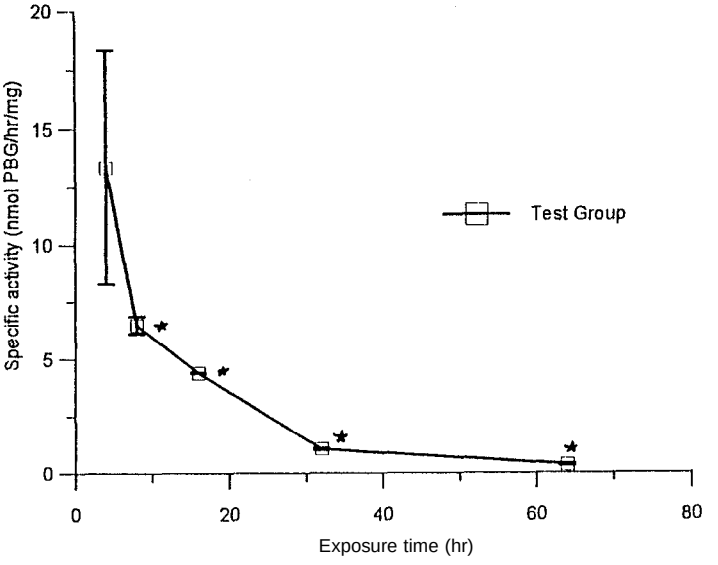

Figure 2. Relationship between the reduction of ALAD activity and exposure time at LC_{50} dose level for *Gammarus pulex.* All results are means ±SD and n=6. Significantly different from corresponding mean of control, *P<0.05. Control value: 18.64±0.04 nmol PBG/hr/mg.

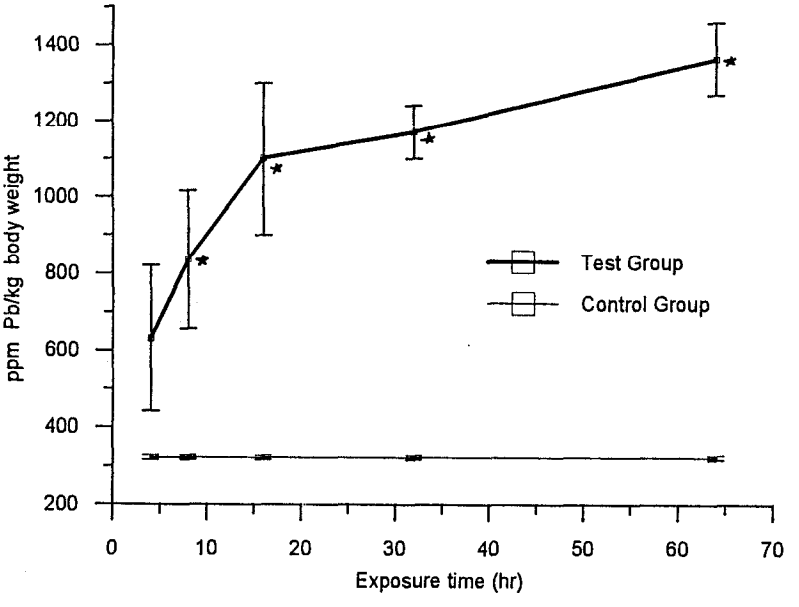

Figure 3. Pb contents of *Gammarus pulex* exposed to Pb at L C_{50} concentration for 64 hr. All results are means ±SD and n=6. Significantly different from corresponding mean of control, *P<0.05.

As shown in Figure 3, lead contents of *Gammarus pulex* exposed to LC_{50} concentration were increased by the time of exposure. After a 64 hr exposure period to LC_{50} concentration, the lead content was approximately 1400 ppm Pb per body weight.

There are few studies which have shown inhibition of ALAD by lead in different groups of organisms (Rodrigues et al. 1989, Conner and Fowler 1994).The present study was undertaken to evaluate the susceptibility of *Gammarus* ALAD to Pb inhibition and also to assess potential environmental toxicity to water ecosystem and to be able to extrapolate from these data to the human population at possible risk from lead pollution. In future studies, the biochemical characterization of amphipod ALAD will be investigated.

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