Evaluation of Chronic Toxicity of Water Lead for Carp Cyprinus carpio Using Its Blood 5-Aminolevulinic Acid Dehydratase

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Carp Cyprinus carpio were exposed for 20 days to test waters consisting of combinations of four lead concentrations (0, 10, 100, and 1,000 ppb) and four levels of water hardness (50, 150, 500, and 1000 ppm as CaCO₃), and 5-aminolevulinic acid dehydratase (ALA-D) activities and lead concentrations in the fish blood were measured. ALA-D activities decreased with increasing blood lead concentrations, and the activities were negatively correlated to the log of the blood lead concentrations ($r = -0.93$). Blood lead concentrations increased with increasing water lead concentrations. However, lead accumulation in the blood lowered with increasing water hardness. ALA-D activities in the lead-contaminated fish blood decreased to about 40% of that of the control fish when carp were exposed for 20 days to test water with a water hardness of 50 ppm CaCO₃ and a water lead concentration of 10 ppb (Environmental Water Quality Standards relating to Human Health in Japan). Judging from the inhibitory degree of carp blood ALA-D, the standard value is the concentration that may cause chronic toxicity to carp.

Key words: 5-aminolevulinic acid dehydratase, water lead, chronic toxicity, water hardness, Cyprinus carpio

The value of the environmental water quality standard for water lead in Japan was revised in March, 1993 and was strengthened from 100 to 10 ppb. However, it is not apparent whether this value is the maximum concentration possible without destroying the desirable living conditions necessary for fish. The concentration which permits a successful completion of the life cycle of fish, that is, the maximum acceptable toxicant concentration, has been determined on the basis of information obtained from chronic toxicity tests. In the past, chronic toxicity tests on the toxicity of water lead to fish were performed using brook trout Salvelinus fontinalis¹ and rainbow trout Oncorhynchus mykiss,² and these tests took years to complete. In addition, the toxicity of water lead to fish has changed along with changes in the chemical characteristics of water, in particular water hardness.³⁻⁴⁻¹⁻⁷⁻⁸

The enzyme 5-aminolevulinic acid dehydratase (ALA-D, EC 4.2.1.24) in fish blood is useful as an indicator of water lead pollution because its activity is inhibited by lead pollution.⁵⁻⁷⁻¹⁻⁸⁻¹⁻⁹⁻¹⁻⁰⁻¹¹⁻¹²⁻¹³⁻¹⁴⁻¹⁵⁻¹⁶⁻¹⁷⁻¹⁸⁻¹⁹⁻²⁰⁻²¹⁻²²⁻²³⁻²⁴⁻²⁵⁻²⁶⁻²⁷⁻²⁸⁻²⁹⁻³⁰⁻³¹⁻³²⁻³³⁻³⁴⁻³⁵⁻³⁶⁻³⁷⁻³⁸⁻³⁹⁻⁴⁰⁻⁴¹⁻⁴²⁻⁴³⁻⁴⁴⁻⁴⁵⁻⁴⁶⁻⁴⁷⁻⁴⁸⁻⁴⁹⁻⁵⁰⁻⁵¹⁻⁵²⁻⁵³⁻⁵⁴⁻⁵⁵⁻⁵⁶⁻⁵⁷⁻⁵⁸⁻⁵⁹⁻⁶⁰⁻⁶¹⁻⁶²⁻⁶³⁻⁶⁴⁻⁶⁵⁻⁶⁶⁻⁶⁷⁻⁶⁸⁻⁶⁹⁻⁷⁰⁻⁷¹⁻⁷²⁻⁷³⁻⁷⁴⁻⁷⁵⁻⁷⁶⁻⁷⁷⁻⁷⁸⁻⁷⁹⁻⁸⁰⁻⁸¹⁻⁸²⁻⁸³⁻⁸⁴⁻⁸⁵⁻⁸⁶⁻⁸⁷⁻⁸⁸⁻⁸⁹⁻⁹⁰⁻⁹¹⁻⁹²⁻⁹³⁻⁹⁴⁻⁹⁵⁻⁹⁶⁻⁹⁷⁻⁹⁸⁻⁹⁹⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻⁺⁻/+
Evaluation of Pb Toxicity by Carp Blood ALA-D

Exposure Tests

The exposure tests and acclimation of carp used in our experiments were performed according to the methods described in our previous paper. After an exposure period of 20 days, ALA-D activities and lead concentrations in the fish blood were measured. Ten fish per test tank were used for the exposure tests. The pH of the test waters was within the range of 7.1–8.1 throughout the exposure tests. The exposure tests were conducted at a water temperature of 16–18°C. The fish used were of 9–11 cm in body length and 20–28 g in weight. The test tanks used were the 60-l box-type tank (600 × 295 × 360 mm, made of glass), and the 90-l circular tank (made of polyvinyl chloride) used in earlier exposure tests as previously reported. In order to determine the effect by the type of test tank on the inhibition by water lead of ALA-D activities in fish blood, exposure tests were performed according to the exposure test described above. Two tanks for each type of test tank were used for the exposure test, and 50 l of test water containing a water lead concentration of 200 ppb were placed in each of the test tanks. The water hardness of the test water was 120 ppm CaCO₃. After an exposure period of one week, the ALA-D activities in the fish blood were measured. As indicated in Table 1, there was no difference in the effect of water lead on the ALA-D activities in the fish blood regardless of the type of tank used in these exposure tests.

Measurement of Blood ALA-D Activity

ALA-D activity was measured by a modified method which does not use HgCl₂ as described in our previous report. In addition, blood collection from fish and measurement of hematocrit also followed methods previously reported. ALA-D activities for five fish for each of the test waters were measured. Relative ALA-D activity was expressed as a ratio of the mean value of lead-contaminated fish to that of the control fish. Or else, the activity was expressed as nmol of porphobilinogen (PBG) which is formed from aminolevulinic acid by 1 ml of erythrocyte (RBC) for 1 h (nmol PBG/mRBC/h), according to the formula of Hodson et al. 

Analysis of Lead in Blood and Water

Lead analysis of fish blood and test waters was conducted as described in our previous report. Blood lead levels for five fish per test water were measured. We filtrated test waters through a 0.45-μm millipore filter before beginning the exposure tests and again after an exposure period of 48 h, and defined water lead of the filtrate as the dissolved lead fraction. Nitric acid was added to the filtrates, and the filtrates were stored at room temperature until analysis.

Statistical Analysis

Data were analyzed for statistical significance by the Student’s t-test. Significant differences were established at 5% level.

Results and Discussion

Dissolved Lead Concentrations of Test Waters

Table 2 indicates the mean concentrations of the dissolved fraction of water lead before the beginning of the exposure tests and again after an exposure period of 48 h. Before the beginning of the exposure tests, the dissolved lead concentrations of test waters with varying levels of water hardness were within the range of 5.1–5.4, 50.2–57.3, and 701.4–738.4 ppb at the nominal lead concentrations of 10, 100, and 1,000 ppb, respectively, with the dissolved lead concentrations being lower than the nominal concentrations. After an exposure period of 48 h, the dissolved lead concentrations were within the range of 1.1–1.7, 17.7–26.3, and 177.4–256.7 ppb, respectively, the dissolved lead concentrations having become remarkably lower. On the other hand, the dissolved lead concentrations of the control waters were within the range of 1.0–1.4 ppb before the beginning of the exposure test and were within the range of 0.7–1.4 ppb after an exposure period of 48 h, the dissolved lead concentrations having barely changed during the exposure test. No relationship between changes in the dissolved lead concentrations and the levels of water hardness of the test waters was observed. Davies et al. stated that the dissolved lead concentrations in waters with high alkalinity which possess high concentrations of HCO₃⁻ and CO₃²⁻, were lower because of the precipitation of lead carbonate. In the present study, it might be possible

Table 1. Effect of each type of test tank on blood ALA-D activity in lead-contaminated carp

<table>
<thead>
<tr>
<th>Test tank</th>
<th>ALA-D activity (nmol PBG/m RBC/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circular tank</td>
<td>865.5 ±224.9⁺(14)⁺,³</td>
</tr>
<tr>
<td>Box-type tank</td>
<td>875.8 ±143.0(14)⁺</td>
</tr>
</tbody>
</table>

The fish were exposed for one week to a water lead concentration of 200 ppb in both a circular tank and a box-type tank.

* The numbers of fish used are shown in parentheses.
* Mean values are not significantly different at 5% level.

Table 2. Levels of the dissolved fraction of water lead recorded in test waters during the exposure tests

<table>
<thead>
<tr>
<th>Nominal Pb level (ppb)</th>
<th>Exposure period (h)</th>
<th>Water hardness (ppm as CaCO₃)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50</td>
<td>150</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>1.3 ± 0.5(6)⁺</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0.8 ± 0.3(5)⁺</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>5.1 ± 1.3(5)⁺</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>1.1 ± 0.4(6)⁺</td>
</tr>
<tr>
<td>1000</td>
<td>0</td>
<td>50.2 ± 8.7(6)⁺</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>17.7 ± 5.4(5)⁺</td>
</tr>
</tbody>
</table>

The numbers of water samples are shown in parentheses.
that the dissolved lead concentrations of test waters decreased because of the precipitation of lead carbonate caused by the reaction of water lead and CO$_3^{2-}$. However, the dissolved lead concentrations did not decrease with increasing levels of water hardness. The reason for this might be thought to be as follows. Since the various levels of water hardness used in the dilution waters were prepared by adding chlorides of calcium and magnesium, the high levels of hardness consisted not of carbonate hardness but of noncarbonate hardness. Water lead concentrations are expressed as nominal concentrations in this paper.

Relation between Water Lead Concentrations and Blood Lead Concentrations

The relation of blood lead concentrations to water lead concentrations after an exposure period of 20 days is shown in Fig. 1. Blood lead concentrations were the mean values for every level of water hardness and for every concentration of water lead. Blood lead concentrations increased with increasing water lead concentrations, but they decreased with increasing levels of water hardness. Fish absorb water lead through their gills, and the absorbed lead accumulates in the blood. The epithelia of fish gills possess negative charges at neutral pH areas. It is assumed that Ca and Mg ions together with Pb ion bind to the gill epithelia. Therefore, blood lead concentrations seem to decrease since the ratios of Pb ion binding to the gill epithelia become lower with increasing levels of water hardness. When carp were exposed for 8 days to test water consisting of a combination of 50 ppm CaCO$_3$ of water hardness together with a water lead of 1,000 ppb, all the fish displayed the symptoms of erratic swimming and loss of equilibrium, with some of the seriously affected fish subsequently dying. No abnormalities were observed in any of the fish belonging to the other test sections. The reason why the toxicity of lead to fish was observed in the test water with a low level of water hardness can be explained by the following, from a mechanism of the effects of water hardness on the lethality of heavy metals to fish reported by Zitko and Carson. Generally, the toxicity of heavy metals is dependent upon the amount of heavy metal binding to the active sites in fish tissues. The binding amounts of heavy metal ions to the active sites increase with increasing blood heavy metal. The blood heavy metal concentrations are affected by levels of water hardness because heavy metal ions compete with Ca and Mg ions for binding to the gill epithelia.

Relation between Blood ALA-D Activities and Water Lead Concentrations

The relation of blood ALA-D activities to water lead concentrations after an exposure period of 20 days is shown in Fig. 2. ALA-D activities are shown as relative values (%) to those of the control fish for every level of water hardness. Although ALA-D activities decreased with increasing water lead concentrations, the depression of the activities was ameliorated with increasing levels of water hardness. ALA-D activity in the lead-contaminated fish decreased to about 40% of that of the control fish when the carp were exposed for 20 days to a water lead concentration of 10 ppb together with a water hardness of 50 ppm CaCO$_3$. At present, the environmental water quality standard of water lead relating to human health of Japan is 10 ppb. Since it is commonly stated that the average level of water hardness of river waters in Japan is 30 ppm CaCO$_3$, blood ALA-D activities in carp, which live in waters where the environmental water quality standard of water lead is 10 ppb, may be remarkably inhibited. If fish blood ALA-D could be used as an effective short-term indicator to estimate the long-term effects of water lead to fish, the water lead concentration of 10 ppb may cause chronically sublethal effects to carp.

Relation between ALA-D Activities and Blood Lead Concentrations

The relation of blood ALA-D activities to blood lead concentrations...
concentrations after an exposure period of 20 days is shown in Fig. 3. ALA-D activities are shown as relative values (%) to those of the control fish for every level of water hardness. On the other hand, blood lead concentrations were the mean values for every level of water hardness and for every concentration of water lead. The relation of blood ALA-D activities to blood lead concentrations became confused when two variables were plotted for every level of water hardness. However, it is apparent that ALA-D activities decreased with increasing blood lead concentrations. As shown in Fig. 4, the ALA-D activities were negatively correlated to the log of blood lead concentrations \( r = -0.93 \) when the relation between these variables was re-plotted on the basis of data for each of the individual fish that were measured.

From the results described above, the effects of water hardness on the inhibition of blood ALA-D activities by water lead are summarized as follows. The inhibitory degree of ALA-D activities decreases with increasing blood lead levels (Figs. 3 and 4). Blood lead levels depend upon the amounts of Pb ion binding to the gill epithelia, namely water lead concentrations (Fig. 1). Since water hardness affects the ratios of Pb ion binding to the gill epithelia, blood lead decreases with increasing levels of water hardness. In addition, judging from the inhibitory degree of carp blood ALA-D, the present water lead standard value of 10 ppb is the concentration that may cause chronic toxicity to carp. Certainly, the toxicity depends upon the chemical characteristics of water. This problem should be further ascertained on the basis of information obtained from chronic toxicity test.

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