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A study on Cu-biotic ligand model for Japanese medaka,Oryzias latipes

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論文題目　：Title: A study on Cu-biotic ligand model for Japanese medaka,*Oryzias latipes*

（メダカ *Oryzias latipes* における銅 biotic ligand model に関する研究）

区　　分　：　甲

論　文　内　容　の　要　旨

Copper (Cu) was an important material ancient activity of humans and is still widely used in industrial activities and in domestic consumption. Cu is primarily discharged into the environment via wastewater from activities such as mining, which can negatively impact aquatic ecosystems. Thus, risk analysis and regulation of Cu is required. It is well known that toxicity of Cu is affected by water chemistry such as hardness, pH and dissolved organic carbon (DOC). Since these conditions differ among watersheds, Cu toxicity might affect. Accordingly, prediction of Cu toxicity in specific watersheds is needed to enable accurate risk assessments. Especially low hardness, ranging from 18.1 to 22.4 mg/L $CaCO_3$, is typical of Japanese surface water. Critical toxicity prediction of Cu in Japan is required.

In a recent study, the biotic ligand model (BLM) for prediction of heavy metal toxicity considering hardness (mainly calcium [Ca] and magnesium [Mg] concentration) was established. Since the model was developed in USA and EU countries, prediction of the toxicities are limited for the biological species living in the countries. The model has not well developed for watershed and species living in Japan. The present study was conducted to elucidate the relationship between Cu toxicity and Ca and Mg concentrations to fish and establishment a BLM model. We used Japanese medaka (*Oryzias latipes*, 1.7 ± 0.05 cm lengths and 0.1 ± 0.025 g in weight) for the test fish species which is most frequently used in chemical risk assessment frameworks in Japan.

First, we performed a series of tests to elucidate the effect of Ca in water on Cu toxicity to medaka. The fish were exposed to Cu at concentrations of 0, 0.025, 0.05, 0.075, 0.1 and 0.2 mg/L for 96 hours in conjunction with various Ca concentrations and the 50 percent lethal concentration (LC50) for 96 hours were calculated. As results, the LC50 values are 0.054, 0.061, 0.081, 0.175 and 0.215 mg/L (at Ca concentrations of 0, 15, 22.5, 30 and 60 mg/L, respectively). These results demonstrated that increasing Ca concentration significantly reduce Cu toxicity.

We also performed a series of tests to elucidate the effect of Mg to toxicity of Cu. At Mg concentrations 0, 10, 20, 40 and 60 mg/L, LC50 values of Cu are 0.054, 0.070, 0.074, 0.054

and 0.080 mg/L, respectively. These results could be interpreted that Mg has weaker effect on the toxicity of Cu compared to that of Ca.

Using obtained results, BLM parameters (ligand affinity constant of Ca and Mg) have been estimated to be 7924 and 1543 (mol^{-1}) respectively. Furthermore, LCx values such as LC10, LC50, and LC90 can be predicted by this BLM. The comparison of LCx by observation and LCx predicted by BLM show that the differences between these two are within a factor of 2 in most cases, demonstrating the predicted values by Cu-BLM on medaka might be acceptable.

In addition, validation tests have conducted with mixture of Ca and Mg with 4 combination concentrations. Observed LC50 values are 0.095, 0.139, 38.2 mg/L and no effect (at Ca and Mg concentrations, 15+10, 30+20, 60+40 and 90+60 in mg/L, respectively). LC50 values predicted by BLM are 0.097, 0.166, 0.182, and 0.258 mg/L. At low hardness mixture (15+10, 30+20 mg/L of Ca and Mg), LC50 values by prediction (0.097, 0.166) are close to those in observed values (0.095, 0.139).

The validation tests using natural waters collected from Kure city in Hiroshima (Ca,5.4-5.6; Mg, 1.7-1.8 mg/L), Nagai city in Yamagata (Ca, 4.3-4.7; Mg,1.3 mg/L), Kurume city (Ca,11.7-12; Mg, 3.6-3.7 mg/L), and Ogi city in Saga (Ca,5.7-6.0; Mg, 1.5-1.6 mg/L). Observed LC50s for Hiroshima, Yamagata, Kurume, and Ogi are 0.120, 0.061, 0.086, and 0.073 and predicted LC50s are 0.221, 0.234, 0.481, and 0.254 respectively. Graphic comparison LC50s of prediction to observation in Hiroshima water the value shows within factor of 2. However, in Yamagata, Kurume, and Ogi water BLM values do not fit well. This might be caused by difference in DOC or other water quality parameters.

In summary, BLM can predict LC50 of Cu on medaka in laboratory water which consists of Ca, Mg, or lower concentration mixture of Ca and Mg. However, at two higher concentrations of mixture Ca and Mg, BLM prediction is unacceptable. For the natural waters BLM prediction is unacceptable except for Hiroshima water. In the Cu-BLM model for Japanese medaka, not only hardness (Ca and Mg) but also pH and DOC should be considered.