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学 位 論 文 要 旨
SUMMARY OF DOCTORAL THESIS

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題目 Title: **Side Effects of Rice Herbicides on Aquatic Plant *Lemna* sp.**
（水生植物 *Lemna* sp. に及ぼす水田除草剤の影響）

Sulfonylurea (SU) are become very popular and widely used to protect agricultural crops. Their increased usage has elicited extensive research into their side effects on nontarget aquatic organisms. For this purpose, we collected fronds of *Lemna* sp. from the pond in Japan, and used in this study.

Toxicity of eight SU was evaluated on *Lemna* sp. and the relative sensitivity was compared with *Pseudokirchenetilla subcapitata*. The most toxic and least toxic herbicide were cyclosulfamuron and nicosulfuron, respectively, for *Lemna* sp. Bensulfonyl-methyl, imazosulfuron, ethoxysulfuron, thifensulfuron and nicosulfuron was more toxic to *Lemna* sp. than *P. subcapitata,* on the basis of EC50. In contrast, *P. subcapitata* was more sensitive to pyrazosulfonyl-ethyl than *Lemna* sp. Difference in sensitivity to cyclosulfamuron and flazasulfuron was small within the species. Difference in sensitivity may be due to different metabolic capability of the two species tested. EC50 of five SU (bensulfonyl-methyl, ethoxysulfuron, thifensulfuron, cyclosulfamuron, and flazasulfuron) are correlated ($\log y = 3.54 \log x - 0.41$, $R^2 = 0.976$, where x and y are EC50 for *Lemna* sp. and *P. subcapitata*, respectively) between the two species tested. A highly significant correlation was observed between toxicity and Log Kow for *P. subcapitata* ($\log y = -2.08 x + 2.48$, $R^2 = 0.987$, where x and y are Log Kow and EC50, respectively). Less water soluble SU have stronger toxicity. The same tendency was observed for *Lemna* sp. when pyrazosulfuron-ethyl and imazosulfuron were removed due to poor correlation of toxicity between the two species used in this study. The equation was: $\log y = -0.367 x + 0.606$, $R^2 = 0.907$, where x and y are Log Kow and EC50 of the six SU, respectively. This could be attributed to the lipophilic nature of the plasma membrane, which makes chemicals with lower Kow easier to diffuse across the membrane into the cytoplasm. Stronger toxicity of SU with lower Kow seems to be explained by the hydrophobic property. Water solubility of SU is pH dependent, in which they are less soluble in lower pH. Therefore, actual environmental

acidity influences the toxicity of SU, and should be considered in risk assessment. Findings from the present study, it is needed to investigate the mechanisms of metabolic and chemical uptake process of the mentioned aquatic species in this study.

Recovery after exposure to chemicals is another important factor to be considered. Relative growth rate (RGR) in exposure decreased in the order of EC50 values at 10 ppb for seven days exposure: $y = 32.3 \log x - 5.04$, $R^2 = 0.901$, where x and y are EC50 and RGR in exposure at 10 ppb, respectively, and frond reproduction was completely stopped at higher concentrations (100 and 1000 ppb). Capability of new fronds production was observed in the recovery test for all the eight SU tested. RGR of six SU were more than 50% at 100 ppb, and four SU at 1000 ppb. There was no correlation between RGR in the exposure and recovery, but when ethoxysulfuron and nicosulfuron are removed, the correlation is stronger ($y = 23.0 \log x - 9.77$, $R^2 = 0.793$). The differences between RGR in the exposure and recovery may be due to different metabolic activity to different chemicals of *Lemna* sp.

Growth was completely inhibited for 7 days but no lethal effect was observed. Effects of exposure period (1, 2, 3, and 4 weeks) and concentration (1, 10, 50, and 100 ppb) on the growth inhibition and recovery, using the most toxic herbicide cyclosulfamuron, showed that RGR was constant within the exposure period of four weeks. In the recovery period, RGR decreased with the longer exposure period. Reproduction was observed within two weeks of exposure at 100 ppb, but no recovery occurred after the exposure for three weeks at more than 10 ppb. *Lemna* sp. can reproduce again in the same rate as that before the exposure if SU is removed within two weeks, even after complete inhibition at 100 ppb and longer exposure beyond three weeks caused no recovery at 10 ppb, and retarded growth at 1 ppb.

In risk assessment, the expected environmental concentrations of SU were reported as 3 – 20 ppb, which are grater than EC50 of *Lemna* sp. for some SU, but recovery of growth is possible when the chemical are dissipated by degradation in the aquatic environment. As SU are mainly used only in the beginning of rice cultivation and rapidly degraded in paddy soils, duration of SU exposure to aquatic environment is supposed to be limited. Therefore, the recovery potential after exposure should be examined in addition to determining EC50, and expected concentration and period of exposure in the environment should be evaluated for the risk assessment. Considering our results and the expected environmental concentrations, it is concluded that cyclosulfamuron does not pose a significant risk to *Lemna* sp. up to two weeks exposure within the expected environmental concentrations.