

## 論文の内容の要旨

論文題目      Comparison of toxicological importance of exposure routes of hydrophobic organic chemicals to a benthic ostracod by semi-static test  
(半止水式試験による底生カイミジンコへの疎水性有機物質の曝露経路の毒性学的重要性の比較)

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Pollutants are present both in dissolved and adsorbed form in the water environment and its ecological impact may not be independent from the exposure pathway. Organic pollutants with high equilibrium partitioning coefficient tend to participate into the solid particles and easy to be ingested by the benthic organisms, causing dietborne toxicity. Previous bioaccumulation studies indicated that the ingestion route was more important to benthic organisms than the aqueous route for hydrophobic organic chemicals (HOCs). It is questionable that if the dietborne toxicity of such chemicals could be dominant in sediment toxicity. However, studies on the toxicity evaluation in different exposure pathways are still limited.

This study aims to determine the relative importance of dietary and aqueous exposure routes in respect of sediment toxicity, which helps decision making in chemical management for HOCs dominated by dietborne toxicity. To achieve this objective, firstly a semi-static toxicity test method with controlled route exposure was proposed based on a standard whole sediment toxicity test ISO 14371 using *Heterocypris incongruens* with three big modifications. Then the dominant exposure route was identified by confirming the consistency of dietary or aqueous dose-response relationships in different exposures. Using this developed method, the toxicity and dominant exposure route of five HOCs was investigated. Finally the effect of partition coefficient on the dominance of dietborne toxicity and mobility was discussed for better chemical management.

The first modification is changing the reference sediment, which is a natural clean river or marine sand with unknown composition, to newly proposed artificial sediment. Because the artificial sediment with clear formula, matching habitat of test organism is generally recommended for standard prospective sediment toxicity testing under laboratory condition (Diepens et al., 2014). Pure quartz sand is not suitable as artificial sediment for *Heterocypris incongruens*. Strong impact of freshwater calcite saturation state on the growth of *H. incongruens* was observed. The lack of carbonate/bicarbonate minerals in samples may lead to growth inhibition up to 20%, but no effect on mortality. A formula of artificial sediment

was proposed for the toxicity test of *H. incongruens*, which consists of 10% (w/w) calcite and 90% (w/w) quartz sand.

The second modification is using contaminated algae instead of spiked sediment to control the exposure route. In the conventional protocol, the target compound is spiked to the sediment, which is difficult to control the exposure condition. In the modified protocol, the test organisms are exposed the contaminated algae through dietary exposure route directly. The exposure condition is easy to control, and the exposure route is clear.

The third big modification is changing the static toxicity test to a semi-static toxicity test with frequent food and water renewal to reduce the variability of the exposure conditions. During the method developing steps, *Desmodesmus subspicatus* was selected from four green algae species as the food algae species for its potential of separating the exposure routes. The soaking time to prepare the contaminated food was developed based on the HOCs adsorption dynamics on suspended *D. subspicatus*. Generally 12 hours is enough to achieve equilibrium for 4tBP, 4tOP and BHT, while 24 hours for 4NP and TEB. The renewal interval of food and water was determined as every 12 hours for 4tBP, BHT and TEB, 24 hours for 4tOP and 4NP based on the HOCs adsorption-desorption dynamics on filtered algae in test condition. The exposure concentration variation during the test was also investigated. During the renewal interval, there is 20% of 4tBP, 14% of 4tOP, 46% of BHT, 3% of 4NP and 15% of TEB releasing from the food to water. The dietborne toxicity test is feasible whereas the waterborne toxicity test is impossible according to the results of adsorption and desorption dynamics.

In this study, two different exposures were set, namely dietary exposure (contaminated food + clean water) and combined exposure (contaminated food + contaminated water). Mortality and growth inhibition of five HOCs under two exposures were calculated as the endpoints. Dose-response curves between endpoint and HOCs in media, which is food or water, were plotted. The dominant exposure route was confirmed by the consistency of the dietary and aqueous dose-response curves in two exposures.

By using this proposed method, the dominant exposure route of five HOCs was identified. For 4tBP, the dietborne toxicity is dominant than waterborne toxicity in the equilibrium condition. The LC<sub>50</sub> of dietborne toxicity was:  $6.3 \times 10^2$  mg/kg (95% CI:  $6.0 \times 10^2$  -  $6.6 \times 10^2$  mg/kg) in combined exposure, and  $7.7 \times 10^2$  mg/kg (95% CI:  $7.1 \times 10^2$  -  $9.3 \times 10^2$  mg/kg) in dietary exposure. For 4tOP, the water exposure route is dominant than dietary route in the equilibrium condition. The aqueous LC<sub>50</sub> was  $4.8 \times 10^{-1}$  mg/L (95% CI:  $4.1 \times 10^{-1}$  -  $5.8 \times 10^{-1}$

mg/L) in combined exposure, and  $4.9 \times 10^{-1}$  mg/L (95% CI:  $4.4 \times 10^{-1}$  -  $5.7 \times 10^{-1}$  mg/L) in dietary exposure. For BHT, it is difficult to distinguish which pathway is dominant. However there is no significant toxicity to *H. incongruens* even higher than the water solubility (0.6 mg/L at 25°C). For 4NP, it is also difficult to conclude if the dietborne toxicity is dominant or not. For TEB, the dietborne toxicity is dominant under the equilibrium condition. The LC50 of dietborne toxicity was:  $2.6 \times 10^2$  mg/kg (95% CI:  $2.0 \times 10^2$  -  $3.2 \times 10^2$  mg/kg) in combined exposure, and  $2.1 \times 10^2$  mg/kg (95% CI:  $1.5 \times 10^2$  -  $2.7 \times 10^2$  mg/kg) in dietary exposure. These successful application cases supported the practicability of the identification strategy coupling the proposed toxicity test method.

Effect of partition coefficient on equilibrium concentration was discussed. Similar measured logK<sub>oc</sub> values were obtained among four algae species. In the species of *D. subspicatus*, the measured logK<sub>oc</sub> of five HOCs under different concentration gradients generally similar to the theoretical values. All of the results support the equilibrium partitioning theory, in which the final concentration is determined to the partition coefficient.

Implementations of dietborne toxicity and mobility on chemical management were discussed. The contribution of dietborne toxicity in combined exposure was quantified by the dose addition model. The dietborne toxicity of 4tBP contributes 82% in combined exposure, 95%-100% for TEB and 13%-38% for 4tOP. For 4NP, it is still impossible to determine the dominant exposure route using the quantification method. In the case of BHT, the dietborne toxicity contributed 0%-35% to the 27% growth inhibition, 36%-42% to the 39% growth inhibition. There is high possibility that the waterborne toxicity of BHT is more important than dietborne toxicity in the equilibrium condition. The contribution of dietborne toxicity is negatively correlated to the partition coefficient in the tested range. Results in this dissertation supported current differentiation threshold of logK<sub>ow</sub> 3 (equal to logK<sub>oc</sub>  $2.8 \pm 0.4$ ) for consideration of sediment toxicity. More data of HOCs dietborne toxicity is needed to derive the upper differentiation threshold.

The mobility of the five HOCs was quantified by the adsorption rate constant, desorption rate constant and residue concentration after being fitted with kinetics models. No significant correlation was found between the mobility and partition coefficient. A proposal of overall implementations for management of HOCs was made considering the partitioning state and dominant exposure route. SQGs of 4tBP and TEB is suggested to be derived considering the dietborne toxicity. 4tBP can be monitored either in water phase or solid phase, while TEB should be monitored in solid phase. For 4tOP and 4NP, the SQGs derivation should consider

both of the aqueous and dietary exposure route, and the monitoring should be conducted in water and solid phase separately. It is not necessary to derive SQG of BHT for the low toxicity.