

## Development of high-throughput and multi-species algal toxicity assay for probabilistic ecological risk assessment of pesticides

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Species sensitivity distribution (SSD) analysis as probabilistic method has been widely used for ecological risk assessment of pesticides. However, more large data is required for the analysis, and especially more than 5 algal species toxicity data is desired for herbicide SSD analysis. In this study, efficient and economical high-throughput algal toxicity assay using 5 riverine periphyton species was developed.

One green algae, three diatoms, and one cyanobacteria were selected as representative riverine periphyton species to reflect actual species composition. Other additional species were also used for toxicity test to verify the selection. Toxicity assays using standard chemicals 3,5-dichlorophenol for periphyton algal species were conducted. 96-well microplate was used as test chamber and algal growth were measured by in-vivo fluorescence.

The EC50s obtained from microplate assay were well consistent with those obtained from conventional Erlenmeyer flask assay. Moreover, EC50s of 3,5-dichlorophenol were within the reported confidence intervals. These results suggest the validity of our microplate assay.

Culture condition was optimized so that 5 algal species can be tested simultaneously under the same condition, such as light, temperature, and medium. All validity criteria of OECD test guideline 201 (algal growth inhibition test) were fulfilled under the light intensity of 3000 lux, temperature of 22 C, and using CSi medium. Consequently, a high-throughput and multi-species algal toxicity assay was enabled.

SSD analysis was conducted using obtained 5 species EC50s of 3,5-dichlorophenol. The SSD was found to be similar to the SSD using additional tested species, suggesting that the SSD using the 5 species largely represents algal sensitivity in natural aquatic ecosystems. Our results provide useful and efficient method for high-tier probabilistic ecological risk assessment using SSD.

### Keyword

Species sensitivity distribution

riverine periphyton

in-vivo fluorescence

3,5-dichlorophenol