Title: Can we apply concentration additive and independent action mixture effect model to species sensitivity distribution? - An experimental validation using herbicides.

Takashi Nagai

National Institute for Agro-Environmental Sciences, Tsukuba, Ibaraki, Japan

Kiyoshi Taya

National Institute for Agro-Environmental Sciences, Tsukuba, Ibaraki, Japan

Ikuko Kitayama

National Institute for Agro-Environmental Sciences, Tsukuba, Ibaraki, Japan

Abstract

Conventional prediction models of chemical mixture, concentration additive (CA) and independent action (IA), have been used for mixture effect assessment. The applications of CA and IA to single species toxicity have a lot of examples of experimental validation. The applications of CA and IA to species sensitivity distribution (SSD) have also been used to calculate multi-substance potentially affected fraction (msPAF) for ecological risk assessment of chemical mixture. However, msPAF calculation has not been validated experimentally. Therefore, we tried to validate the application as a case study using herbicides which are toxic to algae. We tested the mixture toxicity of herbicides on the growth of five periphytic algae, cyanobacterium *Pseudanabaena galeata*, green alga *Desmodesmus subspicatus*, diatom *Achnanthidium minutissimum*, diatom *Nitzschia palea*, and diatom *Navicula pelliculosa*. Toxicity tests were conducted using 96-well microplate for 96 h, and the algal growth was monitored using

fluorescence microplate reader. Five herbicides of the same mode of action (CA model can be applied), pretilachlor, butachlor, mefenacet, cafenstrole, and fentrazamide, were mixed as Mixture 1, and five herbicides of the different mode of action (IA model can be applied), pretilachlor, bensulfuron-methyl, pyraclonil, esprocarb, and simetryn, were mixed as Mixture 2. First, we tested the toxicity of each single herbicide to the five algal species, and then these data were used for SSD analysis. Three effect levels (HC5, HC10, and HC20) of each herbicide were used for further mixture experiments (Mixture 1 and Mixture 2). The fraction of species which were affected more than 50% was defined as experimentally obtained msPAF. In addition, msPAFs were calculated applying CA and IA models to each SSD. One, one, and two species were affected (corresponding to 20, 20, and 40% of msPAF) for HC5, HC10, and HC20 effect levels, respectively in Mixture 1 experiments. CA calculations agreed with the experimental results better than IA. In contrast, one, two, and four species were affected (corresponding to 20, 40, and 80% of msPAF) for HC5, HC10, and HC20 effect levels, respectively in Mixture 2 experiments. IA predictions agreed with the experimental results better than CA. These results suggest mixture effect of chemicals among same mode of action can be predicted using CA model, and that among different mode of action can be predicted using IA model, even in the application to SSD.

Keywords: Ecological risk assessment, Potentially affected fraction, Algae