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Though only occurring rarely, synergistic interactions between chemicals in mixtures have long been a point of focus. Most studies analyzing synergistic interactions used unrealistically high chemical concentrations. The aim of the present study is to determine the threshold concentration below which proven synergists cease to act as synergists towards the aquatic crustacean Daphnia magna. To do this, we compared several approaches and test-setups to evaluate which approach gives the most conservative estimate for the lower threshold for synergy for three known azole synergists. We focus on synergistic interactions between the pyrethroid insecticide, alpha-cypermethrin, and one of the three azole fungicides prochloraz, propiconazole or epoxiconazole measured on Daphnia magna immobilization. Three different experimental setups were applied: A standard 48h acute toxicity test, an adapted 48h test using passive dosing for constant chemical exposure concentrations, and a 14-day test. Synergy was defined as occurring in mixtures where either EC50 values decreased more than two-fold below what was predicted by concentration addition (horizontal assessment) or as mixtures where the fraction of immobile organisms increased more than two-fold above what was predicted by independent action (vertical assessment). All three tests confirmed the hypothesis of the existence of a lower azole threshold concentration below which no synergistic interaction was observed. The lower threshold concentration, however, decreased with increasing test duration from 0.026±0.013μM (9.794±4.897μgL(-1)), 0.425±0.089μM (145.435±30.46μgL(-1)) and 0.757±0.253μM (249.659±63.44μgL(-1)) for prochloraz, propiconazole and epoxiconazole in standard 48h toxicity tests to 0.015±0.004μM (5.651±1.507μgL(-1)), 0.145±0.025μM (49.619±8.555μgL(-1)) and 0.122±0.0417μM (40.236±13.75μgL(-1)), respectively, in the 14-days tests. Testing synergy in relation to concentration addition provided the most conservative values. The threshold values for the vertical assessments in tests where the two could be compared were in general 1.2 to 4.7 fold higher than the horizontal assessments. Using passive dosing rather than dilution series or spiking did not lower the threshold significantly. Below the threshold for synergy slight antagony could often be observed. This is most likely due to induction of enzymes active in metabolization of alpha-cypermethrin. The results emphasize the importance of test duration when assessing synergy, but also show that azole concentrations within the typically monitored range of up to 0.5μgL(-1) are not likely to cause severe synergy concerning Daphnia magna immobilization.

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