

The synergistic effect of piperonyl butoxide (PBO) on the toxicity of pyrethrins to *Hyalella azteca*

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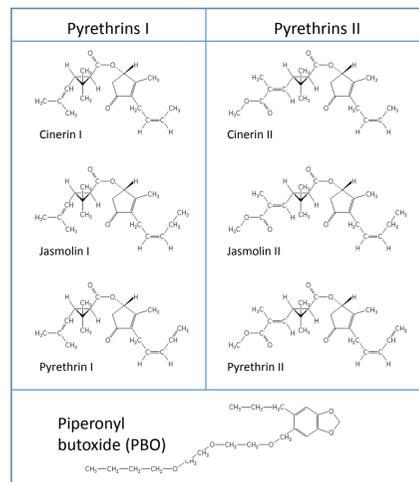


ABSTRACT

Natural pyrethrins and closely related synthetic compounds (pyrethroids) have been used for many years to control insect pests in residential and agricultural settings. Synergists, including piperonyl butoxide (PBO), have been used to substantially reduce the amount of pyrethrins and pyrethroids needed to maintain insecticidal potency. PBO inhibits breakdown of pyrethrins and pyrethroids within the target pest so more parent compound remains available at the site of action. The ratio of toxicity of pyrethrins or pyrethroid alone and with PBO (termed the enhancement factor) is a measure of the synergistic effect. In response to questions from US EPA regarding potential synergism in non-target arthropods, a series of 4-d water-only acute toxicity tests was performed to quantify the enhancement of pyrethrins toxicity by PBO in the amphipod *Hyalella azteca*, one of the most sensitive aquatic species to pyrethrins and pyrethroids. PBO concentrations less than 4 µg/L caused no toxicity enhancement. PBO concentrations between 4 µg/L and 15 µg/L caused pyrethrins toxicity to increase proportionately, with an enhancement factor of 3.2 at 15 µg/L PBO. Simple bridging studies can be used to extrapolate from one insecticide in a chemical class to other chemicals in the same class. These relationships can be used to assess the risk of measured or predicted co-occurring concentrations of PBO and pyrethrins or pyrethroids in surface waters.

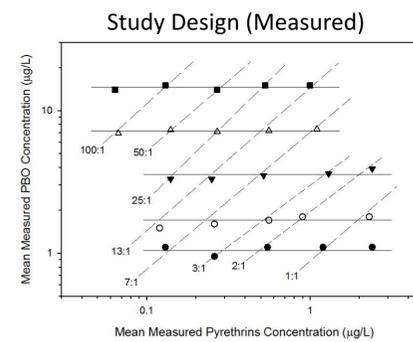
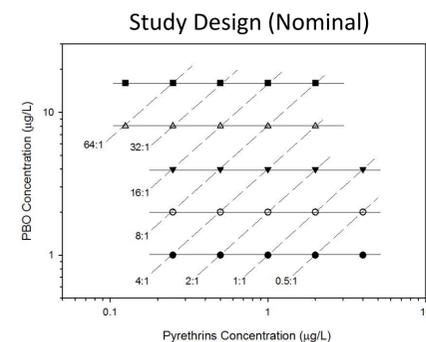
INTRODUCTION

Pyrethrins are a mixture of six naturally occurring plant esters used to control insect pests in public health, residential, and agricultural settings. Pyrethrins are often co-formulated with synergists such as PBO to reduce the amount of pyrethrins required to achieve insecticidal potency. When products containing PBO are applied outdoors, PBO may enter nearby water bodies in spray drift and surface runoff. This raises questions about the potential for synergistic effects of PBO on pesticide toxicity to non-target aquatic species.



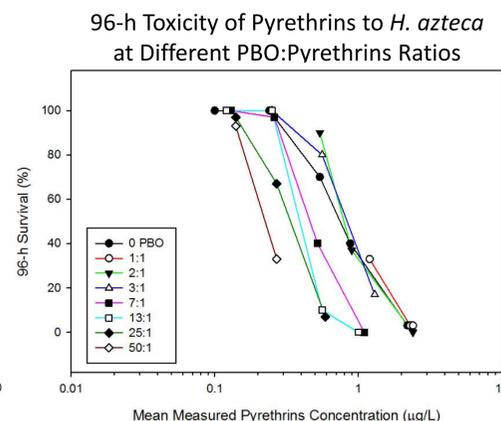
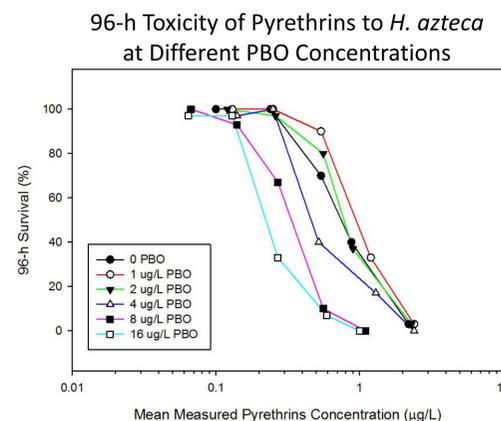
METHODS

Synergism studies with the freshwater amphipod *Hyalella azteca*, a species highly sensitive to pyrethrins and pyrethroids, were conducted as a series of 4-d flow-through water-only tests. In each test the PBO concentration was constant across a range of pyrethrins concentrations. The result of each test was expressed as the LC50 for pyrethrins. The enhancement factor for pyrethrins toxicity at each PBO concentration was calculated based on the ratio of LC50 values, i.e., $LC50_{Py}/LC50_{Py+PBO}$, where $LC50_{Py}$ and $LC50_{Py+PBO}$ represent the results for pyrethrins alone and pyrethrins with PBO, respectively. The study design is shown graphically in the figures below. Nominal (left) and mean measured (right) concentrations of pyrethrins and PBO in each test are indicated by series of symbols connected by horizontal lines. By using a 2-fold dilution factor for pyrethrins in each test and varying PBO concentrations 2-fold between tests, the study design also generated series of identical PBO:pyrethrins ratios across tests, as shown by the diagonal lines.



RESULTS

The concentration-response curves for pyrethrins alone and in combination with PBO after 96-h exposure are presented in the figures below. Left: based on PBO concentrations in individual tests. Right: based on PBO:pyrethrins concentrations from different tests (diagonal lines on study design figure).



DISCUSSION

Synergism of pyrethrins toxicity by PBO was expressed as enhancement factors (see Methods). The LC50s at 1.1 and 1.7 µg/L PBO, and at PBO:pyrethrins ratios of 1.9 and 3.2, were slightly greater than the LC50 of pyrethrins alone (0.76 µg/L), resulting in enhancement factors less than 1 (see tables below). This probably reflects normal between-test variation. The threshold for synergism appears to lie between 1.7 and 3.5 µg/L PBO or between PBO:pyrethrins ratios of 3.2 and 7.0. Clearly defined relationships were observed between PBO concentration, PBO:pyrethrins ratio, and pyrethrins toxicity. Increasing PBO concentration between 3.5 µg/L to 15 µg/L or increasing the PBO:pyrethrins ratio between 7:1 to 50:1 reduced the pyrethrins 96-h LC50 for *H. azteca* by a factor of 3.2 to 3.4.

LC50 values and enhancement factors as a function of PBO concentration (top) or PBO:pyrethrins ratio (bottom)

Mean measured PBO concentration (µg/L)	96-hr LC50 ^a (µg pyrethrins/L)	Enhancement Factor
0	0.76 (0.64-0.92)	1
1.1	0.98 (0.84-1.2)	0.78
1.7	0.82 (0.69-0.97)	0.93
3.5	0.47 (0.34-0.70)	1.6
7.2	0.32 (0.27-0.37)	2.4
15	0.24 (0.21-0.28)	3.2

PBO:pyrethrins ratio (range)	96-hr LC50 ^a (µg pyrethrins/L)	Enhancement Factor
0	0.76 (0.64-0.92)	1
1.9 (1.6-2.0)	0.86 (0.73-1.0)	0.89
3.2 (2.8-3.8)	0.83 (0.64-1.1)	0.91
7.0 (6.2-8.5)	0.48 (0.42-0.55)	1.6
13 (13-15)	0.40 (0.37-0.43)	1.9
25 (24-26)	0.32 (0.28-0.38)	2.3
52 (52-52)	0.23 (0.20-0.25)	3.4

^a95% confidence limits in parentheses

CONCLUSIONS

This series of tests generated clearly defined relationships between PBO concentration, PBO:pyrethrins ratio, and pyrethrins toxicity. These relationships can be used to assess the risk of measured or predicted co-occurring concentrations of PBO and pyrethrins in surface waters.

ACKNOWLEDGMENTS

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