Effects of PBO on propoxur and cyfluthrin on *P. australasiae* and propoxur toxicity in *P. australasiae* and *P. americana*.

Introduction

Periplaneta australasiae, or better known as the Australian cockroach, is a tropical cockroach that is considered a major pest species and a public health problem (Walker, 2007). Insecticides are the main control method for cockroaches and are often formulated with active ingredients like propoxur, pyrethroids and piperonyl butoxide (PBO). Propoxur is a carbamate that inhibits acetylcholinesterase as its main mechanism of toxicity (Kovacic & Somanathan, 2012). Cyfluthrin, a pyrethroid, obstructs nerve function by binding to voltage-gated sodium channels and modifying their kinetics as its main function (Ila et al., 2008). PBO is a pesticide synergist, meaning that it enhances the active ingredient in the insecticide, which can reduce the active dose required to produce a desired effect as the potency of the insecticide increases (Dehkordi et al. 2017). It does this by inhibiting cytochrome P450 and in turn, the metabolism and elimination of the pesticide (Bingham et al. 2011).

In a study done by Valles et al. (1999), they found that pyrethroid insecticide was the most toxic to all the cockroach species tested when compared to propoxur and other insecticides, and that *P. australasiae* was more tolerant of propoxur and pyrethroid than the *P. americana* cockroach. Similar results were reported by Abd-Elghafar et al. (1990), where they found that cyfluthrin was the most toxic insecticide for male and female *B. germanica* (German) cockroaches. These studies indicate that different species of cockroaches can have different levels of resistance to insecticides.

Moreover, Sanchez-Arroyo et al. (2001) observed that in German cockroaches, PBO increased the toxicity of propoxur, reducing its tolerance by 1.8-fold. Furthermore, Scott et al. (1990) reported that adding PBO to the insecticide propoxur and pyrethroid also increased its toxicity, but reported that other insecticides such as bendiocarb and chlorpyrifos showed the opposite effect, a decrease in toxicity.

This study looks to compare the response of the *P. australasiae* with *P. americana* cockroaches to propoxur exposure and explore the differences in their responses. Based on previous studies, it was predicted that the *P. australasiae* would tolerate propoxur more than *P. americana*.

Additionally, we've examined the effect of different pesticides on the *P. australasiae* cockroaches to determine the lethality of various insecticides and the efficacy of PBO as an insecticide synergist on *P. australasiae* nymphs. This was done by investigating the lethal dose (LD_{50}) concentration of propoxur, propoxur + PBO, cyfluthrin, and cyfluthrin + PBO. The cyfluthrin insecticide was expected to be more lethal than propoxur, with the addition of the PBO expected to increase the potency of both insecticides.

Method

Serial dilutions

Serial dilutions were performed using 1% stock solution of propoxur diluted in vehicle to the following concentrations: 0.1%, 0.01%, 0.001%, 0.0001% and 0%. The same process was carried out for 0.1% stock solution of cyfluthrin to the concentrations: 0.01%, 0.001%, 0.0001%, 0.00001% and 0%. Solutions composed of propoxur or cyfluthrin with PBO were made up to the corresponding serial dilutions with the EMK vehicle containing 0.1% PBO.

Cockroach dosing

P. australasiae or *P. americana* cockroaches of mixed ages were anesthetised on ice before 2 µL of previously diluted insecticide was applied to the ventral abdomen. *P. australasiae* was

exposed to all solutions whereas *P. americana* was only exposed to propoxur. Two cockroaches were exposed to each concentration and this process was repeated for all serial dilutions of pesticide solution. The average weight of the cockroaches was measured and the cockroaches were sealed in a ventilated container before the number of dead cockroaches at each concentration was determined 24 hours post exposure.

Results

Figures 1 and 2 summarise the comparative propoxur susceptibilities of two cockroach species, *P. americana* and *P. australasiae*. *P. americana* (LD_{50}=0.81) (Figure 1) was more susceptible to propoxur than *P. australasiae* (LD_{50}=4.22) (Figure 2).

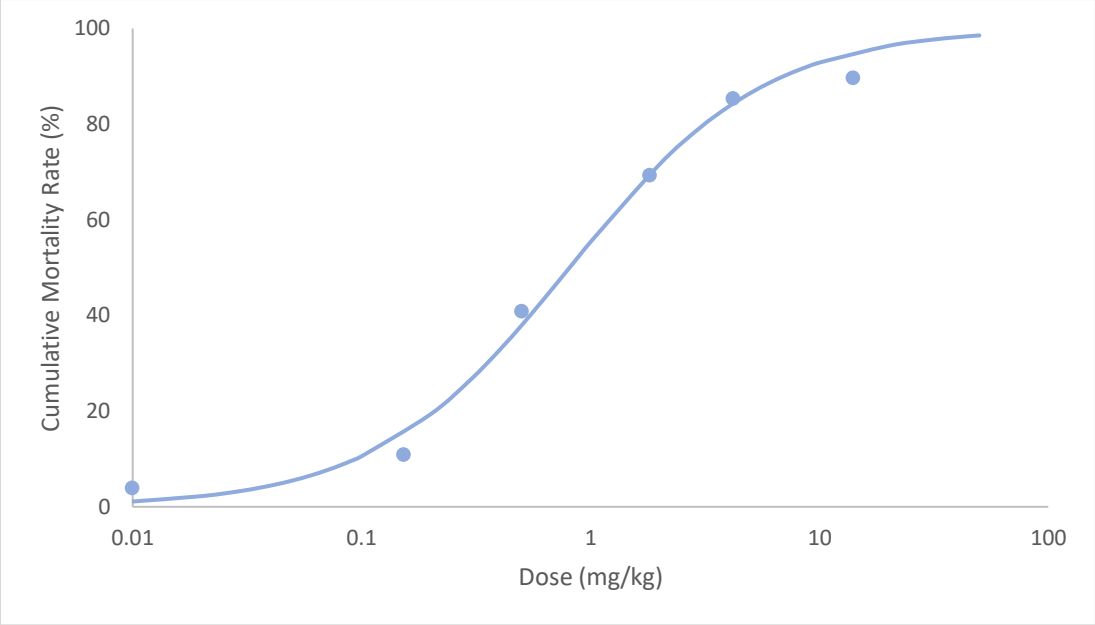

Figure 1. **Quantal dose-response curve of *P. americana* treated with propoxur**. Cockroaches were treated topically with a single exposure to 2µL of varying concentrations of propoxur. The mortality rate was measured 24 hours post exposure.

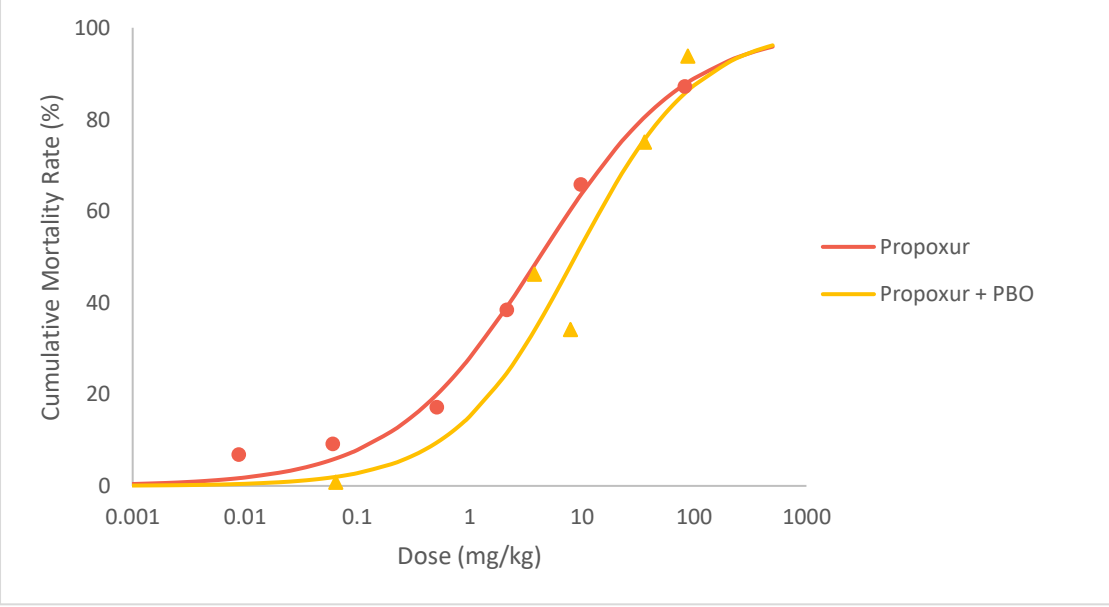

Figure 2. **Quantal dose-response curves of *P. australasiae* treated with propoxur alone or propoxur with PBO**. Cockroaches were treated topically with a single exposure to 2µL of varying concentrations of propoxur with or without 0.1% PBO. The mortality rate was measured 24 hours post exposure.

In addition to propoxur exposure, *P. australasiae* was also exposed to cyfluthrin, which was significantly more lethal (LD_{50}=0.49) (Figure 3) than propoxur (LD_{50}=4.22) (Figure 2).

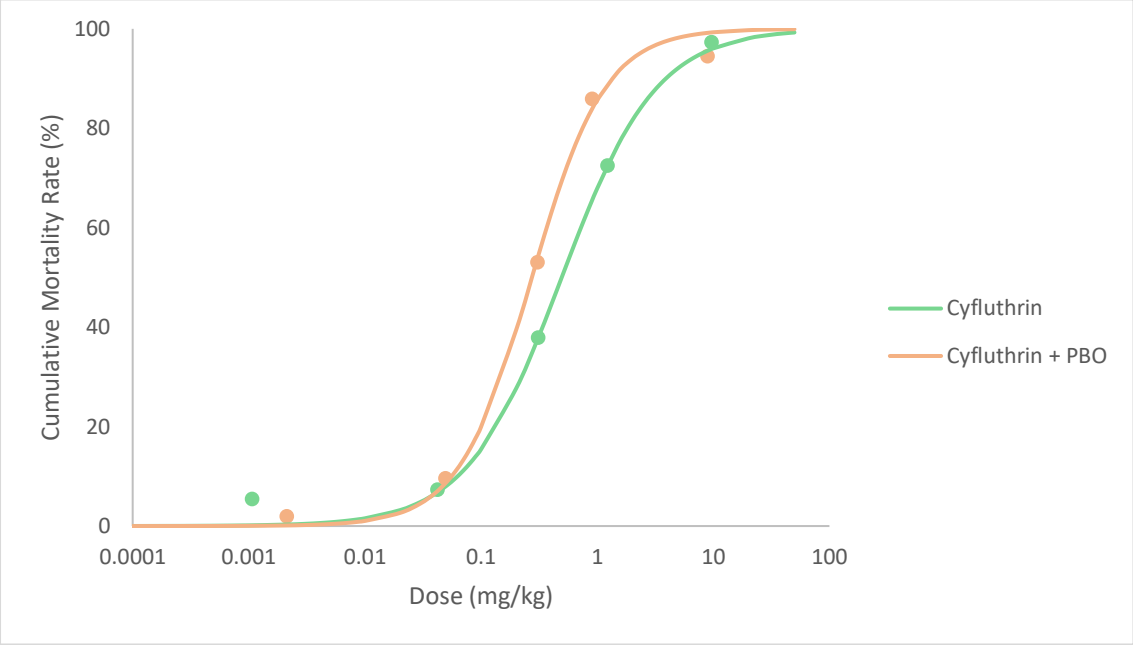

Figure 3. **Quantal dose-response curves of *P. australasiae* treated with cyfluthrin alone or cyfluthrin with PBO.** Cockroaches were treated topically with a single exposure to 2µL of varying concentrations of cyfluthrin with or without 0.1% PBO. The mortality rate was measured 24 hours post exposure.

Studies analysing the effects of PBO on influencing the lethality of both propoxur and cyfluthrin were carried out in Figure 2 and Figure 3. PBO decreased the lethality of propoxur (LD_{50}=4.22 and LD_{50}=8.73 for propoxur and propoxur + PBO respectively) (Figure 2). However, it slightly increased the lethality of cyfluthrin (LD_{50}=0.49 and LD_{50}=0.27 for cyfluthrin and cyfluthrin + PBO respectively) (Figure 3).

Discussion

The present study sought to compare the response of two common domestic cockroaches to propoxur exposure, revealing drastic differences in the susceptibility between the two species. These results imply that large variations in susceptibility to toxicants can be observed between species within the same genus as the LD_{50} is a characteristic of both the toxicant and species (Erhirhie *et al.* 2018). This has major implications on the use of the LD_{50} measurement in insecticide testing as testing of one species is not representative of the whole genus response to the particular chemical. Results were consistent with findings from previous studies by Valles et al. (1999) which indicated greater tolerance in the *P. australasiae* species compared *to P. americana*, although higher LD_{50} values were recorded in the present study in both species. This may be indicative of increasing resistance of cockroaches to insecticides over time as a result of increased use (Wu & Appel, 2017).

Whilst many insecticides have neurotoxic mechanisms (Costa *et al.* 2008), the lethality of these toxicants varies greatly. Several studies suggest that cyfluthrin, and other pyrethroids, have a greater effect on cockroaches than carbamates including propoxur (Valles *et al.* 1999; Abd-Elghafar *et al.* 1990). The present findings support these toxicity results, revealing that cyfluthrin had a lower LD_{50} and was therefore more lethal than propoxur.

PBO is an insecticide synergist and is able to increase the potency of both propoxur and cyfluthrin by inhibiting cytochrome P450 and esterase metabolism of the toxicants in resistant insects with have elevated metabolic levels (Dadzie *et al.* 2017; Bingham *et al.* 2011). Our results showed that cockroaches treated with cyfluthrin in conjunction with PBO showed slight decreases in resistance to cyfluthrin as evidenced by the lower LD_{50} compared to cyfluthrin alone, which is in line with previous studies. Interestingly, PBO had a very different effect when added to propoxur in this study, with results revealing a decreased lethality in propoxur in the presence of PBO. This suggests that whilst *P. australasiae* increases its resistance to cyfluthrin via upregulation of CYP450 metabolism, resistance to propoxur is largely due to some other mechanism (Valles & Yu, 1996). This is incongruent with other studies by Sanchez-Arroyo et al. (2001) which showed increases in potency of propoxur in the presence of PBO when exposed to *B. germanica*, although species differences may account for these discrepancies. Unexpected effects of synergists have also been observed including reductions in penetration rate of propoxur, potentially explaining the results seen (Sanchez-Arroyo *et al.* 2001). It should also be noted that the background mortality rate for the propoxur with PBO group was particularly high compared to other groups, potentially making the solution appear less toxic after adjustment. Despite previous studies supporting the efficacy of PBO against other species (Dadzie *et al.* 2017; Sanchez-Arroyo *et al.* 2001), present findings indicate slight, if any, improvements in potency following the addition of PBO, questioning the efficacy of PBO in reducing resistance in cockroaches, particularly *P. australasiae.*

Comparisons between LD_{50} values are often done by looking for overlapping confidence intervals or using ratio tests (Wheeler *et al.* 2006). However, no statistical analysis was performed in this study, and hence the significance of differences in LD_{50} cannot be inferred, particularly between cyfluthrin and cyfluthrin with PBO. Further, differences in lipid content and distribution in male and female cockroaches in conjunction with other factors are known to affect insecticide susceptibility, thus resulting in differences in LD_{50} (Koehler *et al.* 1993; Munson & Gottlieb, 1953). This study disregarded the sex of the cockroaches, with unknown numbers of males and females in each group, potentially resulting in unmatched LD_{50} comparisons.

Barring these limitations, the analysis of LD50 values of different insecticide formulations on several cockroach species allowed us to compare and judge the efficacy of these different classes of insecticides as well as insecticide synergists, and acknowledge species differences in toxicant response. The results revealed that PBO has limited efficacy when used in conjunction with propoxur and cyfluthrin on modern-day *P. australasiae* cockroaches, potentially as a result of increasing resistance via alternative mechanisms. Thus, further study on other potential insecticide synergists should be performed to improve current insecticide formulations and improve pest control in Australia.

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