# Appendix A. Multi-Active Ingredients Product Analysis Deltamethrin

The Agency does not routinely include, in its risk assessments, an evaluation of mixtures of active ingredients, either those mixtures of multiple active ingredients in product formulations or those in the applicator's tank. In the case of the product formulations of active ingredients (that is, a registered product containing more than one active ingredient), each active ingredient is subject to an individual risk assessment for regulatory decision regarding the active ingredient on a particular use site. If effects data are available for a formulated product containing more than one active ingredient, they may be used qualitatively or quantitatively (USEPA, 2004; USFWS/NMFS/NOAA, 2004).

Analysis of the available open literature and acute oral mammalian $LD_{50}$ data for multiple active ingredient products relative to the single active ingredient is provided below. This data set is limited and a qualitative analysis, in general, does not support any broad conclusions about the interactive nature of deltamethrin in combination with other pesticides.

Deltamethrin has registered products that contain multiple active ingredients; there are 28 multi-active ingredient products containing deltamethrin, which were evaluated in this appendix. Deltamethrin can be formulated with s-bioallethrin, pyrethrins, chlorpyrifos-methyl, imiprothrin, piperonyl butoxide (PBO), oil of thyme and geraniol combination, and PBO and s-bioallethrin combination. There are two products that are co-formulated with PBO, a known synergist. Acute oral toxicity data (*i.e.*, $LD_{50}$ values) from mammalian studies for formulated products that contain deltamethrin and one or more additional active ingredients are summarized in **Table A.1** below. The results of an open literature search for data on formulated products that contain deltamethrin using the EPA ECOTOX database starts on page 7 of this appendix.

Currently, the Agency's guidance for assessing the potential risk of chemical mixtures is limited to human health applications; however, the guidance includes principles for evaluating mixtures to assess potential interactive effects that are generally applicable. Consistent with EPA's Overview Document (USEPA, 2004), the Agency's mixture guidance discusses limitations in quantifying the risk of specified mixtures when there is differential degradation, transport and fate of chemical components following environmental release or application. The $LD_{50}$ values are potentially useful only to the extent that a wild mammal would consume plants or animals immediately after these dietary items were directly sprayed by the product. Increasing time post application, the differential rates of degradation, transport, *etc*. for the active ingredients in the formulation only permit a qualitative discussion of potential acute risk (USEPA, 2004).

A quantitative component-based evaluation of mixture toxicity requires data of appropriate quality for each component of a mixture. In this mixture evaluation $LD_{50}$s, with associated 95% confidence intervals, are needed for the formulated product. The same quality of data is also required for each component of the mixture. Given that some of the formulated products do not have $LD_{50}$ values of the required quality and since $LD_{50}$ values are not available for all the components of these formulations, a quantitative analysis of potential interactive effects is not possible with currently accepted scientific methods. As a screening tool, a qualitative analysis can be used to indicate if formulated products exhibit interactive effects (*e.g.*, synergism or

antagonism). The logic behind the analysis of the multiple active ingredient product analysis, from mammalian toxicity data is that if there are multiple studies with the technical formulation for which confidence intervals (CI) are provided for the $LD_{50}$, then the CI with the smallest lower CI for the $LD_{50}$ (*i.e.*, most toxic $LD_{50}$,) after correcting for %AI, is compared to the $LD_{50}$ upper CI for each formulation, after correcting for %AI. If these confidence intervals do not overlap, then the formulated mixture is considered to be more toxic. When the product $LD_{50}$s, and associated confidence intervals, are adjusted for the percent bifenthrin (a conservative assumption that attributes all of the observed toxicity of the formulated product to deltamethrin); based on this approach, the Health Effects Division (HED) can reach one of three conclusions for the formulations:

- Formulation is no more toxic than single active ingredient
- Formulation is more toxic than single active ingredient
- There is insufficient data to establish difference toxicity

In the case of deltamethrin, a qualitative examination of the trends in $LD_{50}$ values, with the associated confidence intervals, across the range of percent active ingredient, reveals no definitive conclusions. In all but one instance, it was concluded that data was insufficient to establish a difference in toxicity. The exception is product 66330-390, for which it was concluded that for females the formulation was more toxic than single active ingredient.

There are several studies on mixture analysis in the open literature (a screen of the ECOTOX database starts in page 7). Analysis of the multi-A.I. data on both target and non-target organisms indicates that PBO may synergize the effect of deltamethrin in several organisms, including rats (*e.g.*, Ahmad *et al.* 2009; Binham *et al.* 2011, Darnet *et al.* 2011, Fakoorziba *et al.* 2009, Ishaaya *et al.* 1983, Kotze and Sales 1994, Madhumathi and Subbaratnam 2007, Martin *et al.* 2003, Romero *et al.* 2009, Tungu *et al.*, Vijayan *et al.* 2007, Weston *et al.* 2006, Yang *et al.* 2004, and Yavuz *et al.* 2010). For example, Weston and coworkers (2006) have conducted sediment toxicity studies for synthetic pyrethroids. In one study it was found that the presence of PBO in the overlaying water could cause an increase of the toxicity of pyrethroids present in the sediment to the amphipod *Hyalella azteca* (Weston *et al.* 2006). PBO is co-applied with pyrethrins for mosquito control. PBO concentrations of 2−4 μg/L caused a two-fold increase of the toxicity to the amphipod in sediments.

Additionally, there are studies that document synergism between organophosphate insecticides and deltamethrin (*e.g.*, Ahmad 2009, Elhawagy and Nazy 2009, Trevis *et al.* 2010). Interactions of deltamethrin and various oils are also documented (*e.g.*, Pree *et al.* 1996, Vastrad *et al.* 2002, Sridevi and Dhigra 1996). Additionally, carbaryl and carbaryl-PBO combined with deltamethrin are compared in *e.g.*, Tripathi and Agarwal 1997.

Finally, the joint effects of pyrethroids in sediments appear to be additive (*e.g.*, Trimble *et al.* 2009) and in rats (*e.g.*, Wolansky *et al.* 2009).

Based on a qualitative evaluation of the best available data and the Agency's existing guidance, it is reasonable to conclude that these formulations may exhibit a synergistic effect in some instances. Given that the active and inert ingredients would not be expected to have similar

mechanisms of action, metabolites or toxicokinetic behavior it is also reasonable to conclude that an assumption of dose-addition would be inappropriate in some instances. However, the limited size of the data set and the variation in co-formulated pesticides prohibits any definitive conclusions. Consequently, an assessment of deltamethrin potential effect when it is co-formulated with other active ingredients will be based on the toxicity of deltamethrin.

**References:**

USEPA. 2004. *Overview of the Ecological Risk Assessment Process in the Office of Pesticide Programs*. United States Environmental Protection Agency (USEPA). Environmental Fate and Effects Division. Office of Pesticide Programs. Available at http://www.epa.gov/espp/consultation/ecorisk-overview.pdf (Accessed 02/12/2013).

[See other references after Table A1.]

| Table A1. Summary Report Active Mixtures of Deltamethrin | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Product | Current Registration No. | Percent A.I. | Active Ingredient | MRID(s) for Acute Oral Study | Male Oral LD50 (mg/kg) | Male Oral LD50 Lower CI (mg/kg) | Male Oral LD50 Upper CI (mg/kg) | Female Oral LD50 (mg/kg) | Female Oral LD50 Lower CI (mg/kg) | Female Oral LD50 Upper CI (mg/kg) |
| | **LD50 Information on Technicals** | | | | | | | | | |
| 1 | 0G3916 34147-EUP-3 | 99.20% | Deltamethrin | 41651019 | >5000 | | | >5000 | | |
| 2 | 000432-00774 | 98.00% | Deltamethrin | 43340901 | 1090 | 800 | 1480 | 1050 | 840 | 1310 |
| | **LD50 Information for individual products** | | | | | | | | | |
| 1 | 4-461 | 0.02 | Deltamethrin | | | | | | | |
| | 4-461 | 0.05 | S-Bioallethrin | | | | | | | |
| 2 | 149-18 | 0.02 | Deltamethrin | | | | | | | |
| | 149-18 | 0.05 | Pyrethrins (NO INERT USE) | | | | | | | |
| 3 | 264-992 | 3.7 | Deltamethrin | 46140801 | | | | 150.4 | | |
| | 264-992 | 21.6 | Chlorpyrifos-methyl | | | | | | | |
| 4 | 498-192 | 0.02 | Deltamethrin | 44446404 | >5050 | | | >5050 | | |
| | 498-192 | 0.05 | S-Bioallethrin | | | | | | | |
| 5 | 1270-255 | 0.1 | S-Bioallethrin | 44446404 | >5050 | | | >5050 | | |
| | 1270-255 | 0.02 | Deltamethrin | | | | | | | |
| 6 | 4822-518 | 0.05 | Deltamethrin | 44927003 | >5000 | | | >5000 | | |
| | 4822-518 | 0.101 | Imiprothrin | | | | | | | |
| 7 | 4822-562 | 0.1 | Imiprothrin | 44927003 | >5000 | | | >5000 | | |
| | 4822-562 | 0.03 | Deltamethrin | | | | | | | |
| 8 | 8848-72 | 0.02 | Deltamethrin | | | | | | | |
| | 8848-72 | 0.05 | S-Bioallethrin | | | | | | | |
| 9 | 9688-255 | 0.01 | Deltamethrin | | | | | | | |
| | 9688-255 | 0.05 | S-Bioallethrin | | | | | | | |

| Table A1. Summary Report Active Mixtures of Deltamethrin | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Product | Current Registration No. | Percent A.I. | Active Ingredient | MRID(s) for Acute Oral Study | Male Oral LD50 (mg/kg) | Male Oral LD50 Lower CI (mg/kg) | Male Oral LD50 Upper CI (mg/kg) | Female Oral LD50 (mg/kg) | Female Oral LD50 Lower CI (mg/kg) | Female Oral LD50 Upper CI (mg/kg) |
| 10 | 9688-256 | 0.02 | Deltamethrin | | | | | | | |
| | 9688-256 | 0.05 | S-Bioallethrin | | | | | | | |
| 11 | 10807-428 | 0.02 | Deltamethrin | 44974804 | >5000 | | | >5000 | | |
| | 10807-428 | 0.1 | Pyrethrins (NO INERT USE) | | | | | | | |
| 12 | 13283-29 | 0.05 | S-Bioallethrin | | | | | | | |
| | 13283-29 | 0.02 | Deltamethrin | | | | | | | |
| 13 | 28293-332 | 0.02 | Deltamethrin | | | | | | | |
| | 28293-332 | 0.15 | S-Bioallethrin | | | | | | | |
| 14 | 28293-334 | 0.1 | S-Bioallethrin | 44446404 | >5050 | | | >5050 | | |
| | 28293-334 | 0.02 | Deltamethrin | | | | | | | |
| 15 | 28293-336 | 0.05 | S-Bioallethrin | | | | | | | |
| | 28293-336 | 0.02 | Deltamethrin | | | | | | | |
| 16 | 66330-390 | 36 | Oil of thyme | 47673803 | | | | 1750 | 871.6 | 4170 |
| | 66330-390 | 0.75 | Deltamethrin | | | | | | | |
| | 66330-390 | 17.28 | Geraniol | | | | | | | |
| 17 | 73049-177 | 0.02 | Deltamethrin | 44446404 | >5050 | | | >5050 | | |
| | 73049-177 | 0.05 | S-Bioallethrin | | | | | | | |
| 18 | 73049-178 | 0.02 | Deltamethrin | | | | | | | |
| | 73049-178 | 0.15 | S-Bioallethrin | | | | | | | |
| 19 | 73049-180 | 0.05 | Deltamethrin | 44445904 | >5050 | | | >5050 | | |
| | 73049-180 | 0.3 | S-Bioallethrin | | | | | | | |
| 20 | 73049-183 | 0.1 | S-Bioallethrin | 44446404 | >5050 | | | >5050 | | |
| | 73049-183 | 0.02 | Deltamethrin | | | | | | | |
| 21 | 73049-184 | 2.86 | S-Bioallethrin | 44447904 | >5050 | | | >5050 | | |
| | 73049-184 | 1.16 | Deltamethrin | | | | | | | |
| 22 | 73049-210 | 0.25 | Deltamethrin | 45034504 | >5000 | | | >5000 | | |

| Table A1. Summary Report Active Mixtures of Deltamethrin | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Product | Current Registration No. | Percent A.I. | Active Ingredient | MRID(s) for Acute Oral Study | Male Oral LD50 (mg/kg) | Male Oral LD50 Lower CI (mg/kg) | Male Oral LD50 Upper CI (mg/kg) | Female Oral LD50 (mg/kg) | Female Oral LD50 Lower CI (mg/kg) | Female Oral LD50 Upper CI (mg/kg) |
| | 73049-210 | 25 | Piperonyl butoxide | | | | | | | |
| | 73049-210 | 2.5 | S-Bioallethrin | | | | | | | |
| 23 | 73049-354 | 0.572 | Deltamethrin | 45066104 | >5000 | | | >5000 | | |
| | 73049-354 | 2.86 | S-Bioallethrin | | | | | | | |
| 24 | 73049-366 | 0.02 | Deltamethrin | | | | | | | |
| | 73049-366 | 0.05 | Pyrethrins (NO INERT USE) | | | | | | | |
| 25 | 73049-367 | 1.16 | Deltamethrin | 44974604 | >5000 | | | >5000 | | |
| | 73049-367 | 2.86 | Pyrethrins (NO INERT USE) | | | | | | | |
| 26 | 73049-368 | 0.1 | Pyrethrins (NO INERT USE) | 44974804 | >5000 | | | >5000 | | |
| | 73049-368 | 0.02 | Deltamethrin | | | | | | | |
| 27 | 73049-389 | 0.005 | Deltamethrin | 45034604 | >5000 | | | >5000 | | |
| | 73049-389 | 0.1 | S-Bioallethrin | | | | | | | |
| | 73049-389 | 0.5 | Piperonyl butoxide | | | | | | | |
| 28 | 73049-390 | 0.01 | Deltamethrin | 45065705 | >5000 | | | >5000 | | |
| | 73049-390 | 0.05 | S-Bioallethrin | | | | | | | |

## ECOTOX Database literature with mixtures

### *Screen of ECOTOX Records and Papers Providing Data on Multiple A.I. Involving Deltamethrin and Non-Target Organisms*

Ahmad, M. Observed Potentiation Between Pyrethroid and Organophosphorus Insecticides for the Management of Spodoptera Litura (Lepidoptera: Noctuidae). 2009; 28, (3): 264-268, 2009.
Abstract: Mixtures of pyrethroids plus organophosphates were assessed for their potentiation in resistant field populations of Spodoptera litura from Pakistan by using a leaf-dip bioassay method. Cypermethrin, alpha-cypermethrin, bifenthrin, deltamethrin, and cyfluthrin exhibited potentiation by ethion. Mixtures of deltamethrin + triazophos and cyfluthrin + methamidophos also showed potentiation. Cypermethrin in combination with profenofos or chlorpyrifos produced an antagonism. (copyright) 2008 Elsevier Ltd. All rights reserved.

Ahmad, M.; Saleem, M. A., and Sayyed, A. H. Efficacy of Insecticide Mixtures Against Pyrethroid- and Organophosphate-Resistant Populations of Spodoptera litura (Lepidoptera: Noctuidae). MOR. Department of Entomology, University College of Agriculture, Bahauddin Zakariya University, Multan 60800, Pakistan//: ENV,MIXTURE; 2009; 65, (3): 266-274.
**BACKGROUND:** *Spodoptera litura* (F.) is an important pest worldwide, with over 112 host plants, and is exposed to insecticides throughout the year, resulting in the rapid development of resistance. Insecticide mixtures can delay the development of resistance more effectively than sequences or rotations. Cypermethrin, deltamethrin, profenofos, chlorpyrifos and fipronil were assessed separately and in mixtures against laboratory susceptible *S. litura* and two field-collected populations.
**RESULTS:** The field-collected population from Khanewal (KWL) was significantly more resistant to cypermethrin, deltamethrin, chlorpyrifos and profenofos than one collected from Muzaffar Garh (MGH). Mixtures of cypermethrin + chlorpyrifos or profenofos and of deltamethrin + chlorpyrifos or profenofos at 1:1, 1:10 and 1:20 ratios significantly increased ($P < 0.01$) toxicity to cypermethrin and deltamethrin in field populations. The combination indices of cypermethrin + chlorpyrifos at 1:1 and 1:10 ratios and cypermethrin + fipronil at 1:1, 1:10 and 1:20 ratios for the KWL strain and of cypermethrin + profenofos or fipronil at 1:1, 1:10 and 1:20 ratios for MGH were significantly below 1, suggesting synergistic interactions. The inhibitors DEF and PBO largely overcame resistance to deltamethrin, cypermethrin and profenofos, suggesting that resistance to the insecticides was associated with esterase and monooxygenase detoxification respectively.
**CONCLUSION:** Chlorpyrifos, profenofos and fipronil could be used in mixtures to restore cypermethrin and deltamethrin susceptibility. These findings may have considerable practical implications for *S. litura* resistance management.

Ascher, K. R. S.; Eliyahu, M.; Ishaaya, I.; Zur, M., and Ben-Moshe, E. Synergism of Pyrethroid-Organophosphorus Insecticide Mixtures in Insects and Their Toxicity Against Spodoptera littoralis Larvae. MORENV,TOP; 1986; 14, (2): 101-110.
Abstract: Mixtures of one of the pyrethroids cypermethrin, fenvalerate or deltamethrin with one of the OP compounds monocrotophos, profenofos, azinphos-methyl or acephate were assayed at different ratios as 24-h-old dipping residues on alfalfa, which was fed to*S. littoralis* larvae for 48 h. With most of the binary mixtures containing various OP concentrations in excess of those of the pyrethroids, synergism was demonstrated. In the pairs fenvalerate — azinphos-methyl, deltamethrin — azinphos-methyl and deltamethrin — profenofos, however, no synergism was found. In a detailed investigation with pyrethroid concentrations causing 20% mortality and OP concentrations giving a kill of no higher than ;10%, the above findings on synergism were amply confirmed. A cypermethrin-monocrotophos mixture showed synergism also on cotton leaves sprayed in the field.

Barre, N.; Li, A. Y.; Miller, R. J.; Gaia, H.; Delathiere, J. M.; Davey, R. B., and George, J. E. In Vitro and In Vivo Evaluation of Deltamethrin and Amitraz Mixtures for the Control of Rhipicephalus (Boophilus) microplus (Acari: Ixodidae) in New Caledonia. MOR,POP. Institut Agronomique néo-Calédonien, Station de Port-Laguerre, B.P. 73, 98890 Païta, New Caledonia//: ENV,MIXTURE; 2008; 155, (1/2): 110-119.

Abstract: Acaricide resistance is a major problem that hinders the control of the tropical cattle tick, *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini), in many parts of the world where cattle production continues to suffer severe economic losses to tick infestation. Deltamethrin and amitraz have been used alone to control *R. microplus* in New Caledonia for the past decade, and tick populations have developed resistance to both acaricides. A study was conducted to evaluate the effectiveness of deltamethrin and amitraz mixtures, through *in vitro* laboratory bioassays and *in vivo* on-animal efficacy trials, for the control of resistant *R. microplus* on cattle at two dairy farms in New Caledonia. Results of laboratory bioassays using modified larval packet tests (LPT) revealed up to 16.59-fold resistance to deltamethrin, and up to 5.86-fold resistance to amitraz. Significant synergism was observed when amitraz was used as a synergist in deltamethrin bioassays. Amitraz significantly increased deltamethrin toxicity to tick larvae, while deltamethrin was much less effective on amitraz toxicity. Synergism of amitraz by deltamethrin only occurred when the deltamethrin concentration was relatively high. Results of on animal efficacy trials of deltamethrin and amitraz alone and mixtures of both at different concentrations revealed a similar pattern of synergism. Adding amitraz to a deltamethrin formulation led to dramatic increases of percent reduction of both immature and adult ticks. In contrast, adding deltamethrin to an amitraz formulation did not increase control efficacy. Results from this study may lead to the adoption of an acaricide mixture strategy for the control of pyrethroid-resistant *R. microplus* in New Caledonia and elsewhere.

Bingham, Georgina; Strode, Clare; Tran, Lien; Khoa, Pham Thi; Jamet, Helen Pates, and Bingham, Georgina. Can Piperonyl Butoxide Enhance the Efficacy of Pyrethroids Against Pyrethroid-Resistant Aedes Aegypti? 2011 Apr; 16, (4): 492-500.

Abstract: Background Pyrethroid resistance can be considered the main threat to the continued control of many mosquito vectors of disease. Piperonyl butoxide (PBO) has been used as a synergist to help increase the efficacy of certain insecticides. This enhancement stems from its ability to inhibit two major metabolic enzyme systems, P450s and non-specific esterases, and to enhance cuticular penetration of the insecticide. Objective To compare the mortality of a characterized resistant Aedes aegypti strain, Nha Trang, from Vietnam and the susceptible laboratory strain Bora Bora on netting with the pyrethroid deltamethrin (DM) alone and in combination with PBO. Methods Resistance mechanisms were characterized using molecular and bioassay techniques; standard PCR was used to test for the kdr target site mutation. Potential genes conferring metabolic resistance to DM were identified with microarray analysis using the Ae. aegypti'detox chip'. These data were analysed alongside results from WHO susceptibility tests. P450, CYP9J32, was significantly overexpressed in the DM-resistant strain compared with the susceptible Bora Bora strain. Another five genes involved with oxidative stress responses in mosquitoes were also significantly overexpressed. The Nha Trang strain was homozygous for two kdr mutations. WHO cone bioassays were used to investigate mortality with incorporated DM-treated nets with and without PBO. PBO used in combination with DM resulted in higher mortality than DM alone. Conclusion Synergists may have an important role to play in the future design of vector control products in an era when alternatives to pyrethroids are scarce.

Darriet, Freedeeric; Chandre, Fabrice, and Darriet, Freedeeric. Combining Piperonyl Butoxide and Dinotefuran Restores the Efficacy of Deltamethrin Mosquito Nets Against Resistant Anopheles Gambiae (Diptera: Culicidae). 2011 Jul; 48, (4): 952-955.

Abstract: One strategy suggested for the management of mosquito insecticide resistance consists of combining a pyrethroid with an insecticide that has a different mode of action. To restore the efficacy of deltamethrin (pyrethroid) against pyrethroid-resistant strain of Anopheles gambiae Giles (VKPR: homozygous Kdr), deltamethrin was combined with the neonicotinoid insecticide dinotefuran and piperonyl butoxide (PBO). Bednets impregnated with deltamethrin, dinotefuran, and PBO alone and in combination were tested in the laboratory. Knockdown (KD) and mortality were measured using WHO cone tests on susceptible and pyrethroid-resistant adult mosquitoes. The combination of deltamethrin and PBO was synergistic against resistant female An. gambiae (58.2% mortality). Both mortality and knockdown time (KDt50/95 values) of the tricomponent mixture on the VKPR strain were similar to the insecticidal activity of deltamethrin on the pyrethroid-susceptible KIS strain (98.8 and 100% mortality, respectively). The three-compound

mixture of deltamethrin + PBO + dinotefuran showed an insecticidal efficacy greater than the deltamethrin + PBO mixture to the extent of completely restoring the efficacy of deltamethrin on pyrethroid-resistant An. gambiae.

Elhalwagy, M. E. A. and Zaki, N. I. Comparative Study on Pesticide Mixture of Organophosphorus and Pyrethroid in Commercial Formulation. BCM. Pesticide Central Laboratory, Agriculture Research Center, Giza, Egypt,//: ORAL; 2009; 28, (2): 219-224.
Abstract: The marketing of mixtures of organophosphate and pyrethroid insecticides has become very common in developing countries and has resulted in an increase in the prevalence of toxicity. The present study aimed to evaluate the toxic effects of a commercial preparation of the pesticide mixture durasin, which contains 60% diazinon and 0.5% deltamethrin, compared with the individual commercial pesticides of diazinon 30% and deltamethrin 5%. Forty male albino rats weighing 160 ± 20 g were divided into; DA (diazinon 20 mg/Kg b.w.), DA (deltamethrin 2 mg/Kg b.w.), M (durasin 20 mg/Kg b.w.) and control (C); cholinesterase (ChE), malonaldehyde (MDA), glutathione (GSH), glutathione-S-transferase (GST), superoxide dismutase (SOD), total cholesterol (TC), triglyceride (TG) and non-specific esterase's isoenzymes in rat's blood were determined following 7 and 14 days of treatment. The weekly- recorded biochemical results were used as criteria for estimating the joint effects of the tested pesticide mixture. Antioxidant defense mechanisms and lipid peroxidation in rat plasma displayed the same responses with intensities which were related to the different treatments. Biochemical analysis showed that (DA) or (DM) individually cause alteration in lipid metabolism and non-specific esterase, while mixture treatment (M) induced antagonistic effects toward all the tested parameters except total reduced glutathione level, which was synergistic at the 2nd week. In conclusion the commercial mixture (M) under study has potentially greater toxic impact than the components alone in the rat.

Fakoorziba, M. R.; Eghbal, F., and Vijayan, V. A. Synergist Efficacy of Piperonyl Butoxide with Deltamethrin as Pyrethroid Insecticide on Culex tritaeniorhynchus (Diptera: Culicidae) and Other Mosquitoe Species. MORAQUA,MIXTURE; 2009; 24, (1): 19-24.
Abstract: Continuous and indiscriminate use of pesticides, especially in tropical countries for public health or agriculture purpose, has led many vector populations to become resistant to organochlorides, organophosphates, and even to carbamates and pyrethroids. Development of resistance by a vector population has been one of the reasons for the failure of the control measures in many countries. This investigation demonstrates the efficacy of piperonyl-butoxide (PBO) with deltamethrin, as pyrethroid insecticide, against the field-collected mosquitoe larvae of five species, *Aedes aegypti, Anopheles culicifacies, An. stephensi, An. vagus, and Culex quinqufasciatus*, and two morphological variants of *Cx. tritaeniorhynchus* (type A from grand pools of Mysore city and type B from rice fields of Mandya district). For testing the synergistic effect of PBO, stock solutions of deltamethrin and PBO were mixed in 1:6 ratio. The synergistic ratio and the percent suppression in deltamethrin tolerance were calculated by using $LC_{50}$ values. From the results, it is clear that, PBO is an effective synergist with deltamethrin against all of species undertaken in this investigation. So, it is suggested that PBO is a good synergist in this area for decreasing the use of pesticides in environment in vector control.

Hermens, J. and Leeuwangh, P. Joint Toxicity of Mixtures of 8 and 24 Chemicals to the Guppy (Poecilia reticulata). 9272//: 1982; 6, 302-310.
Abstract: The acute median lethal concentrations of equitoxic mixtures of 8 and 24 toxicants with diverse modes of action to guppies were determined. To quantify the joint toxicity, the results are expressed by means of the Mixture Toxicity Index (MTI). The toxicity of the mixtures was near concentration addition. Concentrations of the chemicals of about 0.1 of their $LC_{50}$'s contributed to the toxicity of the mixtures.

Ishaaya, I.; Elsner, A.; Ascher, K. R. S., and Casida, J. E. Synthetic Pyrethroids: Toxicity and Synergism on Dietary Exposure of Tribolium castaneum (Herbst) Larvae. GRO,MORMIXTURE,ORAL; 1983; 14, 367-372.
Abstract: The potency of six dietary pyrethroids, as toxicants and inhibitors of weight gain in first- and fourth-instar *Tribolium castaneum* (Herbst) larvae, decreased in the order of *cis*-cypermethrin

and deltamethrin > *trans*-cypermethrin and *cis*-permethrin > fenvalerate and *trans*-permethrin. Dosages that reduced larval weight also delayed pupation and emergence, probably due to their antifeeding activity. Three oxidase inhibitors (piperonyl butoxide, *O, O*-diethyl *O*-phenyl phosphorothioate, and *O*-isobutyl *O*-prop-2-ynyl phenylphosphonate), at a dietary concentration of 100 mg $kg^{-1}$, had little or no effect on the toxicity of *trans*-permethrin, but strongly synergised the toxicity of *cis*-cypermethrin by about 3-, 3- and 10-fold, respectively. Piperonyl butoxide also synergised the toxicity of *cis*-permethrin, *trans*-cypermethrin and deltamethrin, but not that of fenvalerate. On the other hand, an esterase inhibitor, profenofos, did not enhance the potency of any of the α-cyano-3-phenoxybenzyl pyrethroids. Oxidases appear to be more important than esterases in pyrethroid detoxification by *T. castaneum* larvae.

Kotze, A. C. and Sales, N. Cross Resistance Spectra and Effects of Synergists in Insecticide-Resistant Strains of Lucilia cuprina (Diptera: Calliphoridae). GRO,MORENV,MIXTURE; 1994; 84, (3): 355-360. Rec #: 203595

Abstract: Cross-resistance spectra were determined in strains of the Australian sheep blowfly, *Lucilia cuprina* (Wiedemann), which had been pressured for several years in the laboratory with diflubenzuron, butacarb or deltamethrin. Each strain was highly resistant to its selecting chemical (resistance factors > 1000-fold), however, cross-resistance levels were variable and often low. In particular, strains selected with diflubenzuron and butacarb showed very little resistance to deltamethrin (resistance factors <7-fold). Each strain showed resistance levels to diazinon only slightly higher than the highest levels currently detected in field strain larvae. Piperonyl butoxide and triphenyl phosphate significantly synergized each pressured strain with its selecting chemical, suggesting the involvement of both monooxygenases and esterases in the observed resistances. Synergism ratios in each case were greater with piperonyl butoxide. The lack of any alteration in *in vitro* acetylcholinesterase sensitivity to butacarb inhibition in the butacarb-selected strain, and only low level resistance to DDT in the deltamethrin-selected strain, provided no evidence for target-site insensitivities in these strains. The low-moderate levels of cross-resistance therefore imply the existence of qualitative differences in the detoxification systems in each strain.

Le Patourel, G. N. J. and Singh, J. Toxicity of Amorphous Silicas and Silica-Pyrethroid Mixtures to Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae). POPENV,MIXTURE; 1984; 20, (4): 183-190.

Abstract: The toxicity of some pyrogenic and precipitated amorphous silicas to *Tribolium castaneum* (Herbst) was assessed in admixture with wheat grain. A good correlation ($r^2 = 0.82$, $n = 7$) was found between the 48 h LC50 of the toxic dusts and their adsorption capacity for a saturated hydrocarbon oil. The pyrogenic silicas Cab-O-Sil M5 and Aerosil R972 were used as carriers to prepare dust formulations of permethrin, cypermethrin and deltamethrin at various concentrations, and 48 h (Cab-O-Sil M5 and Aerosil R972) and 168 h (Cab-O-Sil M5) LC50s were determined using the same bioassay system. Low and intermediate concentrations of the pyrethroids substantially reduced the 48 h LC50s of these silicas while high concentrations antagonised their toxic action due to knockdown effects. The 168 h LC50s of Cab-O-Sil/permethrin formulations were approx 15 and 150 × lower than those of talc/permethrin formulations at 2% w/w and 0.1% w/w respectively. The 168 h LC50 of the Cab-O-Sil/cypermethrin formulation was comparable with that of the talc/cypermethrin formulation, and that of the Cab-O-Sil/deltamethrin formulation was about 5 × less than a talc/deltamethrin formulation, at 2 and 0.2% w/w pyrethroid respectively on the dust carrier.

Madhumathi, T. and Subbaratnam, G. V. Determination of Cross- Resistance and Multiple Resistance in Cryptolestes ferrugineus (Stephens). MOR. Department of Entomology, Agricultural College, Bapatla, India//: ENV,MIXTURE; 2007; 19, (1): 63-66.

Abstract: Malathion, dichlorvos and phosphine resistant Machilipatnam strain of *C. ferrugineus* hah dedeloped cross-resistance to fenitrothion, profenofos (47.0 and 3.7 folds at $LC_{99.9}$) and multiple resistance to carbaryl and deltamethrin (6.8 and 4.8 fold at $LC_{99.9}$) in comparison with susceptible or less resistant Charlapalli strain. Synergism due to PBO was found in malathion (SF 7.08), dichlorvos (SF 1.93), profenofos (SF 1.64) and deltamethrin (SF 5.04) at $LC_{99.9}$ level. Except in the case of deltamethrin, the synergistic factors were less than the corresponding resistance factors revealing the possibility of some other mechanisms of resistance in addition to

the detoxification mechanism by MFO.

Martin, T.; Ochou, O. G.; Vaissayre, M., and Fournier, D. Organophosphorus Insecticides Synergize Pyrethroids in the Resistant Strain of Cotton Bollworm, Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae) from West Africa. MORMIXTURE,TOP; 2003; 96, (2): 468-474.
Abstract: *Helicoverpa armigera* (Hübner) populations from West Africa recently developed resistance to pyrethroid insecticides through enhanced metabolism by mixed-function oxidases. The combination index method was used to study the synergism of pyrethroids by organophosphorus insecticides. Several mixtures of insecticides currently registered to control cotton pest complex in West Africa were tested, including: cypermethrin/ethion, cypermethrin/profenofos, deltamethrin/triazophos, deltamethrin/chlorpyriphos, cyfluthrin/chlorpyriphos, and betacyfluthrin/chlorpyriphos. In the resistant strain, the organophosphorus insecticides significantly increased the toxicity of pyrethroids suppressing the resistance effect, either by additive or synergistic effects. Significant synergism was shown for the following mixtures: cypermethrin/ethion, deltamethrin/triazophos, and deltamethrin/chlorpyriphos. The use of synergism from these insecticide mixtures should prove to be an additional tool in the overall resistance management strategy because the pyrethroid resistance in *H. armigera* from West Africa is not yet stable, decreasing between cotton seasons and increasing with treatments. In absence of selection, the susceptibility of *H. armigera* to insecticides should be restored.

N'Guessan, R.; Asidi, A.; Boko, P.; Odjo, A.; Akogbeto, M.; Pigeon, O., and Rowland, M. An Experimental Hut Evaluation of Permanet 3.0, A Deltamethrin-Piperonyl Butoxide Combination Net, Against Pyrethroid-Resistant Anopheles Gambiae and Culex Quinquefasciatus Mosquitoes in Southern Benin.
Abstract: PermaNet 3.0 is a long-lasting combination net with deltamethrin present on the sides and a mixture of deltamethrin and piperonyl butoxide (PBO), an oxidase synergist, on the top panel. An experimental hut trial comparing unwashed and 20 times washed PermaNet 3.0 and PermaNet 2.0, Olyset Net and a conventional deltamethrin-treated net washed three times was conducted in southern Benin. Anopheles gambiae and Culex quinquefasciatus from this area are highly resistant to pyrethroids through kdr and cytochrome P450 mechanisms. The unwashed PermaNet 3.0 killed slightly more A. gambiae (52%) than the unwashed PermaNet 2.0 (44%) (P=0.036), indicating only partial synergism of resistance. After washing there was significant loss of activity to a similar level, with PermaNet 3.0 killing 31%, PermaNet 2.0 killing 29% and the conventional net killing 26%. Blood-feeding rates were partially inhibited for unwashed PermaNet 3.0 and Olyset Net (27% inhibition). Personal protection against A. gambiae derived from PermaNet 3.0 was similar to that from PermaNet 2.0 before washing (50% vs. 47%), and after 20 washes it decreased to 30%. Against C. quinquefasciatus, no treatment killed >24% entering the huts. The synergism from unwashed PermaNet 3.0 was lower than expected, probably due to an unidentified resistance mechanism unaffected by PBO.

Papaefthimiou, C. and Theophilidis, G. The Cardiotoxic Action of the Pyrethroid Insecticide Deltamethrin, the Azole Fungicide Prochloraz, and Their Synergy on the Semi-Isolated Heart of the Bee Apis mellifera macedonica. PHYINJECT; 2001; 69, (2): 77-91.
Abstract: The contraction of the isolated heart of the bee in physiological solution can be monitored for hours, making this preparation suitable for the investigation of the cardiotoxic action of certain compounds. The results of this study have shown that exposure of the semi-isolated heart of the bee to 1, 0.1, and 0.01 μM deltamethrin causes a temporal increase in the frequency and the force of spontaneously generated contractions, which is followed by a decrease in both parameters. The decrease is dose dependent. The action of deltamethrin was not reversible. The fungicide prochloraz applied at the same concentration levels as deltamethrin has an immediate chronotropic and inotropic effect on the semi-isolated heart of the bee, but its effects are more intense than those caused by deltamethrin. Comparison of the dose-response curves clearly shows that prochloraz is more cardiotoxic than deltamethrin. When prochloraz and deltamethrin are combined there is an increase of over 100 times in the cardiotoxicity of deltamethrin and an increase of 10 times in the toxicity of prochloraz. Our suggestion is that this synergistic action could be caused by the action of the two compounds on the same target site,

which in the heart of the bee may be gap junctional intercellular communication, a vital physiological mechanism for the functioning of the heart in both vertebrates and invertebrates.

Pree, D. J.; Stevenson, A. B., and Barszcz, E. S. Toxicity of Pyrethroid Insecticides to Carrot Weevils: Enhancement by Synergists and Oils. ACC,MOR,POP,REPSOIL,ENV,MIXTURE,TOP; 1996; 89, (5): 1254-1261.

Abstract: In laboratory bioassays over 24 h, pyrethroid insecticides, with the exception of tefluthrin, were more toxic than azinphosmethyl or foamer to the carrot weevil, *Listronotus oregonensis* (Leconte). The toxicity of permethrin decreased as post treatment holding temperatures were increased. $LC_{50}$ values for pyre thyroids increased by ≃2- to 3-fold between 24 and 48 h as weevils recovered from treatment. The synergisms piperonyl butoxide and DEF enhanced the toxicity of permethrin by 3- to 5-fold in both 24- and 48-h post treatment observations, but recovery rates were similar to those for permethrin alone. Addition of herbicidal (mineral) oil increased the toxicity of permethrin and deltamethrin, whereas the addition of olive oil was either antagonistic or ineffective. The increased toxicity of the herbicidal oil-pyrethroid mixture appeared associated with more rapid penetration of insecticide. In field trials, cypermethrin was as effective for control of the carrot weevil as the currently recommended insecticide foamer and is, therefore, a suitable alternative where control of both carrot mst fly,*Pails rosae* (F.), and carrot weevil is necessary. Application of cypermethrin in herbicidal oil was not more effective than cypermethrin alone against the carrot weevil at the concentrations tested.

Romero, A.; Potter, M. F., and Haynes, K. F. Evaluation of Piperonyl Butoxide as a Deltamethrin Synergist for Pyrethroid-Resistant Bed Bugs. Journal of Economic Entomology 102(6):2310-2315. 2009

Abstract: An understanding of the mechanisms of insecticide resistance in the bed bug, Cimex lectularius L., has the potential to lead to new approaches for the control of resistant populations. We used the cytochrome P450 monooxygenase (P450) inhibitor piperonyl butoxide (PBO) to assess the role of P450s in deltamethrin resistance in three field-collected bed bug strains, LA-1, CIN-1 and WOR-1. In addition, we exposed two highly resistant strains, CIN-1 and WOR-1 (resistance ratio [RR] >2,500-fold), to dry residues of piperonyl butoxide-synergized pyrethroid formulations to determine the utility of synergism by PBO. Piperonyl butoxide synergized deltamethrin in all three strains, but its impact was variable. The synergistic ratio varied from 40 in CIN-1 to 176 in WOR-1. Because the resistance ratio for each strain after piperonyl butoxide treatment was 174 and 39, respectively, our results suggest that P450s have some involvement in deltamethrin resistance, but other resistance mechanisms must be involved as well. No significant synergistic effect of formulated deltamethrin was observed with the addition of synergized pyrethrins or formulated piperonyl butoxide in the CIN-1 strain, but synergism occurred in the WOR-1 strain. Addition of PBO to pyrethroids is not a comprehensive solution to pyrethroid resistance because strains vary in both overall resistance level and the proportion of that resistance attributable to P450s.

Sridevi, D. and Dhingra, S. Evaluation of Some Non-Toxic Vegetable Oils as Synergists for Different Synthetic Pyrethroids in Mixed Formulations Against Tribolium castaneum (Herbst). MORENV; 1996; 20, (4): 335-343.

Abstract: On the basis of joint action ratios, the type of action of insecticides was determined when three synthetic pyrethroids (deltamethrin, cypermethrin, fenvalerate) were used separately in mixed formulations with each of the four non-toxic vegetable oils (sesame oil, karanj [*Pongamia pinnata*] oil, neem [*Azadirachta indica*] oil, citronella [*Cymbopogon nardus*] oil) and the well known standard synergist piperonyl butoxide (PBO) in four ratios (1:1, 1:2, 1:4, 1:8), and evaluated against a susceptible strain of *Tribolium castaneum* adults by direct spray and film residue methods. Out of 30 combinations tested, additive action was obtained when the oils were used in mixed formulations with cypermethrin and fenvalerate. With the exception of neem oil by the direct spray method, the remaining three vegetable oils also showed additive action with deltamethrin. Antagonistic action, however, was produced by neem oil in the mixed formulation with deltamethrin. Only the well known synergist PBO synergised cypermethrin and fenvalerate.

Szepvolgyi, J.; Nagy, K.; Bedo, M.; Regoly-Merei, A.; Szerletics, M.; Soos, K., and Antal, M. Examination of the

Interaction of Decis and Dithane in Rats. ACC,BCMORAL,MIXTURE; 1988; 53, (1): 107-111.
Abstract: Acute ($LD_{50}$) and short-term (14 days) toxicological examinations were performed in animal experiments on the interaction of a synthetic pyrethroid Decis 2,5 EC (25 g deltamethrin/l) and of ethylene-bisdithiocarbamate/Dithane M-45 (80% mancozeb), using a 1:5 deltamethrin/mancozeb mixture. $LD_{50}$ value of the mixture was similar to that of the more toxic Decis. In the short-term examination, some pathologically high AST and ALT values were observed in the treated groups and the deltamethrin content of fatty tissue samples increased parallel with the increase of Decis consumption. The chymotrypsin and lipase activities in the small intestinal mucosa and γ-GT and LAP activities in the content of the bowels were reduced in several treated groups. The administration of Dithane in a dose in accordance with 20% of the $LD_{50}$ value (3125 mg/kg b.m.) proved to be moer toxic than expected and caused the death of the animals.

Trevis, D; Habr, S F; Varoli, F M; Bernardi, M M, and Trevis, D. Acute Toxicity of the Organophosphorus Pesticide Diclorvos and the Mix With the Piretroid Deltamethrin in Danio Rerio and Hyphessobrycon Bifasciatus. 2010; 36, (1): 53-59.
Abstract: Abstract: Pesticides such as Dichlovos (DDVP) and Deltamethrin (DTM) are commonly used in agricultural production. To evaluate the toxicity of commercial formulations of DDVP, mixed or not with DTM, were realized tests for acute toxicity in fish species Danio rerio and Hyphessobrycon bifasciatus. To the mixture, we used the highest concentrations of pesticides tested, which caused no lethality. The results showed that on the concentrations used, the pesticide DDVP promotes the "all or nothing effect, i.e. 100% of animals dying or 100% survive. In the case of the mixture, the results demonstrated the occurrence of 100% lethality at concentrations of pesticides that did not produce lethal effect if administered alone, indicating a synergistic effect of the pesticide mixture. These data suggest that the use of mixtures of these pesticides may represent an important factor. Finally, the toxicity effect showed on the two species was similar, suggesting that the Hyphessobrycon bifasciatus fish may be used for environmental assessments in Brazilian conditions.

Trimble, A. J. Determining the Occurrence, Fate, and Effects of Pesticide Mixtures Using the Aquatic Amphipod Hyalella azteca. 2009: 198 p. (UMI #3372576).
Abstract: Previous monitoring studies by federal agencies such as the United States Geological Survey have shown that environmental contaminants rarely occur as single compounds but, rather, as mixtures. In aquatic ecosystems, mixtures of these compounds are often complex, sometimes containing dozens of compounds across a number of different chemical classes. Non-target aquatic organisms are frequently exposed to varying levels of contaminants based upon the physical properties of the chemicals, such as water solubility, and life-cycle habits of the individual organisms. In addition to this, past research has indicated that the presence of one class of contaminant may have an influence on the toxicities of other chemical classes. Water-only toxicity testing has historically provided a means by which researchers can rapidly determine the toxic effects of water-soluble compounds such as triazine herbicides and organophosphate insecticides. However, many legacy pesticides, such as organochlorine, and some current-use pesticides, such as pyrethroids, are strongly hydrophobic, and suspended or bedded sediments, rather than water, would generally be more appropriate matrices for monitoring. Yet sampling of sediments and quantification of residues of these pesticides is often lacking. Similarly, there have been few studies examining the toxicity of mixtures of these compounds in sediment. The first goal of this research was to examine the effects of select triazine herbicides on organophosphate insecticide toxicity utilizing water-only toxicity test with the aquatic amphipod Hyalella azteca. The second goal was to analyze an existing database of chemical concentrations using a toxicity-based screening approach in order to estimate the environmental hazard posed by mixtures of pyrethroid, organochlorine, and organophosphate insecticides in sediment to H. azteca. The third goal of this research was to examine the toxic effects of mixtures of different pyrethroid insecticides to H. azteca using compounds identified as most relevant from the screening phase of the study. The fourth goal of this research was to examine how pyrethroid and organochlorine insecticides partition between different size fractions within sediment and detritus, as well as between sediments with differing organic carbon content, and the resulting effects to compound toxicity and bioavailability. The final goal of this research was to examine potential modifications to

bifenthrin sediment partitioning, toxicity, and bioaccessibility resulting from various dissolved salt concentrations in overlying water using H. azteca and Chironomus dilutus as reference organisms. Together, the individual objectives of this study provide a thorough and multi-tiered approach to determining the occurrence, environmental fate, biological effects, and bioavailability of frequently detected and co-occurring environmental contaminants in both agricultural and urban landscapes.

Trimble, A. J.; Weston, D. P.; Belden, J. B., and Lydy, M. J. Identification and Evaluation of Pyrethroid Insecticide Mixtures in Urban Sediments. 2009; 28, (8): 1687-1695.

Abstract: Organochlorine, organophosphorous, and pyrethroid insecticides frequently have been detected together as mixtures in stream sediments. To simplify mixture analyses, additive toxic responses usually are assumed but rarely are confirmed, especially for compounds with similar modes of action. The first objective of the present study was to screen a database of 24 different pesticides and 94 urban-stream sediment samples collected throughout central and northern California (USA) to identify compounds and partial mixtures that dominated sample toxicity to *Hyalella azteca*. Pyrethroids and chlorpyrifos were the most lexicologically relevant compounds in terms of detection frequency, contribution to overall sample toxicity, and co-occurrence in the most common mixture patterns. Organochlorine insecticides were the least toxicologically relevant compounds, with only a small percentage of samples exceeding predefined screening values. The second objective was to confirm that mixtures of type I and type II pyrethroids display additive responses. Ten-day sediment toxicity tests of binary pesticide mixtures were conducted using *H. azteca* as the test organism. Observed dose-response curves were compared to those predicted from concentration-addition and independent-action models. Model deviation ratios (MDRs) were calculated at the median effect level to quantify the magnitudes of deviation between observed and predicted curves. Whereas the concentration-addition model adequately predicted toxicity for all the pyrethroid mixtures (MDRs within a factor of two), dose-response values deviated from additivity enough to warrant further investigation.

Tripathi, A. M. and Agarwal, R. A. Synergism in Tertiary Mixtures of Pesticides. MORWATER,AQUA; 1997; 35, (10): 2365-2374.

Abstract: Two compounds, a pyrethroid, decis and a carbamate sevin both effective molluscicides against the snail demonstrate synergistic activity when mixed in a 1:46 ratio (decis:sevin). The observations show that the LC values decreases sharply. For example, the 96h $LC_{10}$, $LC_{50}$ and $LC_{90}$ of decis decreases 272.72, 112.82 and 10.92 times respectively. When the esterase inhibitor MGK-264 is mixed with the binary mixture in a 1:46:5 ratio (decis:sevin:MGK) it was found that the lethality of the tertiary mixture was much higher than the binary mixture of decis and sevin. It seems that sevin and MGK-264, both being esterase inhibitors prevent the deactivation of the decis in the body of the snail and thereby increase the toxicity of the latter.

Tungu, P.; Magesa, S.; Maxwell, C.; Malima, R.; Masue, D.; Sudi, W.; Myamba, J.; Pigeon, O., and Rowland, M. Evaluation of Permanet 3.0 A Deltamethrin-Pbo Combination Net Against Anopheles Gambiae and Pyrethroid Resistant Culex Quinquefasciatus Mosquitoes: an Experimental Hut Trial in Tanzania.

BACKGROUND: Combination mosquito nets incorporating two unrelated insecticides or insecticide plus synergist are designed to control insecticide resistant mosquitoes. PermaNet 3.0 is a long-lasting combination net incorporating deltamethrin on the side panels and a mixture of deltamethrin and synergist piperonyl butoxide (PBO) on the top panel. PBO is an inhibitor of mixed function oxidases implicated in pyrethroid resistance.

ABSTRACT: METHOD: An experimental hut trial comparing PermaNet 3.0, PermaNet 2.0 and a conventional deltamethrin-treated net was conducted in NE Tanzania using standard WHOPES procedures. The PermaNet arms included unwashed nets and nets washed 20 times. PermaNet 2.0 is a long-lasting insecticidal net incorporating deltamethrin as a single active.

RESULTS: Against pyrethroid susceptible Anopheles gambiae the unwashed PermaNet 3.0 showed no difference to unwashed PermaNet 2.0 in terms of mortality (95% killed), but showed differences in blood-feeding rate (3% blood-fed with PermaNet 3.0 versus 10% with PermaNet 2.0). After 20 washes the two products showed no difference in feeding rate (10% with 3.0 and 9%

with 2.0) but showed small differences in mortality (95% with 3.0 and 87% with 2.0). Against pyrethroid resistant Culex quinquefasciatus, mediated by elevated oxidase and kdr mechanisms, the unwashed PermaNet 3.0 killed 48% and PermaNet 2.0 killed 32% but after 20 washes there was no significant difference in mortality between the two products (32% killed by 3.0 and 30% by 2.0). For protecting against Culex PermaNet 3.0 showed no difference to PermaNet 2.0 when either unwashed or after 20 washes; both products were highly protective against biting. Laboratory tunnel bioassays confirmed the loss of biological activity of the PBO/deltamethrin-treated panel after washing.
CONCLUSION: Both PermaNet products were highly effective against susceptible Anopheles gambiae. As a long-lasting net to control or protect against pyrethroid resistant mosquitoes PermaNet 3.0 showed limited improvement over PermaNet 2.0 against Culex quinquefasciatus.

Vastrad, A. S.; Lingappa, S., and Basavanagoud, K. Vegetable Oils as Synergists of Synthetic Pyrethroids Against Diamondback Moth, Plutella xylostella L. (Yponomeutidae: Lepidoptera).
MORENV,MIXTURE; 2002; 26, (4): 285-290.
Abstract: A study was conducted to determine the effect of vegetable oils (from cotton (*Gossypium* sp.), linseed (*Linum usitatissimum*), safflower (*Carthamus tinctorius*), pundi (*Hibiscus cannabinus*), honge (*Pongamia pinnata*) and sesame (*Sesamum indicum*)) as synergists of synthetic pyrethroids (fenvalerate, deltamethrin and cypermethrin) against *P. xylostella* during 1997-99 in Dharwad, Karnataka, India. Among the oils screened, sesamum, honge and linseed oils greatly synergized fenvalerate. Honge oil alone at 0.3 and 0.4% showed significant insecticidal activity than honge oil at 0.2%. Sesamum oil (0.2%) recorded the highest larval mortality of 81.50 and 99.99% at 24 and 48 h, respectively, and was significantly superior to other oils. The larval mortality with honge and sesamum oils in combination with synthetic pyrethroids increased with increasing rate and interval of observation. At the shortest interval of 24 h, honge oil and sesamum oil at 0.2% synergized all the pyrethroids tested. Both sesamum and honge oils at 0.2% can be utilized to prolong the efficacy of pyrethroids.

Vijayan, V. A.; Sathish Kumar, B. Y.; Ganesh, K. N.; Urmila, J.; Fakoorziba, M. R., and Makkapati, A. K. Efficacy of Piperonyl Butoxide (PBO) as a Synergist with Deltamethrin on Five Species of Mosquitoes.
MORWATER, AQUA; 2007; 39, (3): 159-163.
Abstract: Development of insecticide resistance has been a challenging problem for a long time and new solutions are yet to emerge. In this regard, the use of synergist with the insecticide is thought to play a key role in reducing the resistance levels. Present study demonstrates the efficacy of PBO with deltamethrin against the field collected mosquito larvae of five species of Aedes, Anopheles and Culexfrom in and around Mysore.

Weston, D. P.; Amweg, E. L.; Mekebri, A.; Ogle, R. S., and Lydy, M. J. Aquatic Effects of Aerial Spraying for Mosquito Control over an Urban Area. 2006; 40, 5817-5822.
Abstract: In an effort to combat West Nile Virus, planes dispersed insecticide over Sacramento, CA, treating nearly 50,000 hectares with pyrethrins and the synergist piperonyl butoxide (PBO). Widespread dispersal of insecticide over a metropolitan area, coupled with extensive pretreatment data on the area's urban creeks, provided a unique opportunity to study effects of mosquito control agents on aquatic habitats within an urban setting. There was no evidence of aquatic toxicity from the two active ingredients in the product applied. However, PBO concentrations were high enough to enhance toxicity of pyrethroids already existing in creek sediments from general urban pesticide use. PBO concentrations of 2-4 ug/L were high enough to nearly double the toxicity of sediments to the amphipod Hyalella azteca. Though the increase in toxicity was modest, it was unexpected to find environmental synergy at all. Risk assessments for mosquito control agents have focused on the active ingredients but have failed to recognize the potential for interactions with pesticides previously existing in the environment, which in this case appeared to represent a risk to aquatic life greater than that of the active ingredients themselves.

Wolansky, M. J.; Gennings, C.; DeVito, M. J., and Crofton, K. M. Evidence for Dose-Additive Effects of Pyrethroids on Motor Activity in Rats. Departamento de Quimica Biologica (Area Toxicologia), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria,

Buenos Aires, Argentina//: 2009; 117, (10): 1563-1570.
Abstract: Background: Pyrethroids are neurotoxic insecticides used in a variety of indoor and outdoor applications. Previous research characterized the acute dose–effect functions for 11 pyrethroids administered orally in corn oil (1 mL/kg) based on assessment of motor activity. Objectives: We used a mixture of these 11 pyrethroids and the same testing paradigm used in single-compound assays to test the hypothesis that cumulative neurotoxic effects of pyrethroid mixtures can be predicted using the default dose–addition theory.
Methods: Mixing ratios of the 11 pyrethroids in the tested mixture were based on the ED30 (effective dose that produces a 30% decrease in response) of the individual chemical (i.e., the mixture comprised equipotent amounts of each pyrethroid). The highest concentration of each individual chemical in the mixture was less than the threshold for inducing behavioral effects. Adult male rats received acute oral exposure to corn oil (control) or dilutions of the stock mixture solution. The mixture of 11 pyrethroids was administered either simultaneously (2 hr before testing) or after a sequence based on times of peak effect for the individual chemicals (4, 2, and 1 hr before testing). A threshold additivity model was fit to the single-chemical data to predict the theoretical dose–effect relationship for the mixture under the assumption of dose additivity.
Results: When subthreshold doses of individual chemicals were combined in the mixtures, we found significant dose-related decreases in motor activity. Further, we found no departure from the predicted dose-additive curve regardless of the mixture dosing protocol used.
Conclusion: In this article we present the first *in vivo* evidence on pyrethroid cumulative effects supporting the default assumption of dose addition.

Yang, Y.; Wu, Y.; Chen, S.; Devine, G. J.; Denholm, I.; Jewess, P., and Moores, G. D. The Involvement of Microsomal Oxidases in Pyrethroid Resistance in Helicoverpa armigera from Asia. BCM, MOR MIXTURE,TOP; 2004; 34, (8): 763-773.
Abstract: Five contemporary strains of the bollworm *Helicoverpa armigera* Hübner from China, Pakistan and India, all with high resistance to pyrethroids, were compared with a standard susceptible strain that originated from the Cote D'Ivoire in the 1970s ('SCD'). Two of the Chinese strains ('YGF' and 'YGFP') were derived by laboratory selection from a third, field collected strain ('YG'). The strain 'YG' exhibited 7-, 14- and 21-fold resistance to fenvalerate, cypermethrin and deltamethrin, respectively. After selection with fenvalerate for 14 generations ('YGF'), this increased to 1690-, 540- and 73-fold. Selection with a mixture of fenvalerate and piperonyl butoxide (PBO) for 14 generations ('YGFP') resulted in resistance ratios of 2510, 2920 and 286. The synergistic ratios to fenvalerate that resulted from pre-treatment of PBO were 5-, 462- and 12-fold in YG, YGF and YGFP strains, respectively. Resistance ratios for a Pakistani strain (PAK) were 2320-, 4100- and 223-fold to fenvalerate, cypermethrin and deltamethrin, respectively. The synergistic ratio of PBO to these pyrethroids was 450-, 950- and 11-fold. The strong synergism of pyrethroids by PBO implied that an oxidative metabolism could be involved in pyrethroid resistance in these resistant strains. The activities of cytochrome P450 monooxygenases from midguts of final instar larvae to *p*-nitroanisole (PNOD), ethoxycoumarin (ECOD), methoxyresorufin (MROD) significantly increased in all the resistant strains when compared with the susceptible strain. This further implies that cytochrome P450 monooxygenases are involved in pyrethroid resistance in Asian *H. armigera*. Comparative in vitro studies of the metabolism of $^{14}$C-deltamethrin by midgut microsomes of the resistant PAK and susceptible SCD strains showed that the resistant strain had a much greater capacity than the susceptible strain for the metabolic degradation of deltamethrin. This enhanced metabolic degradation occurred in the presence of NADPH which suggested an oxidative detoxification. In the resistant strains, minor increases in glutathione *S*-transferase activity (to the substrates CDNB and DCNB), and esterase activity (to the substrate α-naphthyl acetate) further suggested that, of the putative metabolic mechanisms, oxidases are the most important. This study provides the first evidence that cytochrome P450 monooxygenases are a major metabolic mechanism responsible for pyrethroid resistance in *H. armigera* from Asia.

Yavuz, O.; Aksoy, A.; Das, Y. K.; Gulbahar, M. Y.; Yarim, G. F.; Cenesiz, M.; Atmaca, E., and Guvenc, D. Repeated-Dose 14-Day Dermal Toxicity of Different Combinations of Some Synthetic Pyrethroid Insecticides, Piperonyl Butoxide, and Tetramethrin in Rats. BCM,BEH,CEL,GRO,MOR.

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Abstract: The aim of this study was to evaluate the repeated-dose 14-day dermal toxicity of different combinations of some synthetic pyrethroid insecticides, piperonyl butoxide, and tetramethrin in rats. A total of 70 adult Wistar rats were randomly divided into 7 (6 experimental and 1 control) groups. Different combinations of insecticides were dermally applied to the rats in the experimental groups for 14 days. Clinical observations were performed daily; hematologic and biochemical parameters were also determined. Gross necropsy and histopathologic examinations were performed systematically, and organ weights were recorded. Although the administered doses of the insecticides were relatively lower than their acute dermal toxicity values, a high mortality rate (27 of 60 experimental animals, 45%) was observed. Furthermore, the insecticide combinations caused decreased body weights and feed consumptions, increased organ weights, and hematologic, biochemical, and common histopathologic changes. As a result, the findings showed that although pyrethroids are considered to be of low acute toxicity, they become more toxic when combined with piperonyl butoxide or tetramethrin in certain doses.