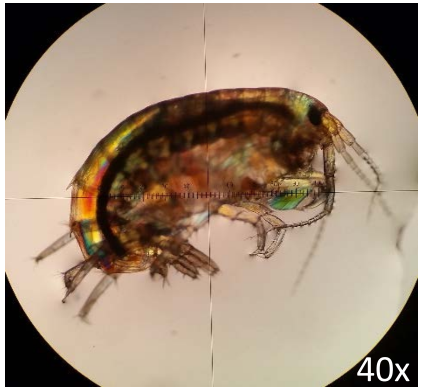

Toxicity, bioaccumulation and trophic transfer of permethrin in pyrethroid-resistant *Hyalella azteca*

Michael Lydy[1] , Kara Huff Hartz,[1] Samuel Nutile,[1] Andrew Derby,[1] Jennifer Heim,[1]
Leslie Muggelberg,[2] Amanda Harwood,[3] Helen Poynton,[4] Kaley Major,[4] Donald Weston[5]

[1]Southern Illinois University Carbondale, Carbondale, IL
[2]College of Albany, Albany, NY
[3]Alma College, Alma, MI
[4]University of Massachusetts, Boston, MA
[5]University of California, Berkeley, CA

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Mosher Slough – Stockton, CA

D-clade resistant
cultured since December, 2013

U.S. EPA Lab – Duluth, MN

C-clade non-resistant
cultured since 2001

Controversy – Adaptation or Acclimation?

SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 34, No. 10, pp. 2250–2262, 2015

Environmental Toxicology

COMPARATIVE SENSITIVITY OF FIELD AND LABORATORY POPULATIONS OF *HYALELLA AZTECA* TO THE PYRETHROID INSECTICIDES BIFENTHRIN AND CYPERMETHRIN

Stephen L. Clark,*† R. Scott Ogle,† Andrew Gantner,† Lenwood W. Hall Jr.,‡ Gary Mitchell,§ Jeffrey Giddings,|| Matthew McCoole,# Michael Dobbs,†† Kevin Henry,‡‡ and Ted Valenti‡‡
†Pacific EcoRisk, Fairfield, California, USA
‡University of Maryland, Queenstown, Maryland, USA
§FMC Agricultural Products, Ewing, New Jersey, USA
||Compliance Services International, Rochester, Massachusetts, USA

- Clark et al. reported LC50 values 49 and 300 times greater for cypermethrin and bifenthrin in field-collected *Hyalella* compared to lab populations
- Lost partial tolerance over time – attributed to non-genetic enzyme activity
- Did not measure genetic VGSC mutations as noted in Weston et al. (2014)

Research Questions

- Is resistance maintained when animals are cultured long-term in a pyrethroid-free environment?
  - Permethrin LC_{50} over time
  - Genotyping the L925 locus
- How much permethrin will pyrethroid-resistant *H. azteca* bioaccumulate?
- Will pyrethroid-resistant *H. azteca* biotransform permethrin?
- Is there potential for transfer of permethrin to higher trophic organisms via consumption of resistant *H. azteca*?

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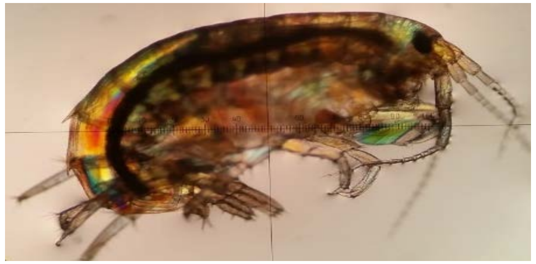
Toxicity Bioassays

- RES *Hyalella* tested after 14, 22 and 30 months in culture
- NR *Hyalella* also tested
- EPA protocol 600/R-99/064
- 96-h static water-only exposures to permethrin
- Lethality endpoint
- Probit analysis to determine LC50



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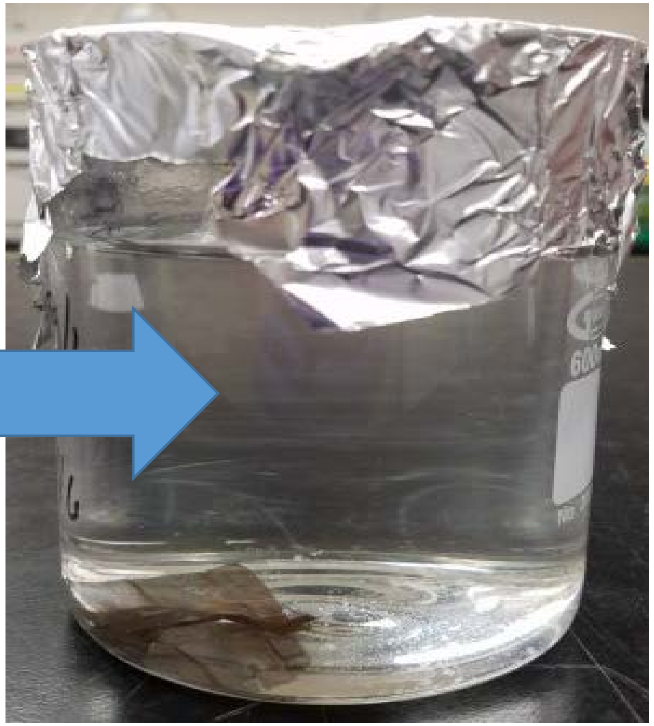
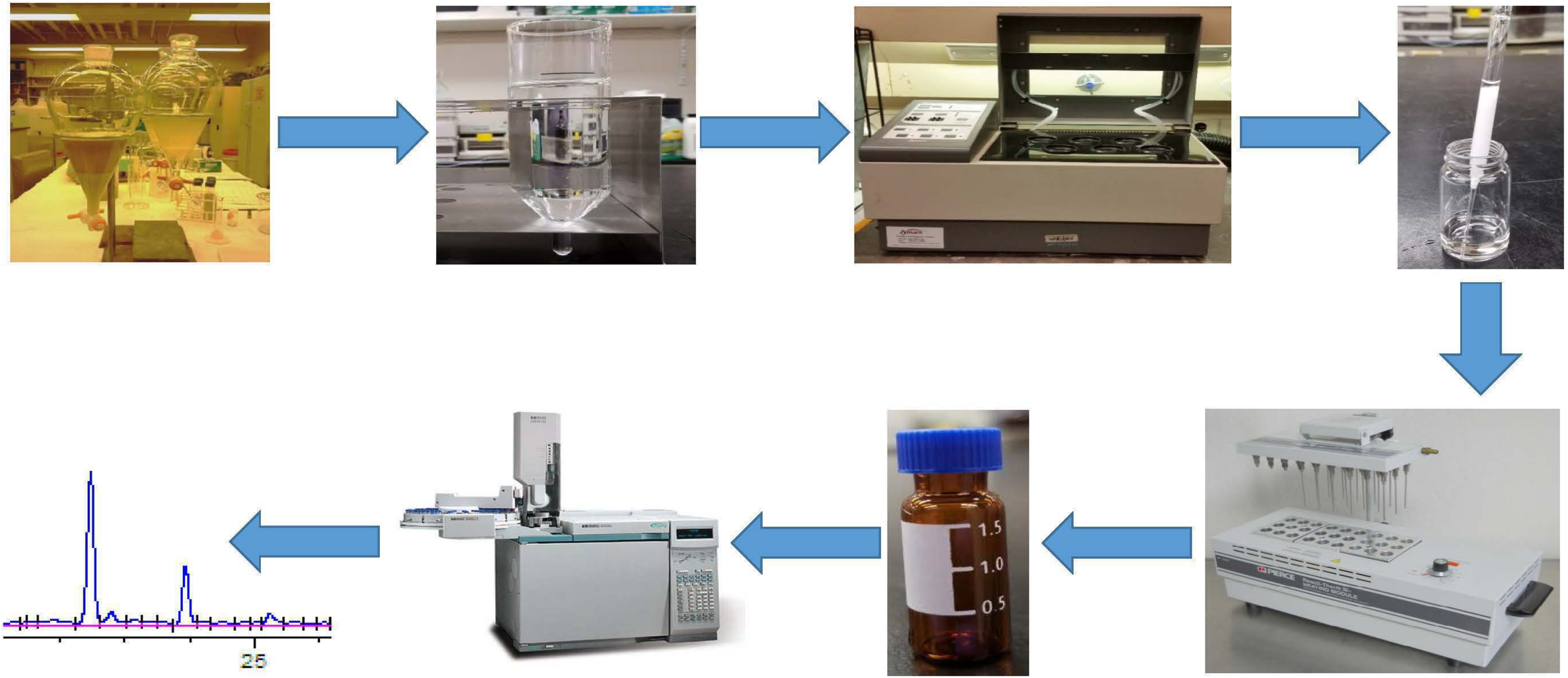


Liquid-Liquid Extraction

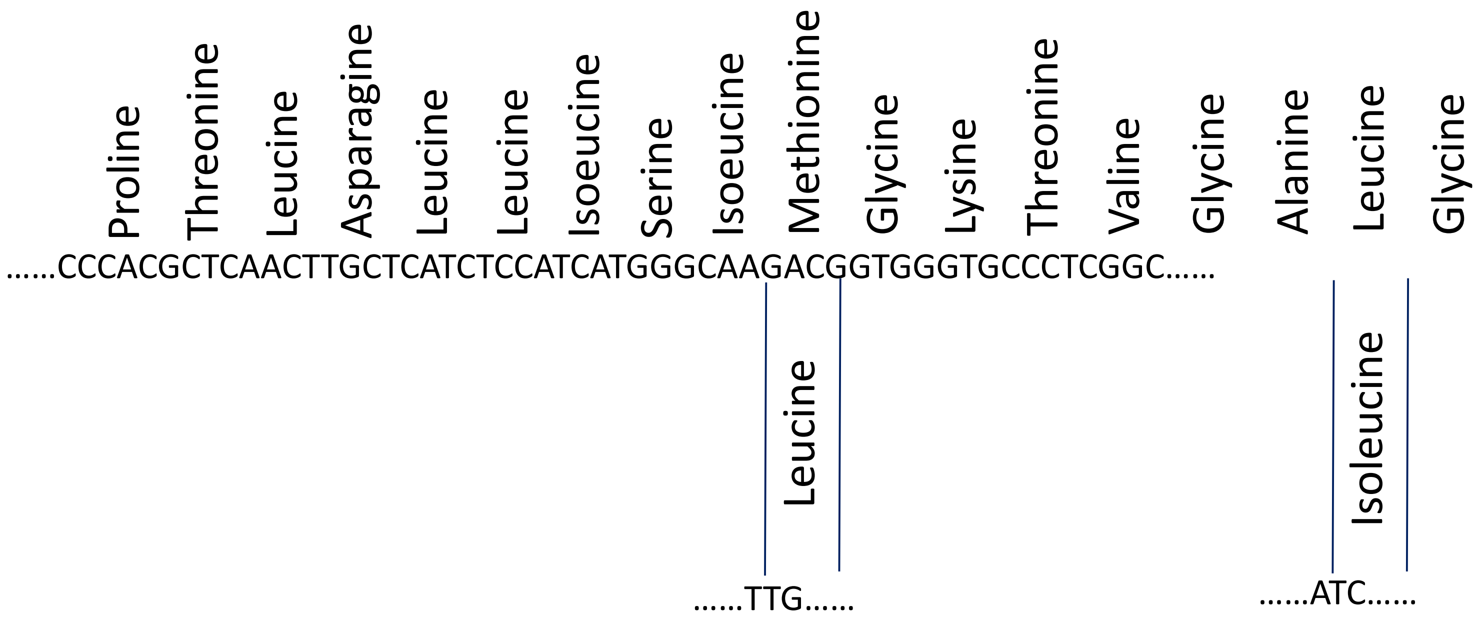


Population	Test Date	LC50 (95% Confidence Intervals)
Non-Resistant	June 2015	34.5 ng/L (31.3 – 38.3)[a]
Non-Resistant	October 2015	31.2 ng/L (26.4 – 36.9)[a]
Resistant	July 2015 (14 months in culture)	1140 ng/L (942 – 1390)[b]
Resistant	February 2016 (22 months in culture)	1670 ng/L (1380 – 2010)[b]
Resistant	October 2016 (30 months in culture)	1418 ng/L (1269 - 1585)[b]

- Approximately 53-fold decreased sensitivity to permethrin that is retained over time.
- So was this result due to the mutation being maintained in the population ?

<u>YES….Resistant *Mosher Slough* population retained the L925 mutation after 30 months !</u>

A portion of chromosome 3, and the encoded amino acid sequence for the voltage gated sodium channel:



918 – Well known mutation in insects known as super-kdr "super-knock down resistance"

925 – Mutation previously found in white fly

The wild-type leucine was replaced with an isoleucine (L925I) or valine (L925V)

Conclusions

- **Is resistance maintained when animals are cultured long-term in a pyrethroid-free environment?**
  - **Permethrin LC_{50} over time**
  - **Genotyping the L925 locus**

- Yes, Mosher Slough *H. azteca* maintain pyrethroid resistance when cultured in a pyrethroid-free environment (LC_{50} at 30 months ~ 53 times non-resistant animals) **Adaptation**
- Mosher Slough *H. azteca* are permanently genetically altered (presence of L925I and L925V) **Adaptation**

Research Questions

- Is resistance maintained when animals are cultured long-term in a pyrethroid-free environment?
  - Genotyping the L925 locus
  - Permethrin LC_{50} over time
- **How much permethrin will pyrethroid-resistant *H. azteca* bioaccumulate?**
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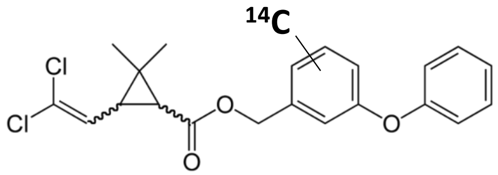
Methods



^{14}C-labeled permethrin

55% cis/45% trans

Water Exposure,
7-14 d old *H. azteca*

Tissue Homogenization

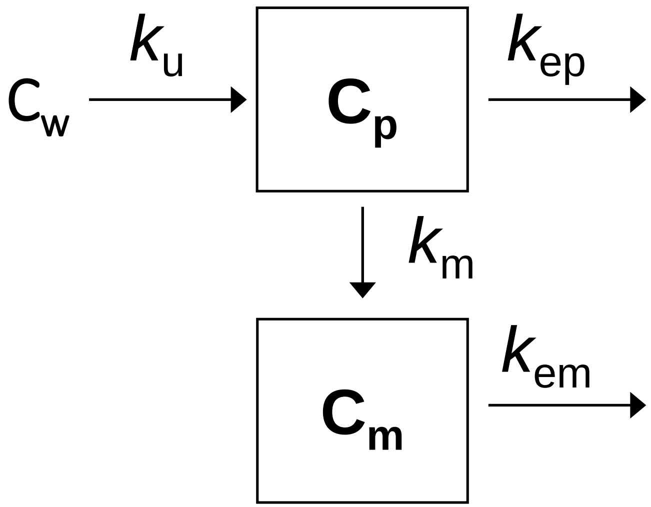
Subsample of tissue

Packard TriCarb 2900TR LSC

Separate fractions for parent permethrin and biotransformation products

Agilent 1100 HPLC equipped with fraction collector

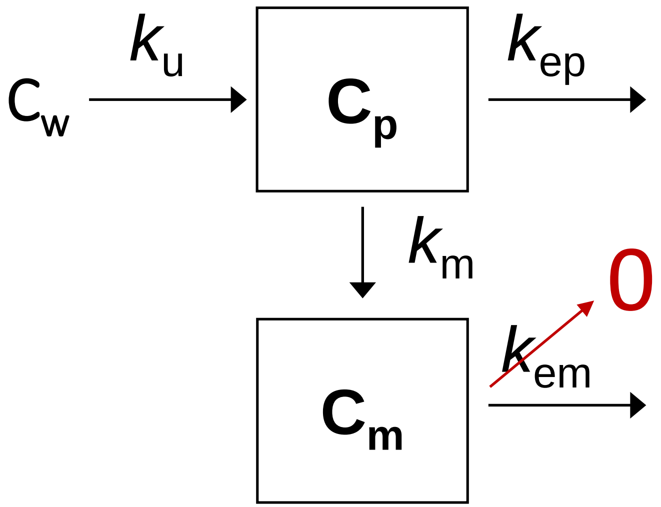
Time to Steady-State Using Toxicokinetics



$$t_{1/2} = \frac{\ln 2}{k_{ep} + k_m}$$

Two-compartment model for *H. azteca* permethrin exposure
C = concentration in water (w) or in *H. azteca* tissue
p = parent permethrin   m = permethrin biotransformation products
k= rate coefficient/ constant for u = uptake or e = elimination

Time to Steady-State Using Toxicokinetics

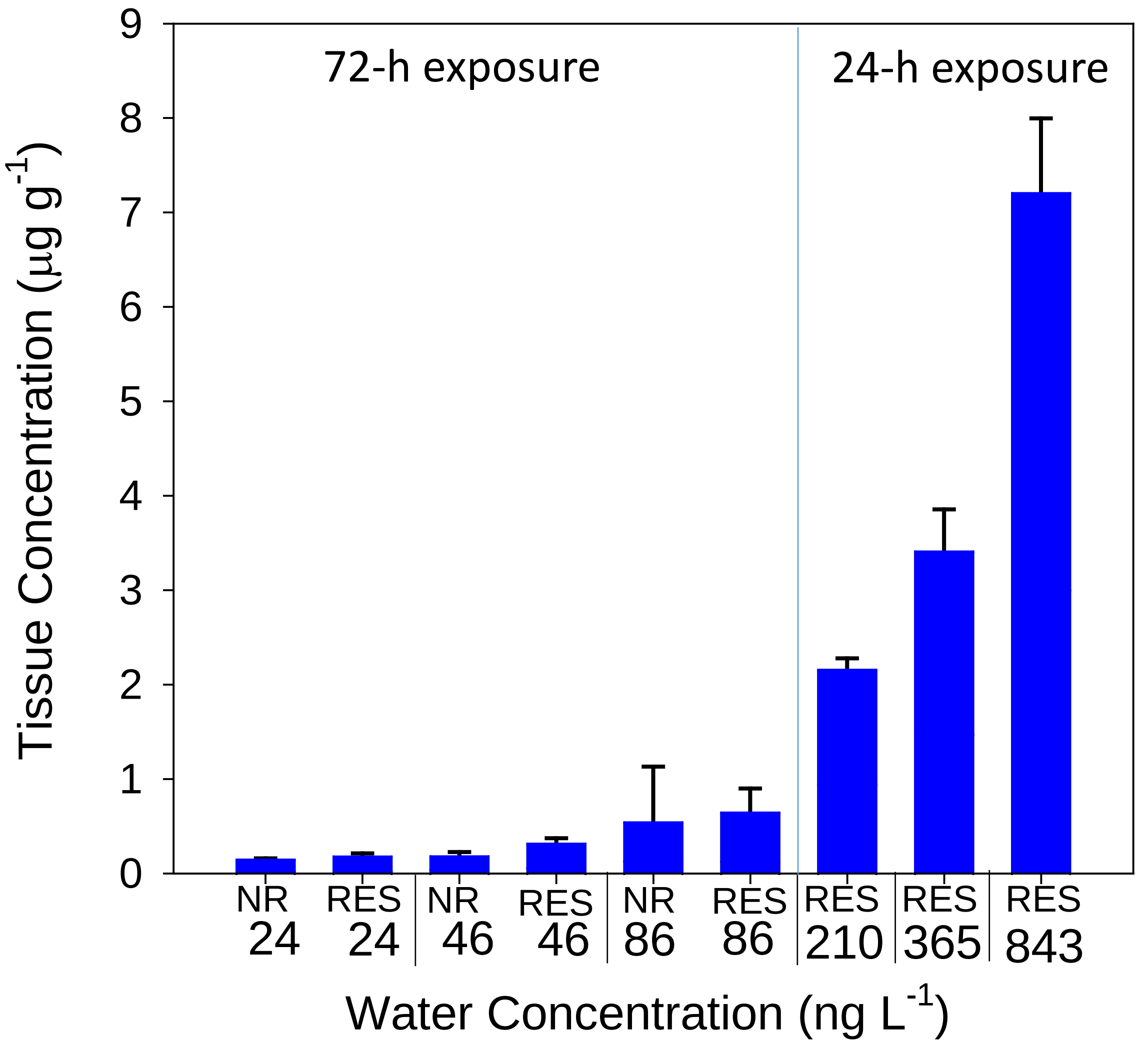


$$t_{1/2} = \frac{\ln 2}{k_{ep} + k_m}$$

Population	5 $t_{1/2}$
Resistant *H. azteca*	17.4 h
Non-resistant *H. azteca*	33.2 h

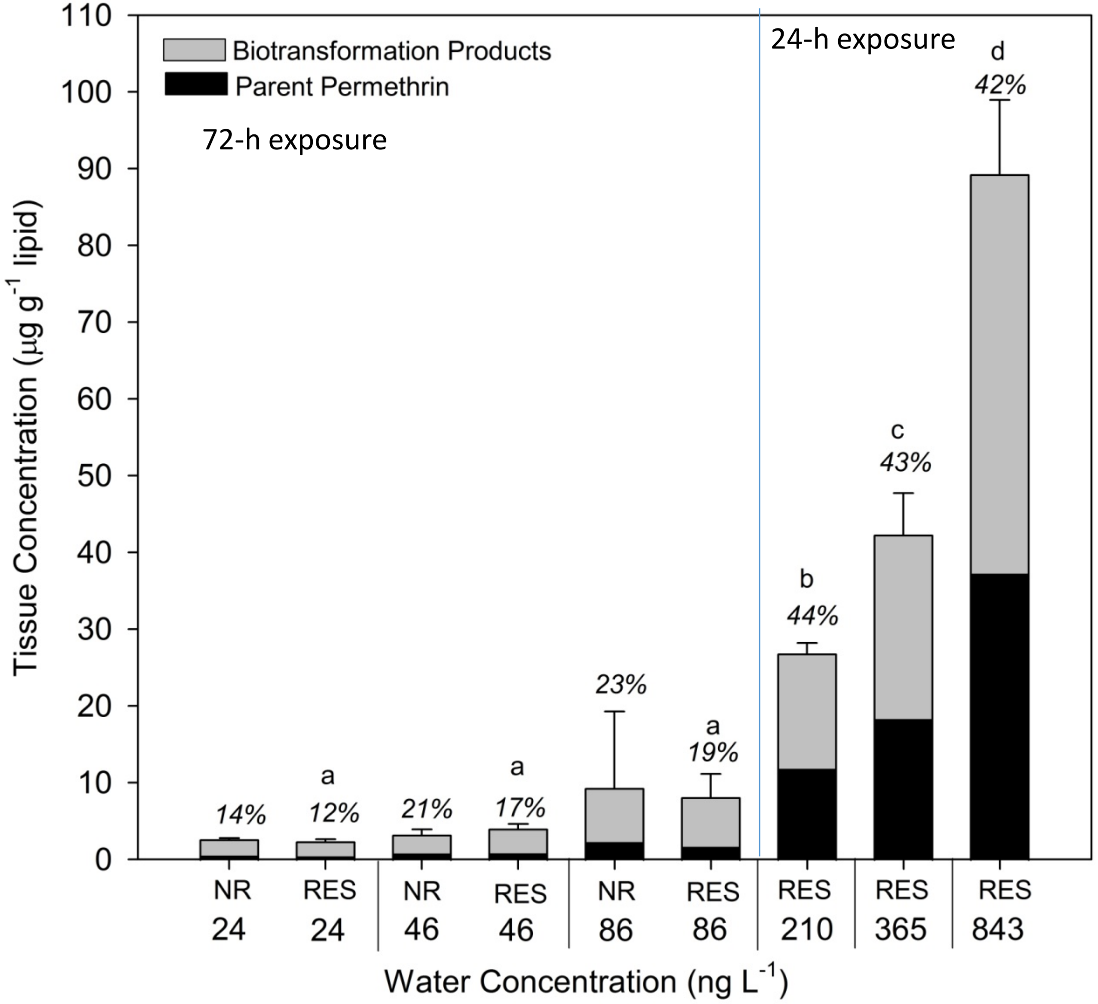
Two-compartment model for *H. azteca* permethrin exposure
C = concentration in water (w) or in *H. azteca* tissue
p = parent permethrin   m = permethrin biotransformation products
k= rate coefficient/ constant for u = uptake or e = elimination

Permethrin Bioaccumulation



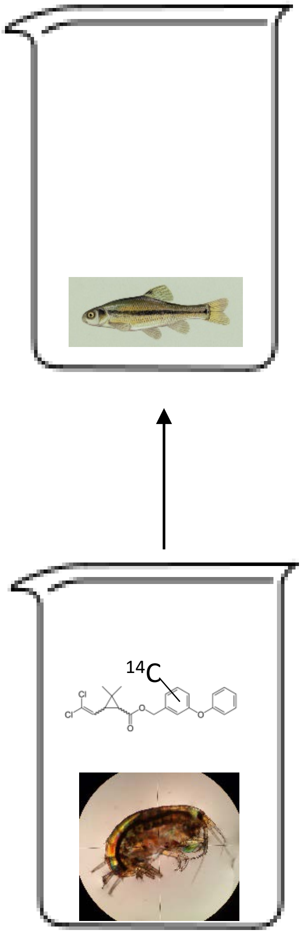
- Similar bioaccumulation of total permethrin in non-resistant (NR) and resistant (RES) *H. azteca*.
- RES *H. azteca* bioaccumulation increased as the permethrin exposure concentration increased.

Permethrin Biotransformation



- Similar biotransformation proportions were noted in non-resistant (NR) and resistant (RES) *H. azteca*
- As the exposure concentration increased, proportion of permethrin biotransformed decreased.

Potential for Permethrin Trophic Transfer

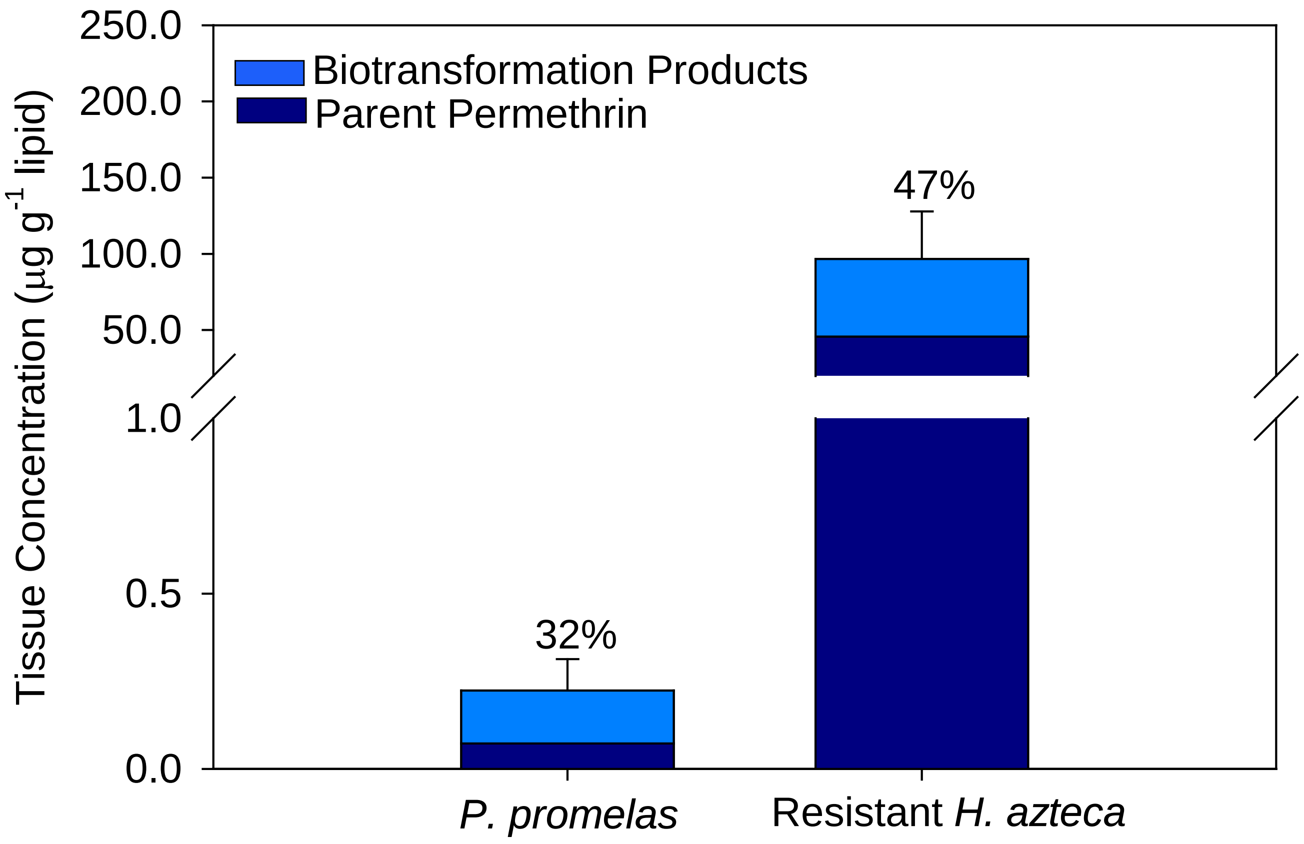


- *Pimephales promelas* fed for 4 days
- Static renewal system

- 15 *Hyalella* fed to each fish/day

- 15 *H. azteca*
- Water spiked at 780 ng/L
- Exposed for 24 h

Potential for Permethrin Trophic Transfer



Conclusions

- We found detectable concentrations of permethrin and biotransformation products in fatheads fed resistant-dosed *Hyalella.*
- Transfer could be parent, biotransformation products or both.
- These body residues are most likely underestimating bioaccumulation potential at steady state.
- Exposure is expected to be even greater if other sensitive species are not able to tolerate the elevated pyrethroid exposures noted at sites…. So fish and birds are more heavily relying on resistant *Hyalella* as a food source.

Take Home Messages

- **Adaptation** has occurred in *Hyalella* due to exposure to terrestrially-applied pyrethroids and this has been confirmed with toxicity and genetic testing.

- Non-resistant *H. azteca* cannot survive high permethrin exposure, thus pyrethroid resistance in *H. azteca* **increases the relative risk** of permethrin trophic transfer.

- Pyrethroid-resistant *H. azteca* increase fish exposure to permethrin and its biotransformation products, by adding an **additional route of exposure**.
  - Especially to fish and birds that depend on *H. azteca* as a food source.
  - Especially to fish and other species with low biotransformation potential.
  - Increased risk of altered sex ratios, & feminization of male fish which could result in **population declines** as estrogenic biotransformation products accumulate.

Environmental Pollution xxx (2016) 1–8

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Do pyrethroid-resistant *Hyalella azteca* have greater bioaccumulation potential compared to non-resistant populations? Implications for bioaccumulation in fish☆

Leslie L. Muggelberg [a, 1], Kara E. Huff Hartz [a], Samuel A. Nutile [a], Amanda D. Harwood [b], Jennifer R. Heim [a], Andrew P. Derby [a], Donald P. Weston [c], Michael J. Lydy [a, *]

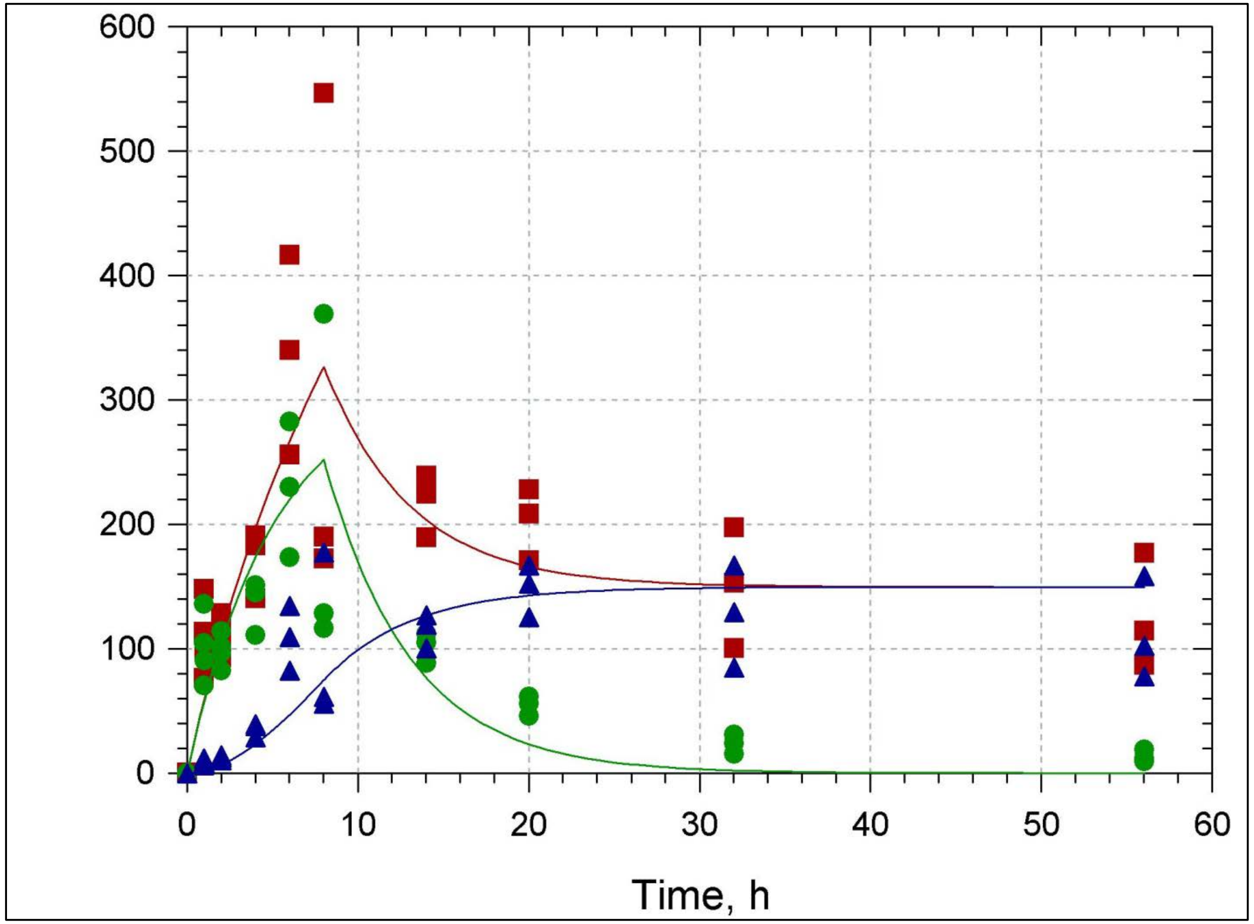
[a] *Center for Fisheries, Aquaculture and Aquatic Sciences and Department of Zoology, Southern Illinois University, Carbondale, IL 62901, USA*
[b] *Biology and Environmental Studies, Alma College, Alma, MI 48801, USA*
[c] *Department of Integrative Biology, University of California, Berkeley, CA 94720, USA*

Please cite this article in press as: Muggelberg, L.L., et al., Do pyrethroid-resistant *Hyalella azteca* have greater bioaccumulation potential compared to non-resistant populations? Implications for bioaccumulation in fish, Environmental Pollution (2016), http://dx.doi.org/10.1016/j.envpol.2016.09.073

Impacts of pyrethroid exposure to fish

- LC_{50} values in the low µg/L range and more toxic at lower temps
- Pyrethroids effect swimming behavior and other behavioral endpoints
- DeGroot and Brander (2014) found that pyrethroid biotransformation products were the main contributors to estrogenic effects in fish
- 3-phenoxybenzyl alcohol and 3-(4’-hydroxyphenoxy)-benzyl alcohol mimic the interaction of 17-β-estradiol with estrogen receptors (Nillos et al. 2010)

Extra Slide 1: Toxicokinetics experiment



Data were fit to two-compartment model using an iterative least-squares procedure using the fourth-order Runge-Kutta approach in the software package Scientist 2.0 (Lydy et al. 2000).

Figure 3. Total permethrin (red squares), parent permethrin (green circles), and metabolites (blue triangles) concentrations in the resistant *H. azteca* over time. Organisms were transferred to undosed water at 8 h for the depuration phase. The r^2 values and coefficients of determination (CoD) were as follows: Total permethrin concentration: r^2 = 0.88, CoD = 0.53; parent permethrin concentration: r^2 = 0.85, CoD = 0.61; metabolite concentration: r^2 = 0.85, CoD = 0.61. The values for k_{em} were not significantly different than zero, thus k_{em} was fixed to zero.

Extra Slide 2: Rate Constants

	k_u mL(water) g(tissue)$^{-1}$ h^{-1}	k_m h^{-1}	k_{ep} h^{-1}
Resistant *H. azteca*	419 ± 41 (340 - 500)	**(5.92 ± 0.85) x 10^{-2}** (4.2 x 10^{-2} – 7.6 x 10^{-2})	**(1.40 ± 0.31) x 10^{-1}** (0.78 x 10^{-1} – 2.0 x 10^{-1})
Non-resistant *H. azteca*	**531 ± 36** (460-600)	(3.98 ± 0.51) x 10^{-2} (3.0 x 10^{-2} – 5.0 x 10^{-2})	(0.64 ± 0.12) x 10^{-1} (0.41 x 10^{-1} – 0.88 x 10^{-1})
P-value	0.0397	0.0505	0.0217

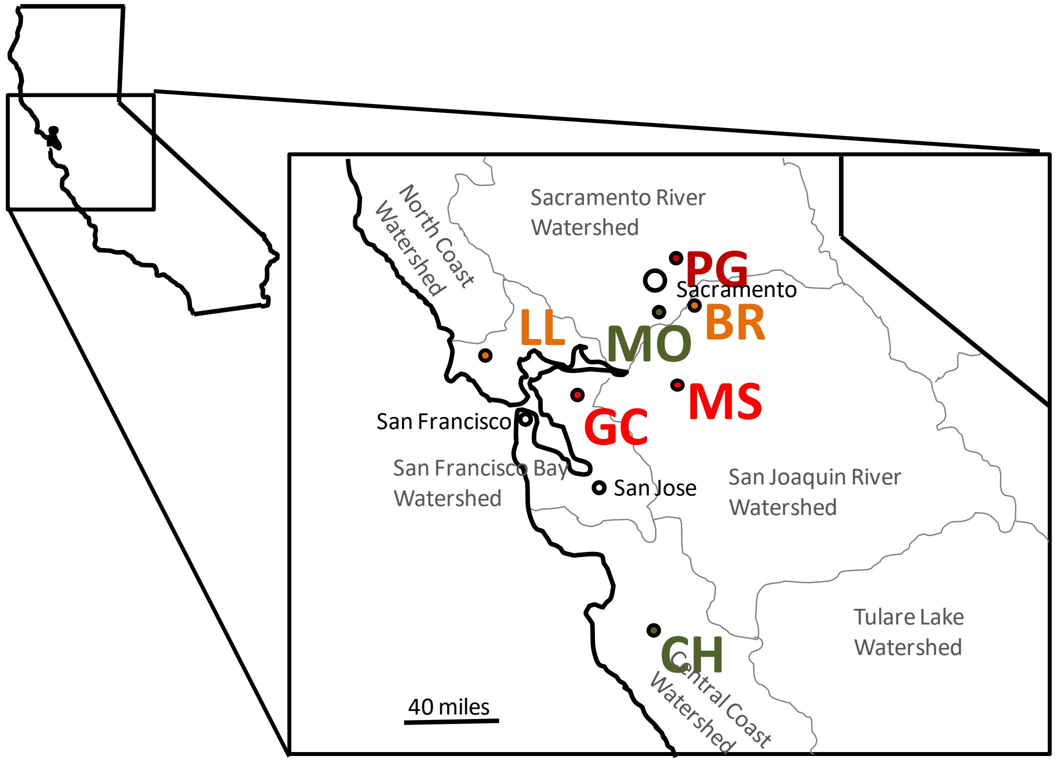
P-value calculated using two sample z-test (Schuler *et al*. 2003)

Extra Slide 3: Lipid Normalized Permethrin Tissue Concentrations[a]

Permethrin Exposure Concentration, ng/L	Total Permethrin in tissue, µg/g wet weight (standard deviation)	Total Permethrin in tissue, µg/g lipid (standard deviation)
24 NR[b]	0.147 (0.025)	2.34 (0.59)
24 Res[c]	0.179 (0.045)	1.68 (0.43)
46 NR[b]	0.182 (0.057)	2.89 (0.91)
46 Res[c]	0.315 (0.069)	2.95 (0.65)
86 NR[b]	0.541 (0.602)	8.90 (9.58)
86 Res[c]	0.645 (0.266)	6.04 (2.49)
210 Res[d]	2.12 (0.13)	61.6 (3.8)
365 Res[d]	3.59 (0.46)	97.4 (13.1)
843 Res[d]	7.04 (0.80)	206 (23)

[a]n = 4, [b]lipid% = 6.3%, [c]lipid% = 10.7%, [d]lipid% = 3.5%

Pyrethroid-resistant *Hyalella azteca*



Locations of *H. azteca* sampling sites. *H. azteca* were collected from seven locations in N. California where sediment pyrethroid concentrations were known. Relative pyrethroid concentrations in sediments are indicated by text color: **Non-detect, Low, Medium, High.** Sediments with medium and high concentrations of pyrethroids were toxic to the laboratory strain of *H. azteca.*

Weston et al. (2013) PNAS

- Permethrin in water LC50 of the resistant population was approximately 53 times higher than that of the non-resistant *H. azteca*
  - Resistant: 1670 ng/L
  - Non-resistant: 31 ng/L