Report as of FY2006 for 2003CA57G: "Distribution and toxicity of sediment-associated pesticides in the Sacramento River watershed."

Publications

- Articles in Refereed Scientific Journals:
  - You, J. and M.J. Lydy. 2006. Determination of pyrethroid, organophosphate and organochlorine pesticides in water by headspace solid-phase microextraction. Inter J. Environ Anal. Chem. 86(6): 381-389.
  - Amweg, E.L., D.P. Weston, C.S. Johnson, J. You and M.J. Lydy. 2006. Effect of piperonyl butoxide on permethrin toxicity in the amphipod Hyalella azetca. Environ Toxicol Chem. 25(7): 1817-1825.
  - Amweg, E.L., D.P. Weston, J. You and M.J. Lydy. 2006. Pyrethroid insecticides and sediment toxicity in urban creeks from California and Tennessee, USA. Environ. Sci. Technol. 40: 1700-1706.
  - You, J., P.F. Landrum and M.J. Lydy 2006. Comparison of chemical approaches for assessing bioavailability of sediment-associated contaminants. Environ Sci Technol. 40: 6348-6353.
  - Weston, D.P., E.L. Amweg, A. Mekebri, R.S. Ogle and M.J. Lydy. 2006. Aquatic effects of aerial spraying for mosquito control over an urban area. Environ Sci Technol. 40: 5817-5822.
  - Amweg, E.L. and Weston, D.P. (In press) Whole sediment toxicity identification evaluation tools for pyrethroid pesticides: I. Piperonyl butoxide addition. Environ. Toxicol. Chem.

Report Follows

Problem and Research Objectives

This research project is an extension of our past studies in which we have proven the analytical feasibility of sediment analysis for pyrethroids, have shown them present in most sediment samples from agriculture-affected water bodies, and have shown pyrethroid-related toxicity to benthic invertebrates in many locations. The study involved far more work on pyrethroids, and particularly on more basic aspects of their toxicology. This study had the following objectives:

1. Conduct bioaccumulation and toxicokinetics studies that examine how pyrethroids are processed by benthic invertebrates.
2. Determine the potential enhancement of pyrethroid toxicity by piperonyl butoxide (PBO), a synergist included in some pesticide formulations and known to be present in surface waters.
3. Examine possible interactions between pyrethroids and organochlorines that may modify the expected independent toxicity of the compounds.
4. Collect sediment from urban creeks and analyze them for pyrethroids and toxicity.
5. Analyze native benthic invertebrates for tissue pyrethroid concentrations.
6. Run chronic toxicity tests on selected sediment samples collected as part of the PRISM project. Dr. Chris Ingersoll, a USGS Scientist located at the Columbia, MO laboratory, will conduct this testing.
7. Measure changes in bioavailability (toxicity tests) and aqueous desorption rates (Tenax beads) of pyrethroids in field-collected soils over time.

Bioaccumulation and toxicokinetics

Methods - Toxicokinetics is the study of the rate processes involved in uptake, distribution, metabolism and elimination of a toxic chemical in an organism. This information is critical when judging the potential for toxicity and bioaccumulation of chemicals, as well as the potential for trophic transfer of toxic substances to predators. This component of the project will determine toxicokinetic parameters such as sediment uptake clearance coefficients (k_s), elimination rate constants for both parent compound (k_{ep}) and metabolites (k_{em}), the biotransformation rate (k_m), biological half life ($t_{1/2}$) and bioaccumulation factors (BAF). Two invertebrate species that differ widely in biotransformation capabilities were used: Chironomus tentans and Lumbriculus variegates.

Findings and status – Previous studies have determined temperature directly influences pyrethroid toxicity. Therefore, we have added a component to examine the temperature dependency of the toxicokinetics. Compounds such as pyrethroids and some organochlorines (i.e., DDT) have decreased toxicity at higher temperatures. The opposite effect is observed in metabolically activated pesticides such as chlorpyrifos. The mechanism for these changes is not completely understood. One means of determining this mechanism is through toxicokinetics at two temperatures (13 and 23 °C). In water only exposures using the midge Chironomus tentans, the pyrethroid permethrin was found to have increased biotransformation at higher temperatures although uptake and elimination rates were not affected. This increase in biotransformation also decreases the bioaccumulation factor (BCF) in the organism. Biotransformation was not observed in Lumbriculus variegatus sediment exposures and uptake and elimination rates were

not altered by temperature. However in preliminary experiments with another pyrethroid, lambda-cyhalothrin, uptake rates were significantly higher at 23°C. While biotransformation was not measured due to low body residues, the concentration in the animal begins to reach steady state at the higher temperature implying biotransformation occurs. This suggests that biotransformation may be one of the underlying factors affecting the toxicity change for pyrethroids.

While the pyrethroids and the organochlorine DDT have a similar mode of action as well as a similar trend in toxicity with temperature, the mechanism for the effect of temperature may be different. Unlike the pyrethroids, neither DDT nor its metabolite DDE exhibited any biotransformation in water only experiments with C. tentans. Like lambda-cyhalothrin, an increase in temperature increased the uptake rates, because there is insufficient biotransformation that leads to an increase in BCF. So for DDT it may be toxicodynamic factors driving the toxicity alteration. In another class of compounds, the metabolically activated organophosphates, temperature has the opposite effect on toxicity with lower temperatures decreasing toxicity.

In similar experiments to those described above, chlorpyrifos uptake rates were significantly increased with temperature as was the biotransformation and elimination rates. For this compound, however, the decreased biotransformation at lower temperatures decreased the formation of the more toxic metabolite. So, similar to the pyrethroids, biotransformation may play an important role in the effects of temperature on toxicity. This data not only provides insight as to the mechanisms of these insecticides, but also may help to explain and validate toxicity identification evaluations (TIEs) for pyrethroids. The kinetics results have been included in two papers that will be submitted within about a month.

PBO synergy

Methods - Piperonyl butoxide (PBO) is a synergist used to enhance the insecticidal properties of pyrethroid insecticides. The function of PBO is to inhibit metabolic degradation of the insecticide by the target pest species. With metabolic detoxification inhibited or completely stopped, the toxic potency of the pyrethroid is increased. PBO is included in many pyrethroid pesticide formulations, and residues of the compound are detectable in surface waters of California. The demonstration of PBO in surface waters raises the possibility that PBO may be having the same synergistic effect on aquatic non-target species as on the pest for which was initially applied, and suggests that literature estimates of pyrethroid toxicity may underestimate the risks since they do not include the possibility of a co-occurring synergist.

Findings and status – We have demonstrated the synergy of PBO and pyrethroids in the laboratory, using permethrin and bifenthrin. Field studies in urban areas and at a pond that had been sprayed for mosquitoes using a PBO-containing product have documented the presence of PBO in the water, but at concentrations far too low to cause synergy. We believe the absence of environmental synergy is the norm. However, a mosquito control application over Sacramento provided an unexpected and unique opportunity to test this hypothesis under worst-case conditions. Due to an outbreak of West Nile Virus, the entire metropolitan area of Sacramento was sprayed from the air for three successive nights. We found PBO in urban creeks at concentrations 100-fold greater than our previous studies. These same creeks are known to

contain pyrethroids from general urban use, and the PBO in the water from the mosquito control effort was in sufficient concentration to approximately double the toxicity of the pyrethroids already in the creeks. Our study had the surprising conclusion that the greatest impact of the spraying was not the insecticide itself, but the interaction of the PBO synergist with pyrethroids pre-existing in creek sediments. Two papers on our PBO work have been published, and a third is in press.

Interactions between pyrethroids and organochlorines

Methods - Our understanding of interactions of pesticides in mixtures is generally limited and restricts our ability to predict impacts of environmental contamination. Little if any research has been conducted examining interactions among pyrethroid insecticides or between pyrethroids and organochlorine pesticides. There is significant potential for interactions as both pesticide groups are neurotoxins with similar modes of action. The objective of this study component is to investigate, for selected species, potential interactions among pyrethroids and among pyrethroids and organochlorines, and classify these interactions as additive, synergistic or antagonistic.

Findings and status – Research conducted to date included examining the toxic effects of binary mixtures of both Type I and Type II pyrethroid insecticides in order to determine if these compounds interact additively. The mixtures that were examined can be categorized in one of three possible combinations: Type I – Type I; Type II – Type II; or Type I – Type II. The Type I pyrethroids used in this study were permethrin and bifenthrin, and the Type II pyrethroids were cypermethrin and lambda-cyhalothrin.

Completed goals of the research are as follows:

1. Conducted 10-d sediment toxicity tests, each consisting of two single-compound components and a binary mixture component;
2. Generated toxicity curves for each of the individual pyrethroids and the concentration-summed mixture;
3. Compared the experimental mixture toxicity curves to those predicted using single-compound data; and
4. Determined if the mixtures deviate from additivity based upon mixture LC50 values, their corresponding fiducial limits, and the slopes of the mixture toxicity curves.

Juvenile (~14-d old) amphipods (*Hyalella azteca*) were used for all toxicity tests. Results indicated that all of the mixtures were additive in nature with the exception of permethrin-bifenthrin and bifenthrin-cypermethrin which were both less than additive. Research examining pyrethroid and organochlorine interactions will be completed this summer. A draft manuscript has been prepared detailing the pyrethroid-pyrethroid interaction data and will be submitted to a peer-reviewed journal this summer. It is expected that an additional paper detailing the pyrethroid-organochlorine interactions will be prepared this fall.

Urban creek sampling

Methods – Our prior work with pyrethroids has focused on agriculture-dominated water bodies. However, urban use of pyrethroids in California is approximately twice that of agricultural use. Despite this, there has been no previous monitoring for pyrethroids in urban water bodies in the U.S.

Findings and status – We had initially proposed using USGS funding to sample three sites in the San Francisco Bay area. Instead, we actually sampled six, and took samples on 2-3 occasions at each one. With matching funds we sampled Sacramento area creeks on four occasions. Briefly, this work has shown pyrethroids are widespread in urban creeks, and in fact, were detectable in every one of our samples. The sediments in nearly all Sacramento creeks are acutely toxic to our tests organism, Hyalella azteca, due to pyrethroids. In the San Francisco Bay area pyrethroids were the primary toxicant in some creeks, but were not in sufficient concentration to explain the toxicity in most of them. One paper on this work has been published. Our initial plans to look at pyrethroid tissue levels in resident macroinvertebrates in the creeks failed to materialize due to lack of sufficient biomass.

Chronic toxicity testing

Methods – Dr. Chris Ingersoll of USGS had intended to do chronic toxicity testing of our urban sediments to supplement the acute testing we had planned.

Findings and status – Dr. Ingersoll was not able to perform the chronic tests because of the demands of other projects in his laboratory.

Changes in pyrethroid bioavailability over time

Methods - USGS support was used to measure relative changes in bioavailability and aqueous desorption rates of pyrethroids in field-collected soils as they age after the initial pesticide application. Acute toxicity tests were used as the measure of bioavailability, while desorption rates were determined using Tenax beads. Soil was collected from agricultural sites at multiple time points up to 270 days post-application.

Findings and status – We tracked pyrethroid bioavailability over time at three farms (pear orchard, tomato, and rice). Results were not conclusive due to both erratic performance of the Tenax extractions and the growers' repeated applications of pesticides on the farms (rather than the single application assumed in the study design). We have, however, continued to pursue Tenax extraction methods, with greater success in these more recent trials, and one paper has been published.

Notable Achievements and Awards

Two graduate students in Dr. Lydy's laboratory (Andrew Trimble, doctoral student; Amanda Harwood, Masters student) have received EPA STAR graduate fellowships to continue this research. These fellowships began August 2006 and are the result in part due to USGS/NIWR funding.

Due in large part to the urban creek findings from this study, as well as our agricultural findings from prior work, the California Department of Pesticide Regulation (DPR) has announced their intent to put pyrethroid pesticides in to the process known as "re-evaluation". It is extremely rare for DPR to put pesticides in re-evaluation based on water quality data, but our studies provided strong evidence of agricultural and urban aquatic toxicity due to the pyrethroids.

Under re-evaluation, DPR will require the registrants to provide additional data on sources, potential mitigation practices, and environmental fate and effects of pyrethroids so that the State of California can manage them more effectively.

We have received four supplemental awards as a result of this study, three from EPA funding via the Sacramento River Watershed Program. The first award of $23,000 provided for sampling at an additional farm site in connection with our studies on changes in pyrethroid bioavailability over time, as described above. The second award, for $91,000, provided for the development of procedures to identify when pyrethroids are the causative agent for mortality when observed in sediment toxicity tests (i.e., Toxicity Identification Evaluation). The third award, for $28,000, was for continued development of pyrethroid toxicity identification evaluation procedures.

A fourth award of $60,000 was just received from the University of California Water Resources Center for research on transport of pyrethroid pesticides in to the coastal waters of Monterey Bay, and their effect on benthic invertebrates of the shelf and deep sea canyon.