Annual Report of Cooperative Regional Research Projects
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I. PROJECT: W-45: Environmental Transformation, Exposure, and Effects of Pesticide Residues.

Reporting for the Period May 1, 1998 - April 30, 1999

II. COOPERATING AGENCIES AND PRINCIPAL LEADERS:

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California Agric. Expt. Station - Davis	B.W. Wilson
University of California - Riverside, Cooperative Extension	R.I. Krieger
Cornell Agric. Expt. Station - New York	A.T. Lemley
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New Mexico Agric.. Expt. Station	T.M. Sterling
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III. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

This report summarizes progress and principal accomplishments under the three objectives of the project: 1) Develop and use advanced analytical methodology to identify movement of pesticide residues between environmental compartments in relation to bioavailability; 2) Elucidate chemical and biochemical mechanisms and pathways of pesticide residue degradation including characterization of degradation products; and 3) Characterize and quantify exposure and effects of pesticides and their degradation products on target and non-target organisms.

1. Develop and use advanced analytical methodology to identify movement of pesticide residues between environmental compartments in relation to bioavailability:

Hawaii Agric. Expt. Sta. - Studies were conducted to develop 'solvent-free' analytical methods which include immunosensors, capillary zone electrophoresis (CZE) and supercritical fluid extraction (SFE) and enzyme-linked immunosorbent assays (ELISAs). We have developed a simple Na_4EDTA-assisted sub/supercritical fluid extraction procedure for quantitative recovery of polar analytes such as 2,4-D and 2,4,5-T in soil. Sensitive ELISAs have been developed for determination of polycyclic aromatic hydrocarbons (PAHs) using monoclonal antibodies.

Washington Agric. Expt. Sta. - Drip irrigation affords the opportunity to apply agrichemicals without runoff, worker exposure, and creation of excessive wastewater. Studies focused on the application of the reduced risk systemic aphicide imidacloprid through drip irrigation systems in hop yards. Studies were completed in four grower yards in the Yakima Valley, WA and at Washington State University agricultural research station in Prosser, WA. Imidacloprid in the grower yards generally remained in the top 3-4 feet of the soil profile. Dissipation of imidacloprid was quite rapid within the first month after application. Although under furrow irrigation imidacloprid did not appear to leach to depths of 5 feet, soil erosion and runoff was obvious from inspection of the furrows. Under drip systems, especially those that optimize irrigation timing to only meet plant growth needs, imidacloprid does not seem to leach.

Plant uptake of imidacloprid was studied at the WSU Prosser station. Residues in leaves rose to several parts per million within one week after application, and they remained at these levels throughout the growing season. Based on past efficacy tests, the residues were sufficient to control aphids. Studies of imidacloprid distribution in soil in conjunction with plant uptake studies show that drip chemigation in certain perennial crops is an effective pesticide delivery technique that does not compromise environmental safety.

2. Elucidate chemical and biochemical mechanisms and pathways of pesticide residue degradation including characterization of degradation products:

Cornell Agric. Expt. Sta. - New York - Degradation product profiles and mineralization studies with ^{14}C labeled compounds for atrazine under Anodic Fenton Treatment (AFT) and Classic Fenton Treatment (CFT) were developed. Degradation pathways were then determined.

Florida Agric. Expt. Sta. - Carbofuran is a widely used insecticide in the US. It has been known since 1981 that repeated field applications of carbofuran will result in enhanced degradation of the chemical. As a consequence of enhanced degradation, insecticidal efficacy will be reduced, resulting in failure of controlling target pests and reduction of crop yield. We studied changes of carbofuran-degrading microbial populations at a site in Florida during three successive annual applications of carbofuran. Soil from this site exhibited enhanced degradation toward carbofuran. We found that carbofuran-degrading

populations in the enhanced soil did not increase during three successive annual applications. Rather enhanced degradation was likely due to an increase in degradative enzyme activity per cell.

Hawaii Agric. Expt. Sta. - Investigations were carried out to examine the environmental effect of phloxine B and uranine as fruit fly control agents in Hawaii. The fate of phloxine B and uranine in coffee fields and a spill site in Hawaii has been studied. Half-lives of phloxine B and uranine were approximately one week in soil and coffee cherries. Phloxine B was rapidly photodegraded in water under sunlight and various light sources in the laboratory.

Hawaii Agric. Expt. Sta. - Both phospholipase A2 and lysophophatidylcholine solubilized phenyl valerate carboxylesterases from chicken embryonic brain in good yield. The changes in brain membrane phospholipids caused by phospholipase A2 and those due to lysophosphatidylcholine were not identical but shared some common features. These findings suggest that the major portion of phenyl valerate carboxylesterases is released from brain membranes upon lysis catalyzed by phospholipase A2. Lysophosphatidylcholines that are formed from brain membranes in this process may support the solubilization due to their superior detergent properties.

Nevada Agric. Expt. Sta. - The inability to discriminate between gas-phase photochemical reactions versus artifact wall interactions has been identified by the USEPA as the major reason for rejecting industry studies submitted under current pesticide registration guidelines. Assessments to characterize wall sorption of test, reference and tracer compounds used in previous photochemical and oxidative studies were performed at various temperatures to procedures outlined herein. In these proportional dilution experiments, the percent of gas for each substance remaining after each dilution was compared to the ideal behaviour of a gas that does not interact on the chamber surface to determine percent gas-wall concentrations using SPME followed by GC-MS. This iterative dilution approach was found to provide a reasonable estimate of the extent test, reference and tracer substances sorb out of the gas-phase onto the chamber wall over the elevated temperature ranges that were used to conduct gas-phase reactivity assessments. Wall sorption, although present, was minimal. The obtained gas-phase sorption data further supports our previous findings in that atmospheric OH model rate prediction models may not be suitable for estimating lifetime of complex multifunctional compounds. For high-use urban and agricultural organophosphorus insecticides, experimental gas-phase determinations are useful and may provide an important safety-net when assessing risks to non-target and ecologically sensitive areas.

Oregon Agric. Expt. Sta. - There is increasing concern that pesticide use practices will result in surface water contamination. The use of constructed wetlands for the treatment of agricultural runoff is gaining in popularity as a relatively inexpensive alternative. Constructed wetlands are commonly used in the treatment of agricultural, municipal, industrial, and storm water waste. Although constructed wetlands have successfully treated many types of wastewater, there has been little evaluation of their treatment of pesticides. During the summer and fall of 1998 field studies were conducted at a container nursery near Gaston, Oregon to evaluate the capability of a constructed wetland to treat atrazine in irrigation runoff. Atrazine was chosen as the test substance due to its wide distribution in Willamette

Valley surface water, and elsewhere. Laboratory studies using wetland mesocosms were also conducted to better understand constructed wetland bioremediation capability and to elucidate important treatment processes for this pesticide. Although this project has the greatest potential to directly benefit the container nursery industry, the study findings should also be applicable to a variety of intensive farming operations across Oregon. In addition, this project may be useful as a demonstration site in pesticide bioremediation and pollution prevention.

USDA/ARS - Riverside - Soil fumigants are important pesticides used for the control of fungi, nematodes, weeds, and insects in high-value crops. Chemicals used as soil fumigants are dispersed through the soil in the gas phase, resulting in large emissions to the atmosphere if containment procedures are inadequate. Because many of these chemicals present a variety of environmental contamination risks, it is imperative that their emissions be minimized. We are working on a number of projects to characterize the mechanisms and rates of fumigant degradation in soil. This information will be used in the development of management practices which incorporate containment (holding the fumigant in the soil) with degradation to result in effectively zero emissions. We have obtained strong evidence that soil fumigants can chemically react with soil organic matter to produce alkylated groups on soil organic matter plus a halide (BrG or ClG) ion. A similar mechanism of degradation occurs when soils are treated with organic amendments or a chemical fertilizer, which dramatically increase the degradation rate of soil fumigants. For some chemicals/amendments, the increased degradation is largely a result of an increase in the chemical degradation rate; for others, biological degradation induced by the addition of organic amendments is the primary mechanism.

Utah Agric. Expt. Sta. - When wood-rotting fungi, such as *Phanerochaete chrysosporium* are grown on a substrate containing cellulose, such as wood, they produce an enzyme that can generate very reactive radicals. This may provide a mechanism to degrade a variety of chemicals, including pesticides. This hypothesis was tested by studying the degradation of representative pesticides (Lindane, DDT and PCP) when the fungus was grown on cellulose. All three pesticides were mineralized. When oxalate, a secondary metabolite of *P. chrysosporium*, was used as an iron chelator for the enzyme purified from cultures of the fungus, it was oxidized to produce a radical which reduced bromotrichloromethane to the trichloromethyl radical. We propose that the oxidative and reductive radicals produced by the enzyme or fungi may be useful for the biodegradation of some chemicals.

3. Characterize and quantify exposure and effects of pesticides and their degradation products on target and nontarget organisms.

California Agric. Expt. Sta. - (1) Our non-invasive EIA assay for testosterone in feces of wild and laboratory mice (Billitti et al, 1998) was applied in a short term project to MTBE, the controversial fuel additive and agricultural and environmental pollutant, as part of a UC Toxic Substances state sponsored study. Little evidence for reproductive toxicity was found with oral dose levels up to 2 g/kg. (2) The guidelines proposed by California Department of Pesticide Registration for blood

acetylcholinesterase (AChE) of farm workers, mixer-loaders and applicators based on research from our laboratory and reported in previous years to W-45 were approved as a state regulation (HS 1752) (3) Two recent models of the portable Test-Mate kit were evaluated for their usefulness as field instruments. Neither kit performed satisfactorily below 25 (old model) or 20 (newer model) ^{o} C, limiting their utility in the field. (4) A finger stick study of AChE levels of residents of migrant housing Centers is nearing completion. The results suggest such assays can be used to monitor exposure to organophosphates providing care is taken with sampling, storage and assaying. (5) A study has begun in collabporation with Pritsos (Reno) on the actions of selenium to growth and oxidative metabolism of birds.

Nevada Agric. Expt. Sta. - Cyanide and arsenic based pesticides interfere with normal mitochondrial function in the cell which can lead to the generation of potentially toxic reactive oxygen species and deplete cellular energy, ATP levels. While these effects can be acutely lethal when these compounds are ingested in large quantities, the biological impact of either acute or sub-chronic ingestion of these compounds in sub-lethal amounts is unclear. During this past year, using the homing pigeon model we developed for testing the biological impact of exposure to environmental contaminants we tested the effect of exposure to cyanide through drinking water. We found that exposure to cyanide in the drinking water resulted in a dose dependent increase in flight time for the birds, suggesting a dose-dependent biological impact. We also began studies on the impact of exposure of a non-target organism to arsenic. We found that exposure to arsenate in drinking water resulted in decreased mitochondrial function, increased oxidative stress and tissue damage.

New Mexico Agric. Expt. Sta. - Understanding herbicide carryover from one season to the next is very important for successful agriculture production because of the potential for injury and yield reduction of the next season's crop. The potential for carryover is dependent on many things including the relative persistence of the herbicide, soil characteristics and crop sensitivity. The two classes of herbicides, imidazolinone and sulfonylureas, are particularly susceptible to carryover because of their ability to persist in soil. The bioassay technique may be the best method for detecting biologically-active, low levels of these herbicides in soils. Therefore, to understand how pH and moisture influence persistence as well as to optimize a technique for predicting carryover, soil and hydroponic (solution culture of plants) bioassays were compared to evaluate the response of corn to these herbicides. In the soil bioassay, there was greater injury to corn with higher pH soil and higher moisture for each herbicide. In the hydroponic bioassay, herbicide activity was greater at higher moisture availability levels but was not greatly influenced by pH. Therefore, factors in the soil other than pH such as organic matter were influencing corn sensitivity. Also, as water availability increased in each bioassay, so did herbicide availability. These bioassays should prove useful for further studies investigating the mechanisms of herbicide availability.

USDA/ARS, Beltsville - Agricultural practices must conserve natural resources and minimize potentially negative effects on non-target organisms and ecosystems to ensure long-term environmental quality. Studies are in progress to evaluate the ecological risks associated with plastic versus vegetative

mulch use in vegetable production. The runoff volume from plastic mulch plots was 2 to 10 times higher compared to vegetative mulch. Runoff from plastic mulch contained 1.5 to 8 times more sediment. Pesticides residue loads in runoff and bound to sediment were more than an order of magnitude greater from plastic mulch plots. Toxicology studies using aquatic organisms showed that only runoff from plastic mulch plots exhibited lethal effects. New studies are being developed to minimize runoff from plastic mulch and to its decrease toxicity.

IV. USEFULNESS OF FINDINGS

Research undertaken by members of W-45 addresses the issue of pesticide persistence in a coordinated effort. By developing analytical measurements of residues and their dissipation in air, water, soil, and plants; by studying factors that influence persistence such as volatility, photolysis, adsorption, and metabolism; and by investigating chemical structure considerations including structure-persistence relationships and the influence of formulation, application methods and climatic factors, the scientists associated with W-45 contribute to the solution of a wide range of problems caused by pesticide persistence. The work of the W-45 project serves to provide information on these processes in order to reduce risk to the public and the environment. This information is critically important for establishment of the safety of current pesticide uses.

V. WORK PLANNED FOR NEXT YEAR

Work planned for the next year will be a continuation of many of the projects described in section m and new collaborative thrusts described under the objectives of the W-45 project. Additional collaborative efforts include cooperative grants being written, cooperative research projects being initiated, and sharing of research approaches and analytical techniques among institutions. The proposal renewal will be rewritten and submitted by January 15, 2000.

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SIGNATURES

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