TITLE: AGROCHEMICAL IMPACTS ON HUMAN AND ENVIRONMENTAL HEALTH: MECHANISMS AND MITIGATION

REQUESTED DURATION: October 1, 2010 through September 30, 2015

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RELATED CURRENT AND PREVIOUS WORK:

Research is needed to elucidate the processes of agrochemical fate and function to maximize efficacy, to determine realistic exposure potentials to biota (including humans) from foliar, soil, water, and airborne agrochemical residues, and to develop exposure mitigation strategies. This research will require (a) more appropriate biomarkers and analytical methods with better sensitivity, accuracy and precision; (b) investigations to mechanistically understand the environmental influences of time, temperature, soil moisture, soil organic carbon content, and structure/function of the microbial community on agrochemical loss and bioavailability; and (c) investigation of sublethal biological effects of low-level chronic exposure.

Analytical Techniques. Immunoassay methods offer a number of advantages in food and environmental analyses, including reduced time of analysis, low limits of detection, high throughput of samples, cost-effective detection, and adaptability to field uses (Tadeo, 2008). University of Hawaii scientists have developed immunoassay methods for a number of pesticides (Gao et al., 2006; Kim et al., 2004, 2006, 2007; Zhao et al., 2006c; Moon et al., 2007; Xu et al., 2007 a,b), antibiotics and animal and plant growth regulators (Shelver et al., 2005a,c; Deng et al., 2008; Sheng et al., 2009a,b), plant natural products (Tan et al., 2008; Zhao et al., 2006a,b; He et al., 2009), and other environmental pollutants (Parrotta et al., 2005; Pelleguer et al., 2005; Shelver et al., 2005b). These assays have been applied for food analysis (Xu et al. , 2007c; Shelver et al. , 2008; Ma et al. , 2009;), pest control and management (Xu et al., 2006, 2009a), fate studies (Rui et al., 2005) and marine pollution (Xu et al., 2009b).

Proteomics not only characterizes the final gene products in a biological system but also provides detailed information about protein abundances, stabilities, turnover rates, functions, structures, post-translational modifications, and protein-protein interactions. Large-scale, high-throughput omics technologies can comprehensively reveal complex protein networks in a biological system (Hendrickson et al., 2008). Among various protein analytical techniques, mass spectrometry (MS) has emerged as the primary method (Cravatt et al., 2007; Siuti and Kelleher, 2007). Proteomic and metabolomic approaches are relatively new in the field of ecotoxicology. A review of proteomic research in general toxicology (Wetmore and Merrick, 2004) identified only three studies associated with ecotoxicology and a recent search yielded only a few more examples (e.g., Olsson et al., 2004; Kim et al., 2005). Similarly, only a handful of metabolomic studies have been conducted with fish or other aquatic organisms (e.g., Ralston-Hooper et al., 2008). For the full value of these technologies to be realized in ecotoxicology, it is necessary that gene, protein and metabolite expression profiles from sentinel species exposed to common environmental contaminants be determined and mined for relevant biomarkers. Such information will strengthen risk assessments utilizing this comprehensive response technique by reducing the uncertainty commonly associated with biomarker data. Scientists at Purdue

University have developed techniques that are ready to be applied for testing a wide range of contaminants.

Previous research on endocrine disrupting compounds (EDCs) in the environment has primarily focused on the exposure of organisms to aquatic sources of EDCs (Payne et al., 2000; Silva et al., 2002; Thorpe et al., 2006; Markman et al., 2007). Methods to quantify exposure in aquatic organisms have been established (Payne et al., 2000; Thorpe et al., 2006), but research concerning the exposure of soil dwelling organisms to EDCs in the soil or sediment is lacking. In addition, most of the research on EDCs in soil has focused on total concentration in soil (Petrovic et al., 2002). However, EDCs adsorb differently to different soils, so the bioavailability of EDCs and risks associated with exposure of organisms to these compounds depends on the properties of the EDCs, properties of the soil, and concentration of EDCs in soil (Duong et al., 2007). Solid-phase microextraction (SPME) was recently used to determine bioavailability of PAHs to earthworms in soil (Jonker et al., 2007). Use of SPME is less expensive and time consuming than the typical *in vivo* earthworm model. Application of this methodology to EDCs would be an important step forward.

Agrochemical Distribution and Fate as Affected by Transport and Partitioning. The wide use of pesticides in agricultural and urban areas has resulted in their frequent detection in surface water (Thurman et al., 1992; Goolsby and Battaglin, 1993; Meyer and Thurman, 1996; Larson et al., 1997; Clarc and Goolsby, 1999), groundwater (Hallberg, 1989; Barbash and Resek, 1996; Meyer and Thurman, 1996), aquatic biota and sediment (Nowell et al., 1999), and the atmosphere (Majewski and Capel, 1995). In the U. S., at least 143 pesticides and 21 transformation products, representing every major chemical class, have been detected in environmental samples (Konda and Pasztor, 2001), which invokes concern for both environmental and human health issues. Understanding of the major factors influencing agrochemical transport, persistence, and bioavailability in a variety of environments including agricultural fields, turf, and atmospheric and aquatic systems will also allow better management of agrochemicals.

The inflow of some pesticides from Eurasia to the Western U. S. via trans-Pacific atmospheric transport has been identified and documented (Primbs et al., 2008; Genualdi et al., 2009). However, our current understanding of the magnitude of the inflow of pesticides to the U. S. through trans-Pacific transport of Eurasian emissions is very limited. Hageman et al. (2006) estimated the relative contribution of regional (within 150 km radius) and long-range (> 150 km radius) atmospheric transport on dieldrin, alpha-HCH, chlordane, and HCB concentrations in annual snow pack collected from remote high elevation sites in seven Western U. S. national parks. These results estimate that 100% of the concentrations measured in the Alaskan parks were due to long-range transport, while 30 to 70% of the concentrations of these POPs measured in the most westernly continental U. S. park (Mount Rainier National Park) were due to long-range transport (including trans-Pacific transport) (Hageman et al., 2006).

Atmospheric transport over a short or intermediate range may also play an important role in the input of pesticides in sensitive ecosystems. For example, Florida has over 40,000 farms totaling 10 million acres and in 2005, the state ranked first in U. S. sales for snap beans, fresh market tomatoes, cucumbers for pickles and fresh market, bell peppers, squash, watermelon, oranges, grapefruit, tangerines, and sugarcane, and second for sweet corn, strawberries, and greenhouse and nursery crops. Much of this production occurs in South Florida where three National Parks (Everglades, Big Cypress, and Biscayne) are all on the top ten most endangered public lands. Significant concerns exist over the potential risks from atmospheric deposition of pesticides to the Everglades National Park from adjacent agricultural production areas.

The use and disposal of natural and synthetic compounds that offer improvements in agriculture, medical treatment, personal care and residential conveniences may result in the contamination of surface waters. These compounds include antibiotics, hormones and pharmaceuticals used for treating humans and domesticated animals; pesticides used for plant and animal protection; and additives to consumer and personal care products (Kolpin et al., 2002; Lee et al., 2004, 2008). In a nationwide survey of 139 rivers in 30 states, Kolpin et al. (2002) reported the occurrence of pharmaceuticals, hormones or other organic wastewater contaminants in 80% of the streams sampled. In order to improve surface water quality and reduce ecological impacts of impaired surface waters, we need to first identify the sources of contaminants. Research is needed to develop tools that will identify contaminant sources so that source reduction and treatment strategies can be designed to help mitigate surface waters.

Soil erosion induces large variation in soil properties with landscape position, resulting in spatially variable pesticide sorption and degradation rates. Herbicide sorption coefficients in surface soil tend to be highest in depressional areas and lowest in upper slope positions (Novak et al., 1997; Liu et al., 2002; Gaultier et al., 2006). Pesticide degradation rates are also spatially variable in both surface soil and subsurface soil (Liu et al., 2002; Charnay et al., 2005; Gaultier et al., 2007). Estimates of pesticide sorption and degradation determined under static conditions in the laboratory may not be relevant under the dynamic field conditions. A recent field dissipation study did not detect differences in transport of bromide (a tracer of water movement) or the herbicide S-metolachlor in an eroded field despite a large variation in soil properties (Papiernik et al., 2009a,b). Additional research is required to discern the relative importance of interacting soil processes in determining the fate and transport of herbicides in spatially variable landscapes.

Over 40 million acres of U. S. land are covered by tended lawn (Milesi et al., 2005). Maintaining the health and beauty of turf often requires chemical fertilization, thatch treatments and the use of pesticides (Smith and Bridges, 1996). Application rates on lawns and golf courses are considerably higher than the rates for agricultural purposes (Gianessi and Anderson, 1995; Barbash and Resek, 1996). Pesticides that are commonly applied to turf grasses have been found in surface waters of urban areas (Hoffman et al., 2000; Gilliom et al., 2006). Creeping bentrass (*Agrostis stolonifers* L.), regularly used for golf course turf, is highly susceptible to snow mold fungi (Wang et al., 2005), which is controlled by application of fungicides including chlorothalonil, iprodione and pentachloronitrobenzene. These compounds have been shown to be moderately to highly toxic to aquatic organisms (PAN, 2009; OSU, 2009). In locations with cold climates, the melting of snow and ice produce springtime runoff that may trigger flooding and greatly contributes to stream flow (USGS, 2009). Little is known about the fate of fall-applied turf fungicides with snowmelt as the influence of winter conditions on their persistence and transport is not well studied. Studies are needed for understanding transport of chlorothalonil, iprodione, and pentachloronitrobenzene in snowmelt runoff from turf.

Agrochemical Toxicity. Carbamate and OP insecticides are designed to inhibit acetylcholinesterase (AChE), which hydrolyses the neurotransmitter acetylcholine in nerve and muscle tissues. Inhibition of AChE causes an increase in synaptic acetylcholine to abnormally high levels, resulting in repetitive stimulation of muscarinic and nicotinic receptors in target tissues. Sufficiently high exposure levels produce clinical signs of acute cholinergic poisoning. Long-lasting neurological damage from acute high level exposure to some OPs is well documented (Karczmar, 1984). California and other states require or recommend blood cholinesterase monitoring for pesticide mixer-loaders, applicators, and farm workers, where inhibition of blood AChE activity is used as a biomarker of exposure to OPs. Although a number of field studies of blood cholinesterase levels exist, the test accuracy has not been scrutinized until recently. Researchers at UC Davis, in collaboration with the Washington State Department of Labor and Industry, have been monitoring blood ChE focusing on fruit orchard workers. The goal is to monitor clinical plasma and red blood cell ChE assays to estimate the extent of exposure of the workers to anticholinergic OP pesticides. One report has been published (Wilson et al., 2009a,b) and another is in preparation. The research has amassed one of the largest ChE data bases ever established for farm workers. In addition to monitoring the reliability of the work, its goals include studying the distribution of blood ChEs in a working population and examining the relative sensitivity of serum ChEs compared to red blood cell AChEs under field conditions.

Pesticide handlers, agricultural workers including harvesters, bystanders, and consumers may be exposed to pesticides via various routes. Pesticide exposure may also occur to children and adults following residential use of pesticides including pet products. Researchers at UC Riverside have been undertaking a range of studies to understand human exposure to pesticides. These studies include handler exposure assessment during cyanide fumigation of citrus; assessment of chloropicrin exposure of bystanders during fumigation; relationships between deposition and distribution of pyrethroids applied indoors and human exposure potential; formation of OP biomarkers in produce and the implication for human exposure assessment; evaluation of the persistence of OP biomarkers in leaves and fruits of strawberries; residential exposure following use of total release from pyrethroid foggers; and development of a new procedure for analysis of a urine biomarker for DDT exposure.

Most toxicity studies using whole organisms generally focus on acute (lethal) toxicities. The impact of non-lethal, low-dose agrochemical exposure on non-target organisms has not been studied extensively. Acute sub-lethal exposures can impact an organism's ability to survive in the wild by causing a loss of energy, disorientation or loss of ability to navigate. With the understanding that compromised flying efficiency and migratory orientation may lead to negative effects on migratory bird species, W-1045 researchers in Nevada have chosen homing pigeons (*Columba livia*) as the test species (Wiltschko and Wiltschko 2003). Recent research has shown that migratory ability in homing pigeons is compromised after exposure to cholinesterase-inhibiting pesticides (Brasel et al. 2007; Moye, 2008) and chemicals found in mining waste (i. e., arsenic and cyanide) (Brasel, 2005; Brasel et al., 2006).

Health risk assessments of chemicals have been traditionally restricted to evaluating the potential risk of a single compound through a single route of exposure (National Research Council 1983).

In the last 15 years, however, a great deal of attention has focused on biological effects of chemical mixtures and the endocrine-disrupting nature of various pesticides. Many recent studies demonstrated that certain herbicides could either synergistically or antagonistically affect the toxicity of certain OPs in the aquatic midge (*Chironomus tentans*) (Belden and Lydy, 2000; Anderson and Lydy, 2001; Jin-Clark et al., 2002, 2008; Anderson and Zhu, 2004; Anderson et al., 2008; Li et al., 2009). Increased toxicity of certain OPs in binary combinations with atrazine correlated to the increased AChE inhibition and increased cytochrome P450 activity (Anderson and Zhu, 2004; Rakotondravelo et al., 2006). W-1045 researchers in Kansas will continue this line of research by developing a cDNA microarray based on the expressed sequence tag data, profiling the gene expression responses in midges exposed to pesticides, and elucidating molecular mechanisms leading to synergistic or antagonistic effects of pesticide mixtures in aquatic midges. Results from this study are expected to provide insights into potential adverse effects of these pesticides at the molecular level, individually and as mixtures, on non-target organisms in aquatic environments.

Pesticide use in Oregon watersheds, particularly those that provide salmon habitat, is of increasing concern. Monitoring studies in Willamette Basing watersheds (Rinella and Janet, 1998; USGS, 2008) suggest that pesticides in surface water may pose a risk to aquatic life fitness and survival, including 26 Evolutionarily Significant Units (ESU) of Pacific salmonids listed as threatened or endangered under the Endangered Species Act (ESA). As required by the ESA as a part of the consultation process, the National Marine Fisheries Service (NMFS) has completed Biological Opinions (NMFS 2008, 2009) for 6 of the 37 pesticide active ingredients that U. S. Environmental Protections Agency (EPA) has determined “may affect” the 26 Pacific salmonid ESUs. In developing the Biological Opinions, NMFS has raised concerns about the paucity of information on pesticide use practices and monitoring data, particularly at the watershed scale. Researchers at Oregon State University will employ both monitoring and modeling methodologies that will allow a more robust evaluation of potential impacts of pesticide use practices on aquatic life including salmon ESUs.

Environmental Transformation Processes and Remediation Technologies. Effective and economical technologies are needed to clean up soil and water contaminated by agrochemicals. Researchers at Cornell (New York) have developed a specialized Fenton system, anodic Fenton treatment (AFT), to study the degradation of ETU, trifluralin and atrazine (Saltmiras and Lemley, 2000, 2001, 2002). A significant improvement to the AFT method was the development of the membrane AFT system (Wang and Lemley, 2002) that uses a semi-permeable membrane to separate the two half-cells. The Cornell group made another significant advance by developing a kinetic model that describes this delivery-controlled AFT (Wang and Lemley, 2001). Membrane AFT was applied to the study of carbofuran and mixtures of six carbamates, and the results were well explained by the kinetic model (Wang and Lemley, 2003a,b). In other work, the Cornell group modified the AFT kinetic model for application to triazines/triazones (Wang and Lemley, 2004), using metribuzin as the primary model compound. A framework was developed for understanding the kinetics of the AFT under flow conditions, an important aspect of future applications (Kong and Lemley, 2006; Zhang and Lemley, 2006, 2007). To further understand the application of this method to soils and soil slurries, several studies were performed on humic acid models (Wang and Lemley, 2006), an actual soil slurry (Kong and Lemley, 2006) and a model soil composed of clay and humic acid (Ye and Lemley, 2009a).

More specific studies on the effect of the interaction of pesticides with clays on AFT degradation were done by Ye and Lemley (2008, 2009b). Recent work has shown that a variety of Fenton approaches with continuous delivery of the iron reagent can successfully degrade candidate pesticides (Zeng and Lemley, 2009).

Sunlight photolysis of chemicals on soils, particularly pesticides, has been examined in previous W-1045 projects (Hebert and Miller, 1990; Miller and Donaldson, 1993). These studies demonstrated that transformations on soils are common and are a significant loss mechanism for a variety of agrochemicals. In the last decade, concerns have arisen over the observation of perchlorate in groundwater and soils distant from any industrial source (Christen, 2003). The low amount of perchlorate in fertilizers (Susarla et al., 1999) is likely only a partial source. Members of the W-1045 project in Nevada are presently investigating whether perchlorate can be formed through photooxidation of chloride on soil surfaces. Preliminary evidence suggests that both semiconductor surfaces (e. g., titanium dioxide) (Serpone et al., 1994) and nitrate (Wallvoord et al., 2003) may be involved in photooxidation of chloride, and further investigation will examine this hypothesis.

The North Shore Restoration Area (NSRA) of Lake Apopka, near Orlando, FL has been reported to contain significant amounts of persistent organochlorine pesticides and their metabolites, particularly DDT, DDD, and DDE (abbreviated as DDx). DDx, especially DDE, are highly resistant to *in situ* remediation (Thomas et al., 2008). While the majority of commercial bioremediation projects use prokaryotic bacteria, other projects involving white rot fungi have proven successful. One disadvantage of using white rot fungi is that the extracellular enzyme production may be inhibited by some soil factors such as high nitrate concentrations. To circumvent this latter problem for NSRA soils, W-1045 researchers in Florida grew the fungi outside the soil system on corncob grits and then washed off the enzymes with tap water. They will further test biodegradation of DDx in microcosms using the enzyme rinsate, and identify conditions under which the removal of DDx is maximized.

Best Management Practices. Movement of pesticides and other agrochemicals from their site of application to surrounding areas reduces the efficacy of targeted pest control and increases risks to non-target organisms. A number of W-1045 scientists are actively involved in research contributing to the development and evaluation of a range of best management practices to maintain agrochemicals at their site of application. For instance, USDA-ARS researchers in Minnesota have been investigating practices to reduce movement of herbicides, sediment, and nutrients from agricultural fields. In Minnesota, 96% of acres planted with corn and soybeans received applications of herbicides (MDA, 2005). Acetochlor and S-metochlor were amongst the top five pesticides sold in 2008 (MDA 2009b). Monitoring studies by USGS and others have reported occurrences of acetochlor, alachlor, metolachlor and their degradation products in surface water and groundwater (Thurman et al., 1991; Holden et al., 1992; De Guzman, 2005). W-1045 researchers have shown that changes in management practices can reduce sediment, nutrient and pesticide losses from agricultural systems (Sur et al., 1992; Rice et al., 2001). W-1045 researchers will continue their investigation to compare tillage practices and drainage practices to determine which management strategies can be used as a best management practice to reduce the offsite movement of chloroacetanilide herbicides and their metabolites.

Fumigants are among the most heavily used pesticides, especially in states such as California, Florida, Hawaii, and Washington. Atmospheric emission of fumigants after soil fumigation is a significant concern for human exposure, as well as a source of air pollution. Starting in 2006, W-1045 researchers in Washington have been working with the grower community in developing innovative field-scale soil fumigation demonstrations with the goal of reducing methylisothiocynate (MITC) air emissions, application rates, and reliance on conventional center pivot fumigant application systems. Data has been collected on a yearly basis during 2006 (LePage et al., 2007), 2007 (LePage et al., 2008), and 2008 (Littke et al., 2009) using near-field receptors to evaluate MITC emissions occurring during and post application by modified reduced emission center pivot (low pressure and reduced drift) and soil incorporated shank field systems. The three year field emission results comparing surface (center pivot chemigated) to soil incorporated shank incorporated fumigant treatments have consistently demonstrated substantial retention of MITC in soil by shank application. This combined effort working with growers, custom applicators, registrants, and regulatory agencies is leading to regional changes in how fumigants are applied, reducing fumigant emissions while retaining product efficacy.

Research to enable better prediction and management of rangeland weed populations is required to minimize negative impacts on surrounding biota. Research by W-1045 scientists in New Mexico and Montana has focused on understanding the mechanisms of herbicide resistance, characterizing the genetic variation in crop and weed species, and determining their role in stress response. Oxidative stress is a major factor limiting plant productivity and results from environmental stresses, including pesticides, which can induce the production of reactive oxygen species capable of severe cell and tissue damage. Mechanisms against oxidative stress include antioxidants and protective enzymes that can be induced during inhibition of photosynthesis or when plants are under stress. By understanding how plants avoid oxidative stress, crops will be better protected from stress and weeds better managed. Studies (Sterling and Lownds, 1992; Sterling et al., 1996; Gibbs and Sterling, 2004) suggest that environmental variation plays a much larger role than genetic variation in differential chemical control. Long-term success in weed control requires integrating multiple management strategies with attention to specificity of biological control agents to avoid selection for resistant genotypes (Sterling et al., 2004).

There is growing interest in the application of GIS towards the epidemiological surveillance of human poisoning incidents of public health significance (Hughes et al., 2007). Poison Control Center (PCC) data can serve an important role in surveillance studies, as they are a toll-free resource covering wide geographic areas across the United States (Bryden et al., 2005; Watson et al., 2005). In a previous investigation (Sudakin et al., 2002), researchers at Oregon State University conducted an analysis of all symptomatic pesticide exposure incidents reported to a regional PCC during a single year. These data were analyzed using spatial and temporal scan statistics to assess for significant clustering of symptomatic exposures. In a more recent study (Sudakin and Power, 2009), these researchers analyzed five years of PCC data to test whether there are significant geographic differences in pesticide exposure incidents resulting in serious (moderate, major, and fatal) outcomes. These analyses may be useful for public health officials to target preventive interventions. Further investigation is warranted to better understand the potential explanations for geographical clustering, and to assess whether preventive interventions have an impact on reducing pesticide exposure incidents.

Results of CRIS Search. This committee and the W-1082 committee (Evaluating the Physical and Biological Availability of Pesticides and Pharmaceuticals in Agricultural Contexts) have addressed the issue of toxics in the environment from very different but complementary perspectives. The two committees have a common interest in the environmental consequences of agricultural practices. However, these projects address different aspects of the environment. W-1082 explores the transport of substances in the soil and places great emphasis on modeling these processes with the aim of developing management tools for the use of agricultural chemicals that will minimize environmental contamination. This project, on the other hand, focuses on the chemical and biochemical transformations of pesticides and environmental contaminants, the toxicological impacts of these chemicals on humans and other life forms, and development of remediation technologies. To enhance the beneficial exchange of information between the two groups, joint members will report relevant information from one committee to the other at annual meetings.