



WATER RESOURCES RESEARCH GRANT PROPOSAL

Title: Evaluating the Effects of Pesticide Mixtures to Freshwater Algae

Focus Categories: TS, SW, WQL

Keywords: Algae, pesticides, pollutants, water quality

Duration: 2 years (9/99 to 8/01)

Federal Funds Requested/Year: \$4,225 (yr. 1); 4,225 (yr. 2); \$8,450 (Total)

Non-federal (matching) Funds Pledged: \$20,255 (yr. 1); \$20,255 (yr. 2); \$40,510 (Total)

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Congressional District: 01

Statement of Critical Regional or State Water Problems

Evaluation and protection of water quality requires an understanding of the effects of contaminants on aquatic biota. While contaminants usually occur as a mixture of chemicals in surface waters, very few studies have examined the effects of such mixtures. Presently, all guidelines and standards for allowable or "safe" amounts of pesticides in surface waters are based on single toxicant studies. Therefore, to assess actual environmental effects of chemicals on aquatic ecosystems, it is necessary to begin evaluating mixtures of toxicants.

Recent reviews of pesticide toxicity to aquatic organisms generally conclude that concentrations found in aquatic systems are not ecologically harmful (e.g., Solomon et al. 1996). However, the possibility that synergistic interactions can occur between agricultural chemicals, such as herbicides and insecticides, have not been thoroughly examined. To date, most investigations of pesticide toxicity to freshwater organisms involve single-species bioassays involving a single compound.

Recently, it has been shown that aquatic midge larvae (*Chironomus tentans*) exposed to atrazine and several organophosphate insecticides exhibit greater than additive toxicity (Pape-Lindstrom and Lydy 1997). This study suggests that the joint action seen between atrazine and these compounds may be due to atrazine induction of detoxification enzymes involved in biotransformation of the insecticides. The synergistic effect observed in this study indicates that oxidative processes involved with activation of the OP molecule may be enhanced in the atrazine exposed midges.

Since pesticides such as herbicides and insecticides are so widely used in Midwestern agricultural systems, there is a likelihood that combinations of these pesticides occur in aquatic environments. It is therefore important that interactions between compounds be examined for potential synergistic effects in aquatic organisms such as freshwater algae. Algae are the most important primary producers in aquatic systems and account for nearly the same percentage of total global net primary production of carbon annually as do cultivated plants. In addition, algae represent the basal component of aquatic food webs, since they are consumed by a variety of invertebrates or directly by fish, both of which are consumed by other fish species. Algae possess a complex system of detoxification enzymes such as glutathione-S-transferases responsible for metabolism (Tang et al. 1998) of both toxic and non-toxic xenobiotics. Therefore, compounds that are relatively non-toxic may act as inducers or inhibitors of these metabolic enzymes which could potentially affect an organism's ability to detoxify other more toxic chemicals during simultaneous exposure.

The present research proposal is designed to measure the joint action of selected pesticides with different modes of action in freshwater algae. Various binary combinations of pesticides will be tested against representative algal species common to Midwestern aquatic environments. Additionally, we will examine the effect of sublethal exposure of selected species on the activity of detoxification enzymes important to pesticide metabolism and potentially to the mechanism of joint pesticide toxicity.

Statement of Results and Benefits

A fundamental goal in the ecotoxicology of surface waters in the Great Plains is to predict the effects of pesticide stressors. Our lack of critical knowledge concerning the impacts of pesticides on Midwestern aquatic communities is at least partially attributable to a general lack of understanding of the potential interactions of multiple contaminants on aquatic biota. Although a number of pesticides have been shown to be relatively innocuous at environmentally relevant concentrations when tested individually, little information exists on the potential synergistic effects when they co-occur in the same environment. This study is designed to investigate the potential synergistic effects between various pesticides important to Midwestern agriculture, and to investigate the underlying mechanisms of the synergism in instances where it is documented. Given the importance of freshwater algae to the integrity of aquatic ecosystems, obtaining more comprehensive information on the potential interaction of contaminating pesticides is clearly warranted and prerequisite to our assessment of pesticide impacts on aquatic systems throughout the region. Results from this investigation will be based on commonly occurring aquatic taxa, thus allowing us to extrapolate our findings to other aquatic systems and to better predict the impact of pesticide interactions on surface water quality in general and on overall ecosystem health.

Nature, Scope, and Objectives

Because of the importance of freshwater algae as basal components of aquatic food webs, we will investigate potential synergism among representative pesticides commonly used

in Midwestern agriculture in representative freshwater algae. Toxicity (algal growth in the presence of pesticide) will be measured by fluorometric determination of chlorophyll a content (Nelson et al. 1999). EC50 values (concentrations that caused 50% reduction in cell doublings/day relative to control treatments) will be calculated for each species. For synergism bioassays, we will compare toxicity of four commonly used pesticides (atrazine, alachlor, chlorpyrifos, and tefluthrin) in binary combinations to determine whether synergistic effects are also observed. Algae bioassays will be conducted at the University of Nebraska Aquatics Lab which possesses an extensive collection of freshwater algal species and the necessary culturing facilities.

Additionally, we will measure the level of detoxification enzymes (specifically glutathione-S-transferase) in the presence and absence of these compounds to determine if there is an induction or inhibition of activity which could be important to the joint pesticide action. Enzyme activity determinations will be conducted at the University of Nebraska Insecticide Toxicology Lab which is equipped and has the necessary expertise to conduct such assays.