MAIN AND INTERACTIVE EFFECTS IN A WETLAND MESOCOSM EXPERIMENT: ALGAL COMMUNITY RESPONSES TO AGRICHEMICAL RUNOFF

Bryan W. Brooks, Julie A. Chambers, Bruce S. Libman and Stephen T. Threlkeld
Department of Biology
University of Mississippi

INTRODUCTION

The negative impacts of agrichemical pesticides on various components of aquatic communities have received increased attention in recent years. The potential for interactive effects between multiple agrichemicals is always present, but investigations of multiple agrichemical effects are limited (Parott and Sprague 1993; Sexton et al. 1996). The toxic effects of the suite of agrichemicals in watershed runoff samples will invariably differ from laboratory studies which focus on the independent effects of the chemicals. The need for an experiment to quantify the effects of a mixture of pesticides on multiple endpoints at different trophic levels of wetland ecosystems gave impetus for our present study.

Atrazine, chlorpyrifos, and monosodium acid methane arsonate (MSMA) are pesticides used widely in the southeastern and central United States. Methyl mercury is an organic metal which enters the environment primarily through anthropogenic activities (Slemr and Langer 1992). The nature of these chemicals (herbicide, insecticide, or metal) and the mechanisms of their independent effects have been previously studied. Atrazine is a selective systemic herbicide applied to agricultural fields to control weeds. Atrazine toxicity is exerted on plants because it inhibits photosynthesis by blocking the electron transport chain of photosystem II located within in the cell. This results in the destruction of chlorophyll and increased cellular levels of CO_2. Atrazine is relatively non-toxic to animals but has been suggested to induce sublethal responses (Solomon et al. 1996). Effects of methyl mercury on aquatic plants can include death and sublethal effects including inhibition of mitosis, plant senescence, and decreased chlorophyll levels. Methyl mercury accumulates in organisms by mimicking the amino acid methionine and may then be transported across cell membranes (Clarkson 1994). The compound will then bind to thiol containing proteins such as enzymes. Upon binding with proteins, methyl mercury can change or inhibit the activity of proteins by modification of their structures. When methyl mercury is converted to inorganic mercury, increased hydrogen peroxide production, disruption of the electron transport chain and decrease of chlorophyll can result (Lindqvist 1991; Clarkson 1994). Chlorpyrifos is an organophosphate non-persistent insecticide. It exerts irreversible toxicity to animals by inhibiting acetylcholinesterase activity. While chlorpyrifos is extremely toxic to aquatic invertebrates, chlorpyrifos is considered to be relatively non-toxic to plants based on its mode of action (Menzer 1991; Sorrano et al. 1995). MSMA is an organoarsenic herbicide commonly used as a selective contact grassweed herbicide. By mimicking phosphate in absorption, translocation, and metabolic pathways, MSMA exerts its toxicity by interfering with the normal metabolism and growth of plants. MSMA is typically considered to be non-toxic to animals, however, organic arsenicals have been suggested to be responsible for several toxicological consequences including neurologic, reproductive, carcinogenic and genetic potentials (Menzer 1991; Tomlin 1994).

MATERIALS AND METHODS

Research Facility Set-up

Sixty-six 500-liter mesocosms at the University of Mississippi's Biological Field Station were filled with 15 cm of sand and a 5 cm thick top layer of sediment from a nearby pond. Six baskets of Juncus effusus were planted in each mesocosm. The mesocosms were then filled with water from a spring pond. Chemicals were added to the mesocosms on 10 June 1996 (day 0). Atrazine, chlorpyrifos and MSMA were present at ambient levels (non-detectable) or added at nominal levels of 192, 51, and 219 ppb, respectively, as expected from an Expected Environmental Concentration (EEC) model for wetlands downstream of agricultural fields receiving runoff two days after an agricultural application (EPA 1995). Methyl mercury was present at 0.2 mg/kg or was added to bring total mercury in the upper 1 cm of sediments to a nominal level of 0.4 mg/kg wet weight.

Design and Analysis

Treatments were randomly assigned to sixty-six mesocosms according to a center-point enhanced 2^4 factorial design. Mesocosms were sampled on days 1, 2, 4, 8, 16, and 32 after dosing on day 0 (10 June 1996). One third of the mesocosms were sampled on days 1 and 8, another third were sampled on days 2 and 16, and the final third were sampled on days 4 and 32. Data was analyzed by ANOVA using the SAS system (SAS 1987). Statistical significance was determined at $P < 0.10$.

Sample Collection and Measurement

Measured response variables included gross and net primary productivity, algal fluorescence, turbidity, and chlorophyll a from phytoplankton. Primary productivity was determined by changes in diel measurements of dissolved oxygen concentration using the single curve method (Hall and Moll 1975; Wetzel and Likens 1991). Algal fluorescence was measured by fluorometry on a Sequoia Turner model 450 fluorometer with SE 665 and NB440 filters. Turbidity was determined using the Hach 2100P turbidimeter. Biomass of phytoplankton was measured as chlorophyll a (Wetzel and Likens 1991).

RESULTS

Atrazine reduced algal fluorescence (Figure 1a), turbidity (Figure 2a), and chlorophyll a (Figure 3a) on day 1 through day 8. An apparent recovery by the algal community was detected on day 16 and continued through day 32 as atrazine did not reduce algal fluorescence, turbidity and chlorophyll a. A significant atrazine x methyl mercury antagonistic interaction which was present on day 1 through 8 for algal fluorescence (Figure 1a & 1b), turbidity (Figure 2a & 2b), and chlorophyll a (Figure 3a &3b) was not present on day 16 and day 32.

Atrazine significantly reduced gross (Figure 4a) and net primary productivity (Figure 5a) through day 32. A significant atrazine x methyl mercury antagonistic interaction was present for gross primary productivity on each sample day except on day 16 (Figure 4a & 4b) and was present for net primary productivity on each day except day 2 and day 16 (Figure 5a & 5b).

Chlorophyll b, glucose mineralization and bacterial abundance were not significantly affected by methyl mercury, atrazine and atrazine x methyl mercury treatments (Hwang and McArthur, unpublished data).

Chlorpyrifos and MSMA did not significantly affect algal fluorescence, turbidity, chlorophyll a, or gross and net primary productivity (all $P > 0.10$).

DISCUSSION

The design of this study enabled us to examine various treatment combinations of three agrichemicals and an organic metal. Although atrazine is a herbicidic agrichemical and methyl mercury is an organic metal, it is well established that both are toxic to aquatic algal communities. We would expect the independent effects of atrazine and methyl mercury on the algal community to be additive when the two chemicals are present together in our treatments. Our data indicate that in the presence of mercury, the effect of atrazine is reduced for the response variables examined. Explanation of the antagonistic interaction observed between atrazine and methyl mercury is difficult because limited information exists about interactions between herbicides and metals. Methyl mercury and atrazine may interact by a chemical-chemical means in the water column or in the sediment. Based on their chemical structures, this seems unlikely. They also may interact on the cellular level if mercury reduces the uptake of atrazine or alters the effect of atrazine on the metabolic processes of photosystem II. Hwang and McArthur found that bacterial abundance and glucose mineralization did not exhibit the same antagonistic atrazine x methyl mercury interaction as the algal community (Hwang and McArthur, unpublished data). Our measurements of chlorophyll b also were not affected by the atrazine x methyl mercury interaction. Given that the bacterial community does not photosynthesize and respond to atrazine in the same manner as the algal community, a methyl mercury alteration of atrazine toxicity on photosystem II appears possible.

The effects of methyl mercury on glutathione levels in phytoplankton and algae have been examined demonstrating a methyl mercury dose dependent induction of glutathione levels (Howe and Merchant 1992), while glutathione has typically been shown to decrease when exposed to methyl mercury (Clarkson 1994). Glutathione is a tripeptide that can be responsible for the detoxification of herbicides. Plants with high levels of a specific glutathione-S-transferase (an enzyme that catalyzes the conjugation of glutathione) are tolerant to atrazine (Marrs 1996). A mechanism may exist such that methyl mercury stimulates glutathione-S-transferase which induces glutathione levels in the algal community and mediates increased tolerance to atrazine. This possible mechanism warrants future investigation and, if confirmed, could clarify the antagonism of atrazine toxicity by methyl mercury observed in the present study.

ACKNOWLEDGMENTS

This research was supported by EPA award R 821832-01-0 to The University of Mississippi, Stephen Threlkeld, PI, with subcontracts to Jackson State University, Mississippi State University, and The University of Southern Mississippi Gulf Coast Research Laboratories.

The authors would like to thank Ms. Kathryn Valentine, Ms. Hope Belk, Ms. Ginger Powers, Mr. David Felder, Mr. Ken Overstreet, and Mr. Bo Solomon for help with data collection. Mr. Robert Baca, Mr. Jeff Steevens, Dr. Beth Ahner, and Dr. Bill Benson made helpful comments and suggestions.

REFERENCES

Clarkson, T. W. 1994. The toxicology of mercury and its compounds. Chap. in Mercury Pollution: Integration and Synthesis. Boca Raton, FL: Lewis Publishers.

Hall, C. A. S. and R. Moll. 1975. Methods of assessing aquatic primary productivity. Chap. in Primary Productivity of the Biosphere. New York: Springer-Verlag.

Hoagland, K. D., R. W. Drenner, J. D. Smith, and K. R. Cross. 1993. Freshwater community responses to mixtures of agrichemical pesticides: effects of atrazine and bifenthrin. Environmental Toxicology and Chemistry. 12:627-637.

Howe, G. and S. Merchant. 1992. Heavy metal-activated synthesis of peptides in *Chlamydomonas reinhardii*. Plant Physiology. 98:127-136.

Hwang, H. and N. McArthur. 1997. Unpublished data. Jackson State University: Department of Biology.

Lindqvist, O. 1991. Mercury in forest lake ecosystems - bioavailability, bioaccumulation and biomagnification. Water, Air and Soil Pollution. 55:131-157.

Marrs, K. A. 1996. The function and regulation of glutathione s-transferases in plants. Annual Revue of Plant Physiology and Plant Molecular Biology. 47: 127-158.

Menzer, R. E. 1991. Water and soil pollutants. Chap. in Casarett and Doull's Toxicology: The Basic Science of Poisons. New York: McGraw-Hill.

Parott, J. L. and J. B. Sprague. 1993. Patterns in toxicity of sublethal mixtures of metals and organic chemicals by Microtox and by DNA, RNA, and protein content of fathead minnows. Canadian Journal of Fisheries and Aquatic Sciences. 50:2245-2253.

SAS Institute Inc. 1987. SAS/STAT User's Guide, Release 6.03 Edition. Cary, NC.

Sexton, K., B. D. Beck, E. Bingham, J. D. Brain, D. M. Demarini, R. C. Hertzberg, E. J. O'Flaherty, and J. G. Pounds. 1995. Chemical mixtures from a public health perspective: the importance of research for informed decision making. Toxicology. 105: 429-441.

Slemr, F. and E. Langer. 1992. Increase in global atmospheric concentrations of mercury inferred from measurements over the Atlantic Ocean. Nature. 355: 434-437.

Solomon, K. R., D. B. Baker, R. P. Rechards, K. R. Dixon, S. J. Klaine, T. W. La Point, R. J. Kendall, C. P. Weisskopf, J. M. Giddings, J. G. Giesy, L. W. Hall, and W. M. Williams. 1996. Ecological risk assessment of atrazine in North American surface waters. Environmental Toxicology and Chemistry. 15:31-76.

Sorrano, R., F. Hernandez, J. B. Pena, V. Dosda, and J. Canales. 1995. Toxicity and bioconcentration of selected organophosphorus pesticides in *Mytilus galloprovincialis* and *Venus gallina*. Archives of Environmental Contamination and Toxicology. 29: 284-290.

U. S. Environmental Protection Agency. 1995. The Generic Expected Environmental Concentration Program (GENEEC), Version 1.2. Washington, D.C.: Environmental Fate and Effects Division, Office of Pesticide Programs.

Tomlin, Clive, ed. 1994. The Pesticide Manual. British Crop Protection Council, Surrey, United Kingdom.

Wetzel, Robert G. and Gene E. Likens. 1991. Limnological Analyses. New York: Springer -Verlag.

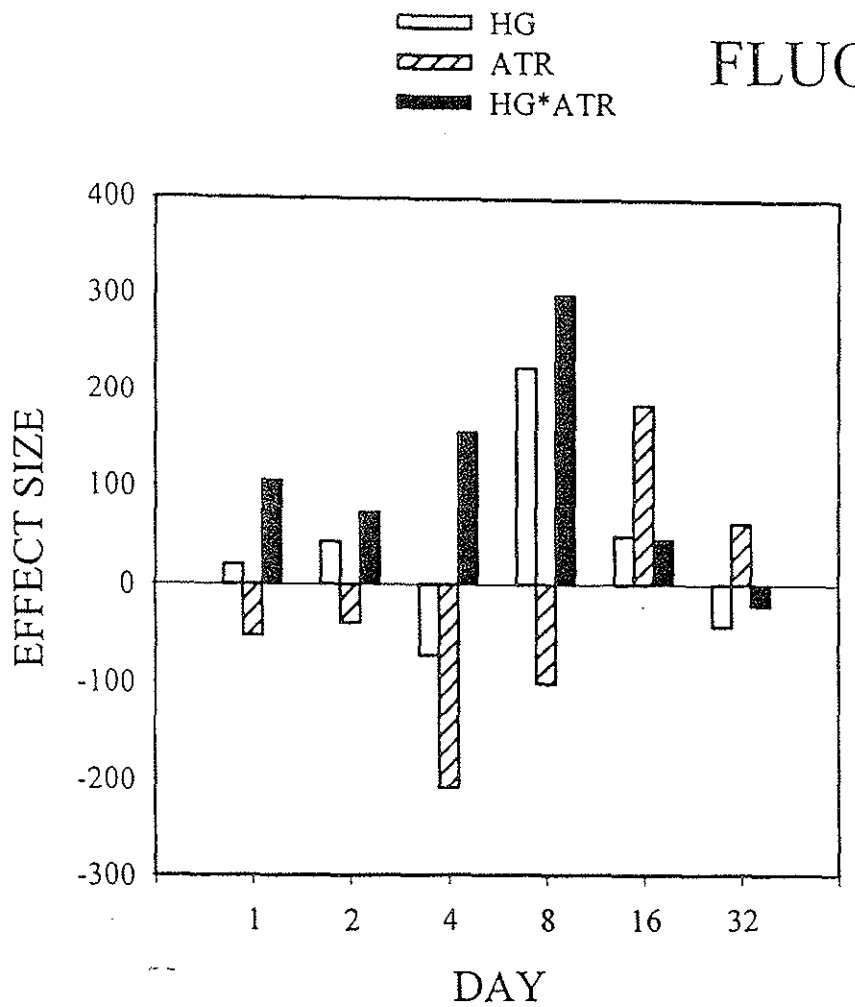

Figure 1a. Changes in effect sizes of methyl mercury, atrazine and methyl mercury x atrazine interaction for fluorescence over the 32 day sampling period.

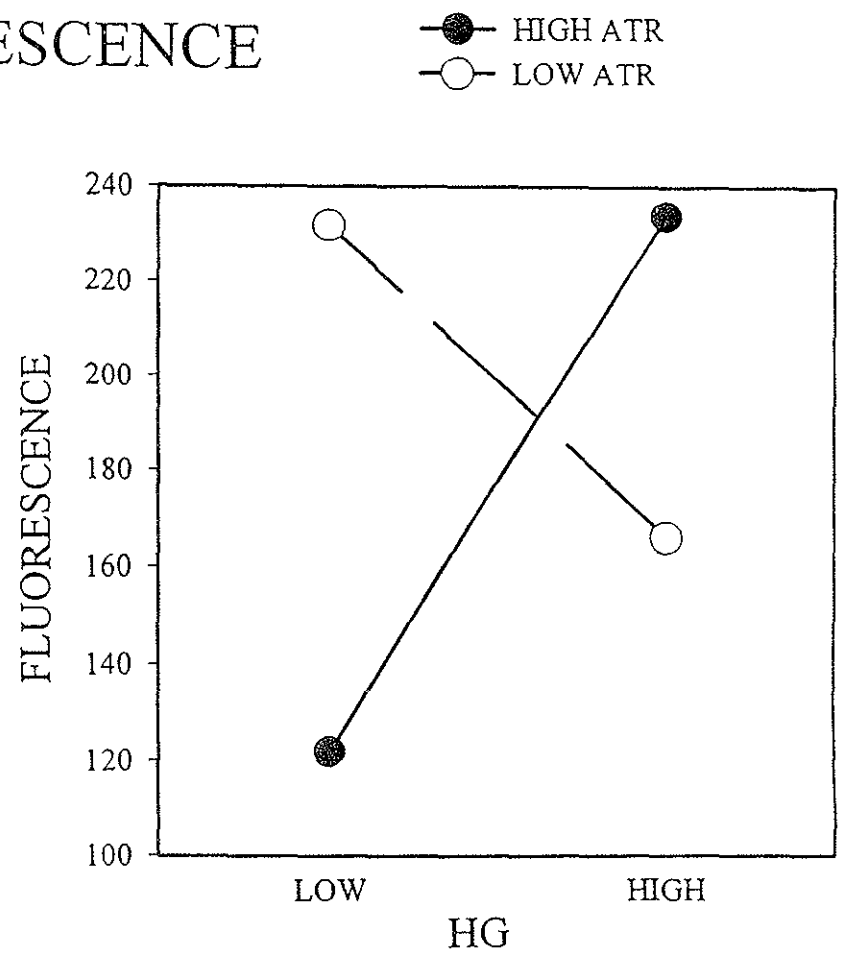

Figure 1b. Interaction of methyl mercury by atrazine for fluorescence over the 32 day sampling period.

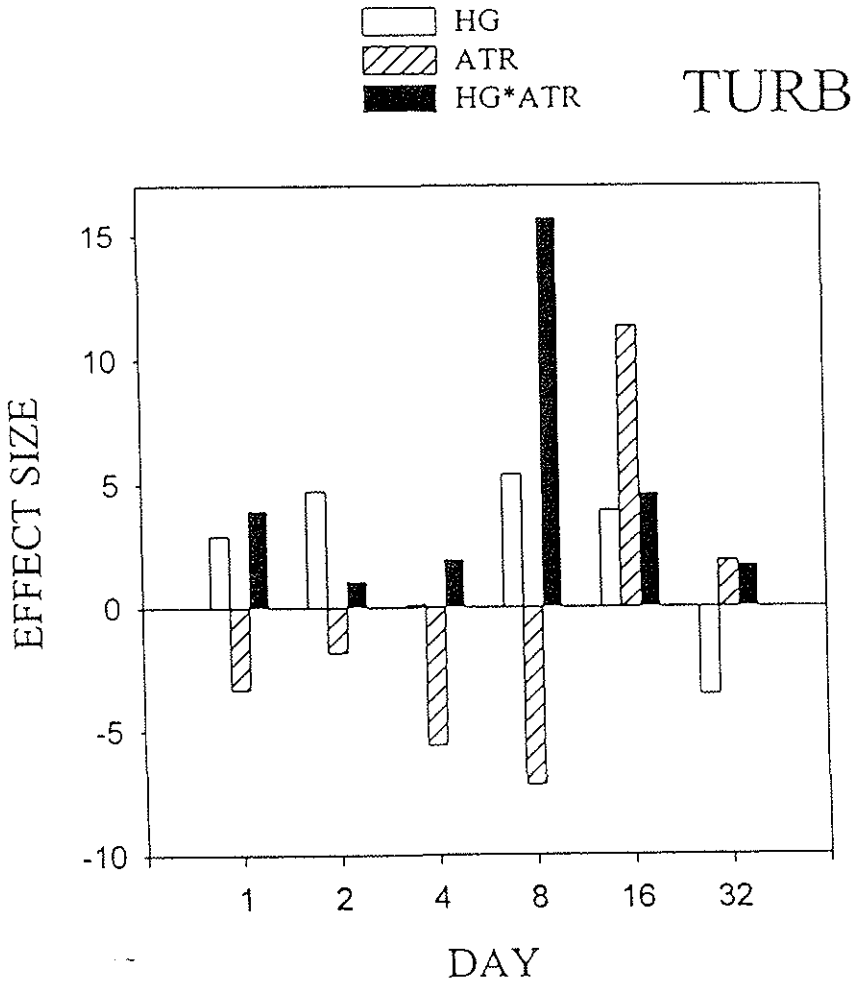

Figure 2a. Changes in effect sizes of methyl mercury, atrazine and methyl mercury x atrazine interaction for turbidity over the 32 day sampling period.

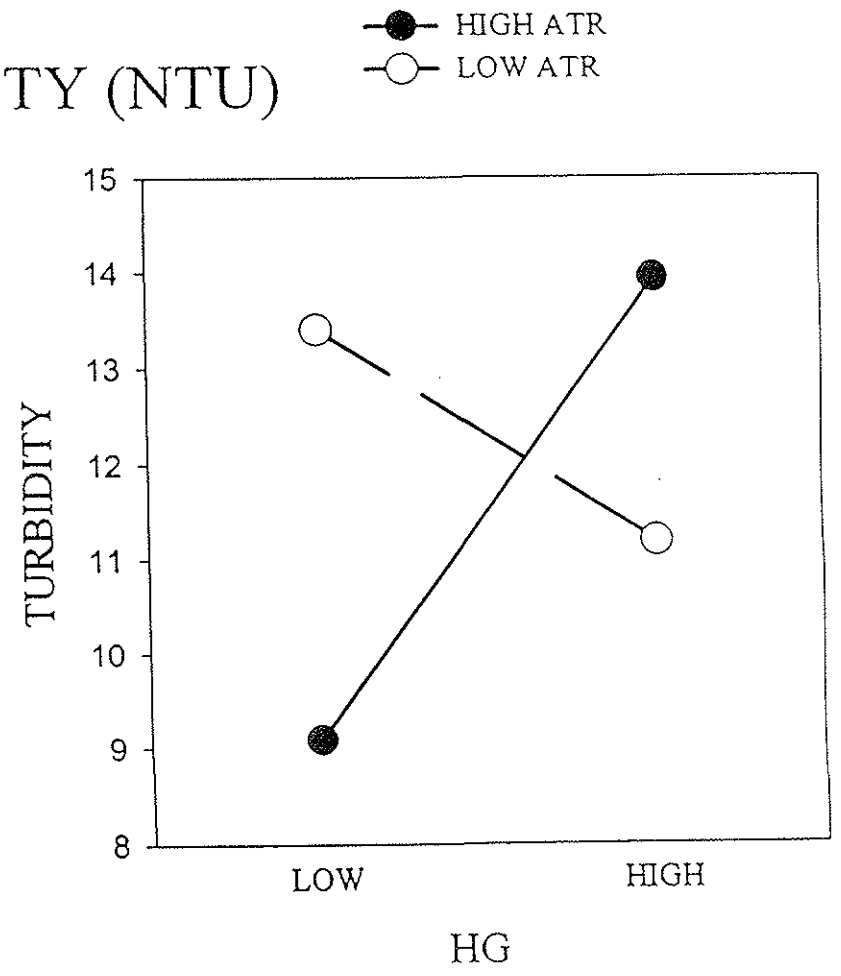

Figure 2b. Interaction plot of methyl mercury by atrazine for turbidity over the 32 day sampling period.

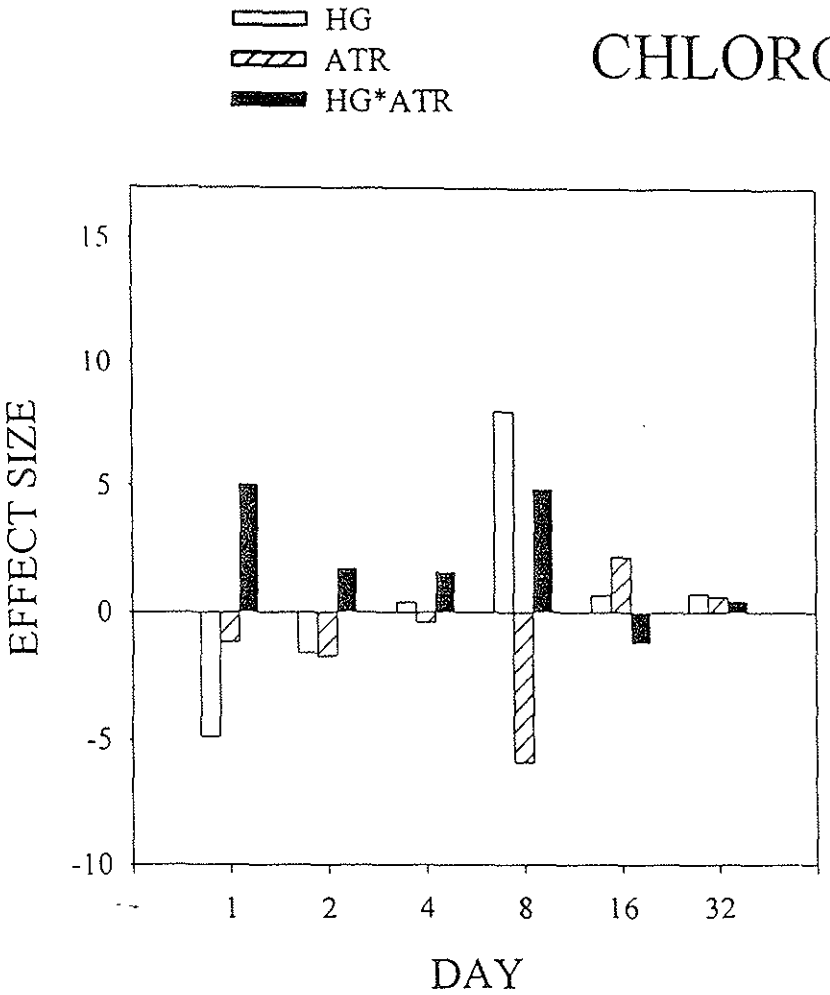

Figure 3a. Changes in effect sizes of methyl mercury, atrazine and methyl mercury x atrazine interaction for chlorophyll a over the 32 day sampling period.

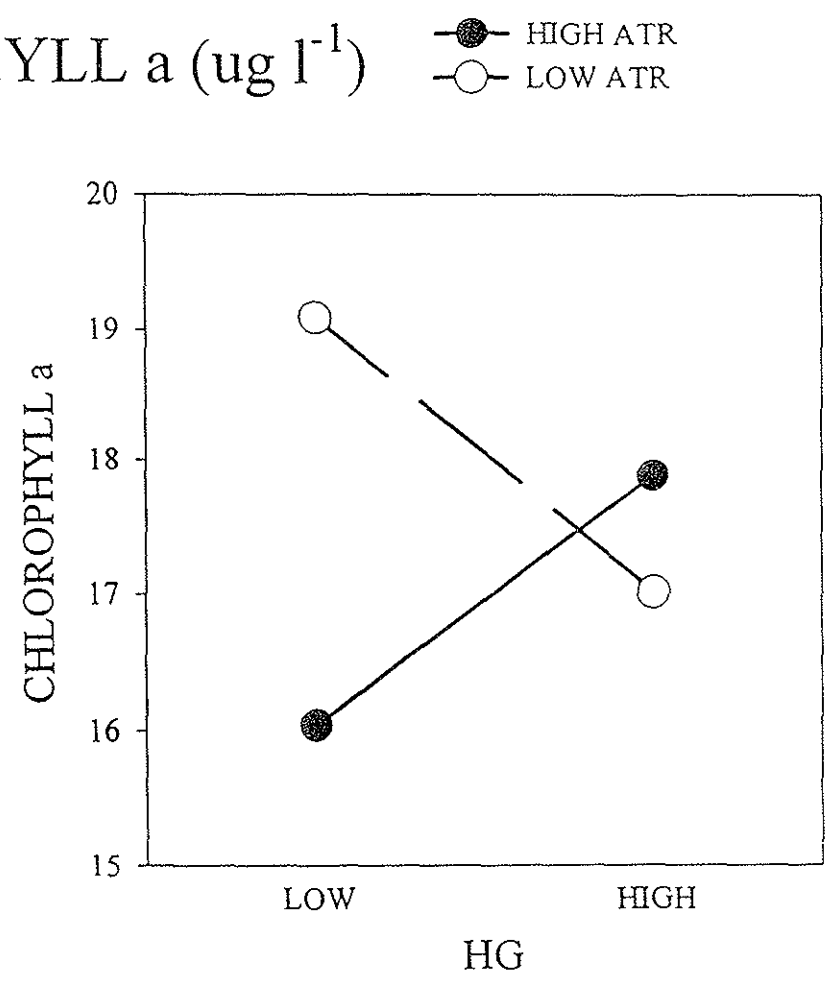

Figure 3b. Interaction plot of methyl mercury by atrazine for chlorophyll a over the 32 day sampling period.

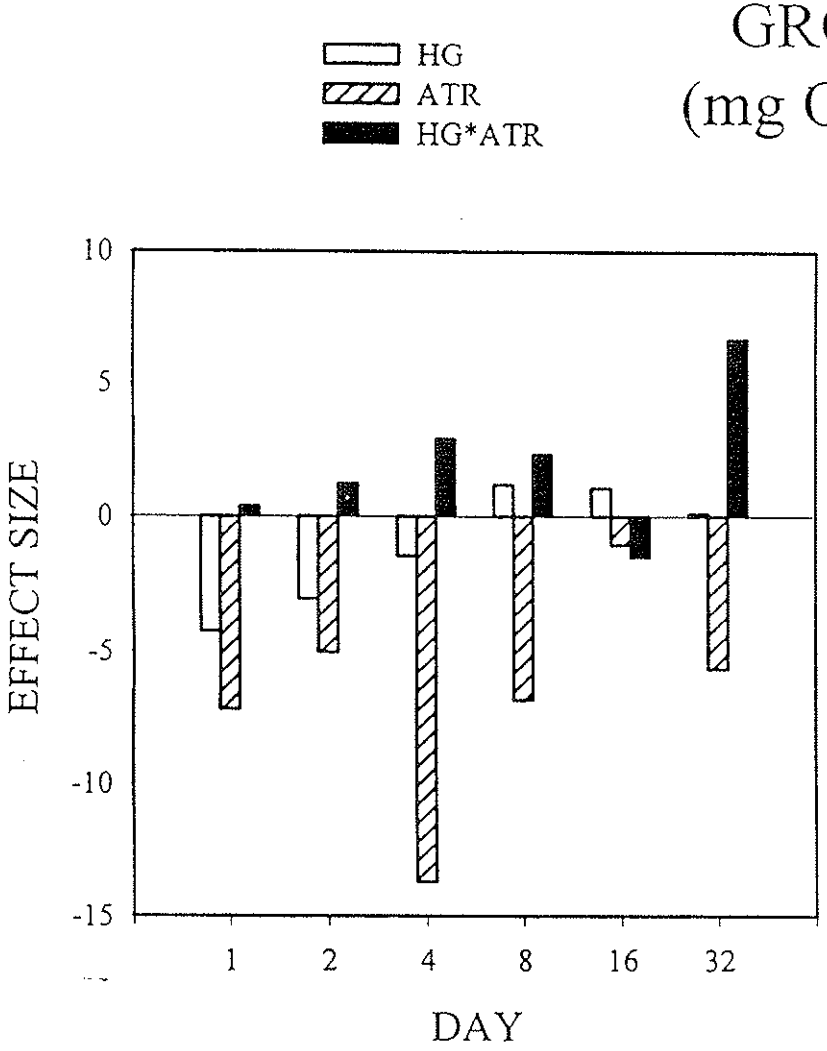

Figure 4a. Changes in effect sizes of methyl mercury, atrazine and methyl mercury x atrazine interaction for gross PPR over the 32 day sampling period.

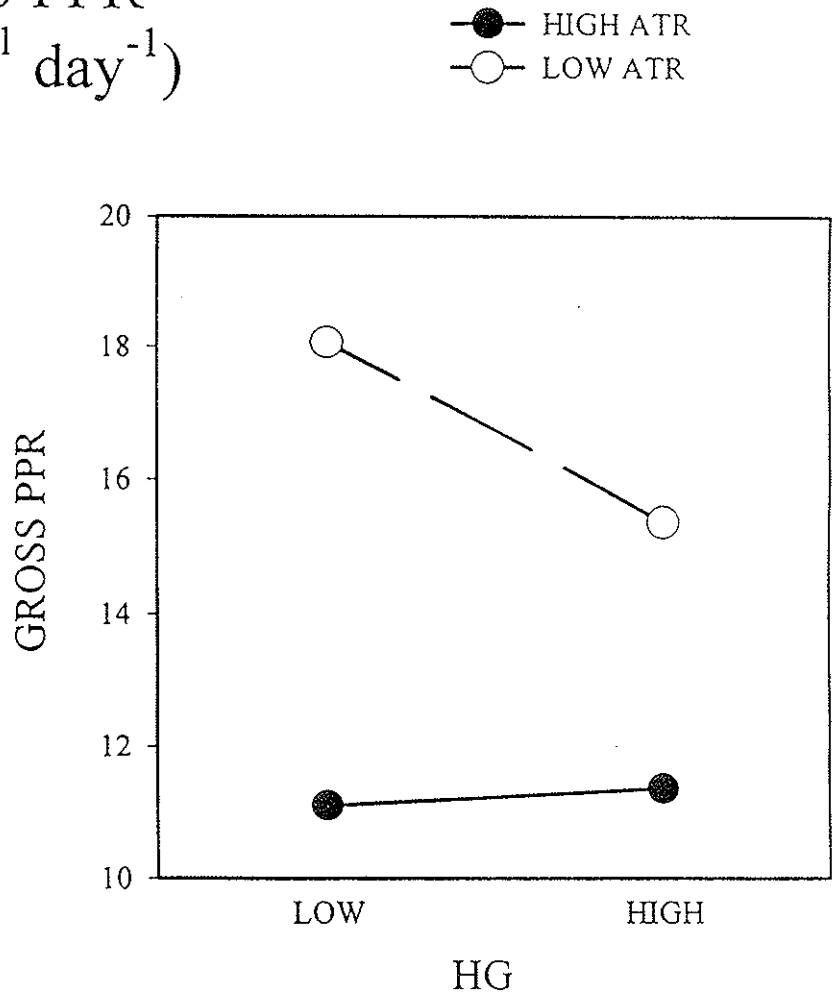

Figure 4b. Interaction plot of methyl mercury by atrazine for gross PPR over the 32 day sampling period.

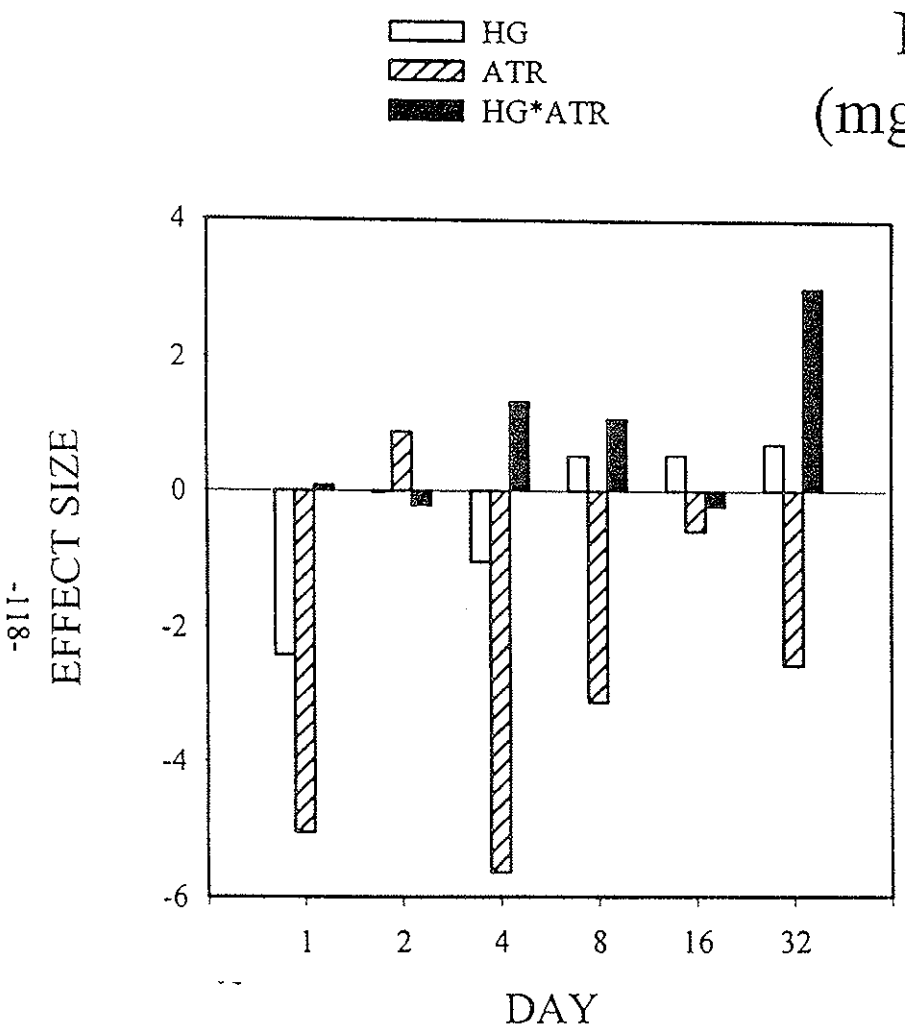

Figure 5a. Changes in effects sizes of methyl mercury, atrazine and methyl mercury x atrazine interaction for net PPR over the 32 day sampling period.

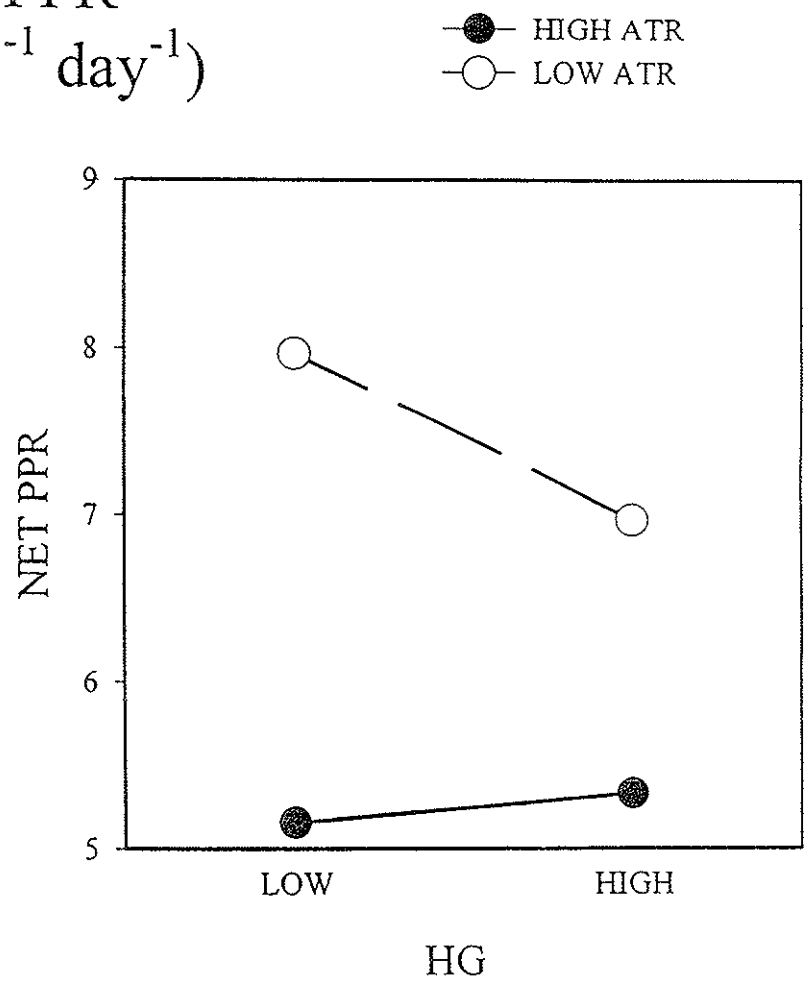

Figure 5b. Interaction plot of methyl mercury by atrazine for net PPR over the 32 day sampling period.