THE EFFECTS OF TEBUTHIURON ON AQUATIC PRODUCTIVITY

by

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A THESIS

IN

WILDLIFE SCIENCE

Submitted to the Graduate Faculty
of Texas Tech University in
Partial Fulfillment of
the Requirements for
the Degree of

MASTER OF SCIENCE

Approved

Accepted

May, 1985

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No. 52
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ACKNOWLEDGEMENTS

Funding for this research was provided by the Noxious Brush and Weed Control program at Texas Tech University.

I wish to thank my committee chairman, Dr. Brian Murphy, for his advice and support throughout the study. Committee member Dr. Dennis George provided helpful suggestions regarding the thesis. Committee member and good friend, Dr. Ed Cheslak, made a significant contribution to my aquatic biology background and to my graduate education in general.

Sincere appreciation is expressed to Tim Schlagenhaft, Dave Terre, and Todd Lawson for their assistance in field and laboratory work. I am especially indebted to Tim Berry, without whose hard work and extra effort this project never could have been completed.

Elanco Products Company provided residue analysis and input to the design and analysis of this experiment. In particular, I wish to recognize Dr. Roger Meyerhoff for his help and encouragement throughout the study and review of the manuscript.

I extend my gratitude to John Byrd for his stimulating questions and use of his personal library. Special thanks to Dave Wester for his patience and invaluable help with data analysis.

My wholehearted thanks to my wife, Kathy; her understanding and support smoothed the rough edges. We were a good team. Also, I extend thanks to my parents. The attainment of this degree would not have occurred without their love, wisdom, and support.

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ABSTRACT

Tebuthiuron (N-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]
=N,N'-dimethylurea) appears to control the riparian shrub saltcedar
(Tamarix spp.); however, its use is restricted since the fate and
effects of this herbicide in aquatic systems are unknown. Possible
tebuthiuron impacts on aquatic production were examined in ten
2846-L microcosms. Each microcosm contained sediment, water, algae,
micro- and macroinvertebrates, and fish. The following doses of
tebuthiuron were used: 0 (control), 10, 70, 200, 500, and 1000
µg/L. The control and the 200 µg/L treatment level were replicated
(n=3) to allow for specific statistical comparisons of treatment
effects. Data generated from all treatment levels was used in
tebuthiuron fate analysis and in correlation analysis between the
microcosm variables. The adsorption of tebuthiuron to sediments
contained in ten microcosms was described by the Freundlich
equation, $x/m = 3.24c^{0.68}$. Of the system variables investigated
(phytoplankton primary production, chironomid density and biomass,
and fish biomass), only chironomid larvae density exhibited
reductions at the 200 µg/L treatment level. The pathway by which
density depressions occurred is unknown. Possible explanations
include a direct toxic effect on the chironomids and/or a species
shift in the algal community resulting in a greater percentage of
"unpalatable" biomass. The changes in chironomid density suggest
that system effects may occur at the 200 µg/L treatment level.

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CHAPTER I

INTRODUCTION

There are many water allocation and supply problems that western land resource managers must address (Hyra 1978; Johnson 1978). An issue of particular importance is that of saltcedar (Tamarix spp.) control. Saltcedar was introduced into the southwestern United States from Europe, Africa, the Middle East, and the Far East in the early 1800s for use in windbreaks and as shade and ornamental trees (Smith and Rechenthin 1964). It has spread profusely, currently occupying about one million acres of western floodplain lands (Larner et al. 1974).

Saltcedar has a substantial water consumption rate of 28,042 m³ per hectare per annum (U.S. Dept. of Interior 1959). Busby and Schuster (1973) studied a 112-km section of the Brazos River in Texas and estimated that this species complex consumes 54,279,245 m³ of water annually. Considering concomitant usage of nutrients and space, saltcedar can significantly impact forage production, water supplies, and populations of terrestrial and aquatic organisms.

Although no satisfactory method of saltcedar control is in use at present (Hughes 1966), tebuthiuron (N-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl] N,N'-dimethylurea), has been shown to be a potentially viable saltcedar regulator (Jones et al. 1978).

Tebuthiuron is a substituted urea herbicide that has widespread application for the control of many weed and brush species in the United States. It has not yet been approved or registered by the

Environmental Protection Agency for near streams and lakes, effectively precluding its use on saltcedar. Part of the reason for this status is the lack of information on the fate and effects of tebuthiuron in aquatic systems that may receive runoff from treated saltcedar-dominated riparian zones. The purpose of this study, then, was to investigate possible tebuthiuron impacts on aquatic systems.

Literature Review

Values of Riparian Systems to Fish, Wildlife, Livestock, and Outdoor Recreation

The Council on Environmental Quality in 1978 concluded that no ecosystem is more essential to the survival of the nation's fish and wildlife resource than are riparian systems (American Fisheries Society 1980). Terrestrial organisms are usually in greater diversity and abundance in riparian areas as compared to upland sites (Waldrip and Malespin 1979). One reason for this phenomenon is that streamside vegetation serves as an ecotone between aquatic and upland systems (Odum 1978).

To an even greater extent, riparian ecosystems are of paramount importance to the continuance of aquatic biota in the West (Behnke 1977, Brown et al. 1977, Hubbard 1977, and Schmidly and Ditton 1978). Viable streamside vegetation provides shade, stream structure habitats (e.g., undercut banks), detrital nutrients and terrestrial insects to the aquatic food chain, and serves as a

buffer to protect watercourses from silt loads originating from upland sites (Brinson et al. 1981).

Riparian systems are often valuable areas for livestock production in the West and Southwest. Cattle are attracted to bottomlands because they prefer the quantity, quality, and variety of forage (Schmidly and Ditton 1978). Another attribute is that many riparian zones serve as a dependable water source. Since rangeland red meat production is increasing in importance (Harris 1977), it follows that riparian systems are likewise gaining in national food production importance.

Finally, riparian areas as compared to upland sites receive a disproportionate amount of consumptive and nonconsumptive natural resource use by the Western populace (Boer and Schmidly 1977). Riparian areas are an integral part of this recreational opportunity.

Degradation of Riparian Systems

Unfortunately, riparian ecosystems in the West and Southwest are being degraded (Brinson et al. 1981). Principal deteriorative causes that must be addressed by riparian system management include overallocation of water, indiscriminate riparian vegetation removal, overgrazing, and invasion of saltcedar (Anderson et al. 1977; Davis 1977; Behnke and Raleigh 1978).

Water allocations in many parts of the West approach or exceed the supply. For instance, $20 \times 10^9 \text{ m}^3$ per annum have been allocated from the Colorado River despite flows averaging less than 17×10^9

m³ annually between 1922-1966 (Johnson 1978). These water allocations are composed of many competing demands (e.g., agricultural and municipal). Additionally, groundwater withdrawal is so excessive that supplies over large areas are made unavailable to riparian systems (Fox 1977; U.S. Department of the Interior 1982). Since many states do not formally recognize a required minimum flow for aquatic and terrestrial biota (Nelson et al. 1978), the various surface and subterranean water withdrawals are free to deplete streamflows to the point where the environmental values of the streams are seriously impaired (Hubbard 1977). To help counter this possibility, techniques have been developed to assess required minimum stream flows for biotic maintenance (Stalnaker and Arnette 1976; Dewsnap and Jensen 1977; Nelson et al. 1978; Wesche and Rechard 1980; Orth and Maughan 1982). Required stream flow assessments, in combination with favorable legislation and increased water yields gained through other aspects of efficient riparian management (Horton and Campbell 1974), will help assure minimum flow for viable stream/riparian habitat maintenance.

The increasing demands for water have impelled the development of methods that purportedly maximize water harvest from stream systems. Since it is known that phytotranspiration can reduce surface and subsurface waters in the West (Brown et al. 1977; Richard and Cushing 1982), many studies have been conducted to investigate the annual loss of water to woody riparian vegetation. Robinson (1965) estimated that 3,083,814 m³ of water were lost

annually by evaporation or transpiration on 6,070,500 ha of phreatophytes (brushy species that transpire "excessive" amounts of groundwater) in 17 western states. An investigation by Ffolliat and Thorud (1974) indicated that water yield from riparian sites in Arizona might be increased as much as 74,012 m³ per annum by total clearing of woody vegetation. This estimate may be extremely inflated because several total clearing projects have documented little or no significant water yield increases (Campbell 1970; Horton 1972; Brown et al. 1977; Johnson 1978). Factors such as increased surface evaporation in treated areas have negated much of the predicted yield increases. In addition, total clearing projects require indiscriminate clearing and thus destruction of riparian habitats.

Justification for total "phreatophyte-removal" projects should be based on the value of additional water harvested and the cost-benefit analyses of affected land uses, particularly fish and wildlife production, livestock production, and recreation. Riparian ecosystems, however, can be managed to increase water yields and still maintain or improve existing habitat (Horton 1977). Projects aimed at this multiple use objective would require selective, rather than complete, removal of riparian vegetation (Campbell 1970).

Overgrazing results in a wide spectrum of detrimental impacts to riparian ecosystems that include deterioration of fish and wildlife habitat and forage production (Gunderson 1968; Carothers 1977; Winegar 1977; Behnke and Raleigh 1978; American Fisheries

Society 1980; Brinson et al. 1981; Rickard and Cushing 1982). Behnke (1977) stated that the best opportunity for increasing resident fish populations in the West was to improve stream/riparian habitats that have been adversely affected by grazing. To accomplish this improvement, cattle exclusion from all riparian areas is often recommended (Brown et al. 1977). Although permanent or temporary cattle exclusion may be warranted in particular cases, management solutions to the overgrazing problem are more complex and are not easily found. In actuality, proper grazing may have no significant impact on some riparian systems. Heede (1977) used controlled grazing in combination with other measures to restore a damaged (overgrazed) riparian system. Thus, livestock production under proper grazing management should be considered a legitimate use of some riparian areas (Behnke and Raleigh 1978). The American Fisheries Societies' (1980) Riparian Habitat Committee concurred with this philosophy and stated that proper grazing could become an important riparian management tool benefiting fish and wildlife habitats.

Saltcedar (Tamarix spp.) deteriorates bottomlands (Anderson et al. 1977). This species complex can outcompete native riparian species (Johnson 1978; Boer and Schmidly 1977), many times forming dense stands that replace forage (Horton 1977). Reduced avian and mammal populations often occur in saltcedar invaded areas versus bottomlands relatively saltcedar free (Anderson et al. 1977; C. Edwards unpub. data). Livestock production is also affected by

reduced forage and water. In addition, extensive thickets of saltcedar make physical management of livestock difficult.

Aquatic biota are subject to the direct effect of water reduction resulting from the water-use rate of saltcedar. Elimination and subsequent replacement of saltcedar by native riparian vegetation would possibly decrease water usage rates and at least result in increased fish and wildlife habitat viability and livestock forage production.

Saltcedar usually occurs in lower elevation ($< 1,067$ m) riparian areas (Pase and Layser 1977). Horton and Campbell (1974) proposed that phreatophyte control would be most effective on lower elevation flood plains which support dense stands of phreatophytes. Davis (1977) suggested that southwestern water yields could be increased and also improvements made to the condition of the riparian habitat if control efforts were concentrated on the upper forested watersheds and the floodplains below 1,067 m with dense stands of mesquite or Tamarix spp.

To illustrate, after the majority of the Rock Creek, Arizona, chaparral dominated watershed burned, subsequent herbicide treatment of the watershed and riparian areas prevented the rejuvenation of the nonriparian chaparral community. The sparsely forested vegetation along the drainage was converted to a dense mixed broadleaf deciduous forest. Habitats are now provided to species such as bear and turkey, neither of which had used the area before treatment (Brown et al. 1977).

Tebuthiuron Usage Benefits

Considering the formidable impediment saltcedar is to terrestrial and aquatic habitats, water supplies, and forage production, much research has been conducted on saltcedar control. Recently, tebuthiuron has been shown to be a potentially viable saltcedar regulator (Jones et al. 1978). This substituted urea herbicide was first developed in the early 1970's. Since then it has been applied experimentally in various ecosystems within the United States and Canada. At high application rates (5.6-10.0 kg/ha, active ingredient) total vegetation kill resulted. It has been applied in this dosage range for the control of vegetation on railroad rights-of-way, along highway guardrails, and on industrial sites (Christensen et al. 1974; Ford 1974; Lade et al. 1974). Application rates of up to 13.4 and 17.9 kg/ha (a.i.) have been used for the control of hardwoods in pastures (Steinert and Stritzke 1975) and southern pine forests (Peevy 1975).

At lower application rates (2.2 kg/ha, a.i.), selective control of annual broadleaf and grassy weeds, and some brush, can be achieved (Christiansen et al. 1974; Stritzke 1976; Jones and Pettit 1978). In this regard, the use of tebuthiuron in rangeland ecosystems for the selective control of noxious brush and weeds is expected to increase dramatically in the near future (Dr. R. E. Sosebee pers. comm.). Potential areas of use for tebuthiuron have been identified in 24 states. Treated acres in 1982 and projected treated acreage in 1986 are approximately equal to 0.06% (113,316

ha) and 0.23% (404,700 ha), respectively, of the total grazeable hectares in the 24 states (Elanco Products Company 1982).

Although tebuthiuron is expected to be widely used, there is a lack of data on the potential comprehensive ecological effects of this herbicide. Groundwork, however, has been laid to study these ecological effects.

Fate of Tebuthiuron in Treated Watersheds and Receiving Waters

Tebuthiuron is moderately soluble in water (2.5 g/L) and therefore has potential for movement within and from the terrestrial environment (Elanco Products Company 1982). Moreover, this herbicide is nonvolatile (2×10^{-6} mm Hg at 25°C; Weed Science Society of America 1979); consequently, runoff and leaching are the primary transport mechanisms of tebuthiuron from terrestrial to aquatic systems. To characterize the extent of tebuthiuron movement within and from terrestrial systems into receiving waters, several experiments evaluating the fate of tebuthiuron in various components of the watershed and subsequent transport to adjacent water bodies have been performed. A maximum of 4.5% of the tebuthiuron applied to watersheds (2.24 kg a.i./ha) was found to run off into adjacent water bodies (Elanco Products Company 1982). This result is similar to that found for fluometuron (Baldwin et al 1975), which has a soil mobility that resembles that of tebuthiuron (Chang 1977).

After watershed treatment, tebuthiuron concentration in receiving lotic waters exhibited a range of nondetectable to 0.083 mg/L (Elanco Products Company 1983). Downstream of the study sites,

no traces of tebuthiuron were found. In two studies (Elanco Products Company 1983) using catchment ponds as receiving waters the maximum concentration of tebuthiuron detected was 0.18 mg/L (4.5% of the applied herbicide). Tebuthiuron concentrations in the catchment ponds declined to 0.04×10^{-2} and 0.03 mg/L six months post treatment.

Eaton et al. (1976) demonstrated the loss of tebuthiuron by uptake and metabolism in some plant species as well as the direct loss from soil through the formation of volatile metabolites. Additionally, tebuthiuron degradation through microbial action and photodecomposition is thought to be significant (Elanco Products Company 1983). Much of the applied tebuthiuron, however, remains for various time periods within natural ecosystems in soil and vegetation. Garcia and Gontarek (1975) found that 47 to 85% of the pellet-applied tebuthiuron (2.2-, 4.5-, and 9.0 kg/ha) persisted in the soil 3 months after application, primarily in the upper layers (0 to 15 cm). They hypothesized the residues were adsorbed to and/or absorbed by the humus in the soil. By the fourteenth month after treatment, the soil residues had declined to 2 - 12% of the original dose. Soil analysis after application of ^{14}C labeled tebuthiuron has indicated that the herbicide leaches slowly and that groundwater contamination is unlikely (Elanco Products Company 1982). Small amounts (<112 g/ha) of herbicide were found in coarse soil at a maximum depth zone of 45-60 cm. This detection was made 799 days after treatment, following 231.7 cm of precipitation.

Following tebuthiuron application in semi-arid areas on sandy soils, woody species continually defoliate and refoliate before root mortality occurs (Sosebee 1978). This phenomenon occurs even though soil residues of only parts per billion persist after 14 months (Garcia and Gontarek 1975). In an effort to explain this phenomenon, Garcia and Lee (1979) hypothesized the existence of a tebuthiuron cycle, as follows: Absorption of the herbicide from the soil by vegetation decreases the soil residues. Since precipitation is minimal in these areas, lesser amounts of the chemical are lost to leaching and surface runoff than in more humid climates. Ultimately, the tebuthiuron in the plant attains a lethal concentration after which defoliation and mortality occur. Tebuthiuron and/or its metabolites are then again available for plant absorption after decomposition of the leaf and stem litter, thereby beginning another cycle. This cycle would set up the possibility for multiple exposures of tebuthiuron to the aquatic system after a single application (the usual mode of treatment). These exposures would likely decrease in maximum concentration through time due to degradation of the compound.

Finally, some portion of the tebuthiuron taken up in plants probably will be ingested by animals. In one study, various mammals were given a single oral dose of 10 mg tebuthiuron/kg body weight. Approximately 90% of the original compound and its metabolites (primarily derived from demethylation and hydroxylation) were excreted in the feces and urine of all subjects by 96 hours (Morton

and Hoffman 1976). Other studies have shown very similar results with usually less than 5% of the administered dose excreted as intact tebuthiuron (Elanco Products Company 1983).

Single-Species Acute and Chronic Toxicity Studies

Toxicity of tebuthiuron to selected aquatic plants, invertebrates, and fish has been investigated (Elanco Products Company 1983). Nitrogenase activity of the blue-green alga Anabeana flos-aqua was monitored over a 48-hour exposure to 0.0, 0.5, 3.0, or 10.0 mg/L tebuthiuron. Only at the 10.0 mg/L concentration was a reduction in activity noted (Elanco Products Company 1983). Populations of Selenastrum capricornutum were temporarily depressed at tebuthiuron concentrations above the no-effect level 0.013 mg/L (Elanco Products Company 1983).

The 96-hour LC50's for crabs (Uca pugilator) and pink shrimp (Penaeus duorarum) exposed to tebuthiuron were 48 mg/L and greater than 320 mg/L, respectively. The Daphnia magna 48-hour EC50 (effective concentration), based on immobilization, was 297 mg/L of tebuthiuron (Elanco Products Company 1983).

Both acute and chronic studies have been conducted with fish. Bluegill (Lepomis macrochirus) and rainbow trout (Salmo gairdneri) 96-hour LC50 values were determined as 112 mg/L and 144 mg/L, respectively (Elanco Products Company 1975). Bluegills were exposed to aquaria concentrations of 0, 1, 10, or 25 mg/L tebuthiuron for 12 days. No elevation of liver microsomal enzyme activity occurred (Morton and Hoffman 1976). Although bluegill converted tebuthiuron

to several metabolites (Morton and Hoffman 1976), 92.2 and 94.6% of the 10 and 25 mg/L dose, respectively, remained unchanged in the aquaria water. A rainbow trout embryo-larvae test exhibited a no-observed-effect herbicide level at 26 mg/L (45 day duration). Embryos and larvae of the fathead minnow (Pimephales promelas) are less tolerant than any life stage of any fish species studied so far, having a no-observed-effect level of 9.3 mg/L after 33 days (Elanco Products Company 1983).

Tebuthiuron Tissue Residues in Fish

A residue investigation with bluegill exposed to ^{14}C -labeled tebuthiuron was performed at concentrations of 0.01 and 1.0 mg/L. During the 28-day exposure period, maximum tissue herbicide concentration was found to occur within 7 days and decline thereafter. This concentration of tebuthiuron and metabolites was nearly 10 times the concentration found in water samples taken concurrently (Meyerhoff 1981).

Multispecies Association Toxicity Tests

A "mini-ecosystem" study has been performed (Elanco Products Company 1983). Fathead minnows, snails, midges (Diptera), algae, and hydrilla were exposed to 0.01, 0.03, and 0.10 mg/L tebuthiuron in water for 20 months. Populations of fathead minnows and snails were not affected by treatment. Similarly, no treatment influence on midge emergence or on hydrilla occurred. Diatoms and blue-green algae were recruited at all exposure levels.

Research to date has determined acute and chronic tebuthiuron toxicity levels for selected fish, invertebrate, and aquatic plant species (Elanco Products Company 1983). No examination of impacts on integrated multispecies/multitrophic level systems, however, has been undertaken. The objective of this study was to determine the effects of tebuthiuron on the productivity of phytoplankton and the standing crops of invertebrates and fish in multispecies microcosms. The potential impacts of this chemical brush control agent on aquatic food chains can then be evaluated.

CHAPTER II

MATERIALS AND METHODS

Microcosm Description

Ten multispecies microcosms were established outdoors on the Texas Tech University campus. Microcosms used in this study were constructed to simulate, as much as was possible under the prevailing time constraints, isolated pools in west Texas streams. This natural situation was of interest since these streams do not usually flow year-long, forcing the aquatic biota to occupy isolated pools. Moreover, tebuthiuron concentration may be appreciably higher in isolated pools as opposed to shallow runs or pools with inflow and outflow (i.e., a flowing stream). Consequently, the potential for herbicide effects on aquatic organisms may be greatest in these isolated pools.

Round galvanized steel tanks (2846-L capacity) were partially filled with six centimeters of local topsoil (90.3% sand, 3.0% silt, 6.7% clay, 7.81 pH, 1.51% organic matter, and 6.12 meq/100 g soil cation-exchange-capacity). Four-0.25 m² Hester-Dendy invertebrate samplers (Hester and Dendy 1962) were placed on the sediment surface and four sediment sampling devices (1-L plastic bags attached to 185-cm² metal frames) were buried in the sediment of each tank.

Tanks were filled with water from a local playa lake, and dechlorinated municipal water was used to maintain the water level at a depth of 0.55 m (2083 L volume). Thus a relatively high water

surface to volume ratio was created in each microcosm, similar to that found for stream pools.

The outdoor location allowed exposure to area weather conditions and colonization by indigenous macroinvertebrates and algae. Additionally, each system was inoculated with 45 L of water from a local eutrophic pond. Phytoplankton and invertebrate communities were allowed to develop naturally through colonization and succession for 30 days before tanks were dosed with tebuthiuron; this increased the opportunity for the microcosms to be functionally similar to, and thus more predictive of, isolated stream pools (Bowling et al. 1980).

One day (22 July 1982) before treatment, 65-1.6 cm fathead minnow (Pimephales promelas) larvae were stocked in each tank to allow the assessment of potential tebuthiuron impacts on a typical west Texas stream fish species. The addition of this level of organization was also important because macro-secondary consumers frequently influence the rest of an ecological system (Pilson and Nixon 1980).

Treatment Design

The following doses of tebuthiuron (97.5% technical material) were used in this study: 0 (control), 10, 70, 200, 500, and 1000 $\mu\text{g/L}$. Since 200 $\mu\text{g/L}$ approximates the highest transient concentration of tebuthiuron found in surface waters (180 $\mu\text{g/L}$, Elanco Products Company 1983), the control and the 200 $\mu\text{g/L}$ treatment level were replicated (three tanks each) to allow for

specific statistical comparisons of treatment effects. The remaining four treatment levels were not replicated. Data generated from these nonreplicated treatment levels, although not statistically evaluated for tebuthiuron impacts, was presented both as supplemental data and combined with replicated treatment data and used in tebuthiuron fate analysis. Moreover, all treatment level primary production, chironomid standing crops, and fish standing crop component data, were employed in correlation analysis between the various components.

Water and Sediment Residue Analysis

Water samples (1 L) were collected eight times (-1, 1, 3, 7, 14, 34, 64, and 108 days after treatment [DAT]) from each microcosm for analysis of tebuthiuron concentrations. Each tank sample was a composite of 250-ml aliquots collected from the surface of each tank quadrant. Sediment samples were collected four times (-3, 7, 64, 108 DAT) from each tank for tebuthiuron analysis. Tebuthiuron concentration in water was determined by gas chromatography using a flame photometric detector. Spiked water samples of 10 $\mu\text{g/L}$ and 1000 $\mu\text{g/L}$ indicated recoveries of 93% (CV=7.44%) and 98% (CV=1.96%), respectively. Sediment tebuthiuron residues were determined using gas chromatography-flame photometric methods for the -3 DAT and 7 DAT samples and gas chromatography-mass spectrometric methods for the 64- and 108 DAT samples. These two methods were found to be linearly correlated ($r^2 = 0.96$, $P \leq .001$) and hence were considered to supply essentially identical analyses. Spiked sediment samples

of 100 $\mu\text{g/kg}$ and 500 $\mu\text{g/kg}$ resulted in recoveries of 58% (CV=12%) and 54% (CV=18%), respectively. All analyses were conducted by the Analytical Chemistry Group of Lilly Research Laboratories at Greenfield, Indiana, according to inhouse analytical procedures (Loh et al. 1978).

The adsorption of herbicide on the sediments as a function of herbicide concentration in the water was described by the Freundlich equation (Peck et al. 1980):

$$x/m = k [c^{1/n}] \quad (1)$$

where x/m is the amount of tebuthiuron absorbed per unit mass of sediment ($\mu\text{g/kg}$), c is the concentration in water ($\mu\text{g/L}$), n is a constant, and k is the Freundlich adsorption coefficient. After $\log x/m$ versus $\log c$ data are plotted, a significant or nonsignificant correlation is determined by the least squares method (Steel and Torrie 1980). If a significant relationship is found, proceed to solve for the k and n variables using the regression prediction equation. This equation is of the form:

$$\log x/m = a + b(\log c) \quad (2)$$

where a is the intercept and b the slope. The Freundlich equation variable n is $1/b$. The Freundlich adsorption coefficient (k) is a . All variables have then been obtained and the Freundlich equation can be constructed.

Water and sediment tebuthiuron residue-versus-time data were plotted and fitted through the use of several mathematical functions (linear, exponential, logarithmic, and power). Each function was

converted to its linear form and then fit by least-squares regression. The best-fit curve describing tebuthiuron fate over time in water and sediment was chosen using the highest r^2 value as the criterion of selection.

Primary Production Analysis

Primary production in each microcosm was assessed 15 times (-9, -7, -1, 1, 2, 3, 5, 7, 11, 14, 35, 42, 57, 64, 106 DAT) using the light bottle-dark bottle technique (Vollenweider 1974). Initial dissolved oxygen levels were measured and duplicate light and dark bottles were placed at mid-depth (0.3 m) in each tank from 1000 h to 1600 h. After removal, bottles were immediately placed in ice, transported to the lab, and analyzed for dissolved oxygen content with a meter.

Macroinvertebrate Density and Biomass Analysis

Density and biomass of aquatic invertebrate species were measured four times (-3, 7, 64, 106 DAT). Hester-Dendy samplers were removed from each microcosm and all attached material scraped and rinsed from the plates. This material was preserved in 70% ethanol for later identification, separation by taxa, enumeration, and biomass determination (Lind 1979).

Chironomid larvae (Diptera: Chironomidae) were the only macroinvertebrates analyzed for the following reasons: 1) chironomids are abundant in most natural aquatic and semiaquatic habitats and usually account for at least 50% of the combined

macroinvertebrate species composition (Coffman 1978); 2) chironomids are very valuable as fish food organisms (Odum 1971; Wirth and Stone 1956); and 3) the chironomid population was assumed to directly reflect phytoplankton primary production. The hypothesis that chironomid population parameters are good indicators of phytoplankton production was formulated on two premises. First, there was little coarse detritus (e.g., leaves, branches) in the microcosms, thereby reducing the likelihood of significant numbers of macroinvertebrate shredders (particle size range of food greater than $10^3 \mu\text{m}$). Also, Canton and Chadwick (1983) have found multiplate artificial substrates to select against scrapers (periphyton and vascular hydrophyte herbivores) and favor filterers and gatherers (particle size range of food less than $10^3 \mu\text{m}$).

Fish Biomass Analysis

Fathead minnows were sampled via electrofishing six times (3, 7, 14, 41, 56, 116 DAT). Three fish/tank were randomly targeted for sampling at each date except at the study termination; all remaining fish in each microcosm were then removed (116 DAT). Individual live weights were measured to estimate total biomass in each system.

Statistical Analysis

Pretreatment samples were taken to characterize the initial state of the primary production and chironomid standing crop components. The fish biomass component did not require pretreatment analysis since it was gnotobiotic. The average coefficients of

variation (CV, Steele and Torrie 1980) were estimated from this component pretreatment data for the control and the 200 $\mu\text{g/L}$ treatments to determine whether these microcosms satisfied the replicability criteria of Giddings and Eddlemon (1979). Since the average CV was found to be above the range considered acceptable (10 to 30%), analysis of covariance (Steel and Torrie 1980) was employed (completely randomized splitplot design). This method adjusted the productivity values of all tanks as if they began at equal initial levels of primary production and chironomid density and biomass. Since all tanks began at equal fish biomass, fish biomass data was described using the analysis of variance over a completely randomized splitplot design (Steel and Torrie 1980).

Mean values of the primary production, chironomid density and biomass standing crops, and fish biomass standing crop components of the control and 200 $\mu\text{g/L}$ treatments were compared over time and within each sampling period. Linear regression analysis (Steele and Torrie 1980) was used to relate two components (e.g., chironomid density versus primary production) by using values from all microcosms ($N = 10$). These values were not adjusted by the covariate.

CHAPTER III

RESULTS AND DISCUSSION

Tebuthiuron Fate

Tebuthiuron concentration declined in the water column of all microcosms over the course of the experiment. This loss was best described by negative exponential mathematical models (Table 1). With the exception of the two lowest doses, tebuthiuron was found in the sediments of each treated tank. The dynamics of tebuthiuron in the microcosm sediment components was described by logarithmic mathematical models (Table 2).

The relationship between tebuthiuron concentration in microcosm water and sediment was described by the Freundlich equation (Peck et al. 1980). This equation was found to be valid since a significant linear relationship was found when the log of the tebuthiuron concentration in sediments was plotted against the log of the herbicide concentration in water (Figure 1). The Freundlich equation, as determined by this investigation, is $x/m = 3.24c^{0.68}$ ($R^2=0.83$).

At the final sample date (109 DAT) 14.9 to 32.0% of the initial inoculum was unaccounted for in each system. Although not volatile (2×10^{-6} mm Hg at 25°C), tebuthiuron has been found to be susceptible to breakdown through both metabolism and direct photodecomposition (Elanco Products Company 1983). Since residue analysis was performed for the parent compound only, biodegradation and

Table 1. Fate of tebuthiuron in the water columns of microcosms receiving five treatment levels. Data in $\mu\text{g/L}$ are fit with an exponential function of the general formula: $y = ae^{-bt}$

Detected concentration ($\mu\text{g/L}$) in Tebuthiuron water at last sample treatment date (109 days-after ($\mu\text{g/L}$) treatment)		Coefficient of determination Prediction equation ^d (r^2)	
10	7 ^a	$Y = 10e^{-.004x}$	0.69
70	53 ^{a,c}	$Y = 69e^{-.004x}$	0.87
200	133 ^b	$Y = 221e^{-.005x}$	0.74
500	350 ^a	$Y = 556e^{-.005x}$	0.83
1000	790 ^a	$Y = 1,043e^{-.003x}$	0.77

^aData from one microcosm/treatment level.

^bData averaged from three microcosms/treatment level.

^cDetected concentration ($\mu\text{g/L}$) in water at 64 days-after-treatment

^dEstimated tebuthiuron concentration ($\mu\text{g/L}$) in water at x days-after-treatment.

Table 2. Fate of tebuthiuron in the sediments of microcosms receiving five treatment levels. Data in $\mu\text{g/L}$ are fit with a logarithmic function of either one of two general formulas:
 $y = a - b \ln x$

Tebuthiuron treatment ($\mu\text{g/L}$)	Concentration ($\mu\text{g/kg}$) in sediment (109 days-after- treatment)	Prediction Equation ^d	Coefficient of determination (r^2)
10	---- ^a	-----	----
70	---- ^a	-----	----
200	80 ^b	$Y = 200 - 30 \ln x$	0.39
500	210 ^c	$Y = 200 - 2.71 \ln x$	0.02
1,000	390 ^c	$Y = 150 + 50 \ln x$	0.99

^aConcentration in sediment was not detected.

^bData averaged from three microcosms/treatment level.

^cData from one microcosm/treatment level.

^dEstimated tebuthiuron concentration ($\mu\text{g/kg}$) in sediment at X days-after-treatment.

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photodecomposition of the parent compound may account for a significant portion of the loss of the herbicide.

Tebuthiuron Effects

Mean primary production, chironomid biomass and density, and fish biomass values of all 10 microcosms under six tebuthiuron treatment levels are listed in Table 3. Primary production, chironomid biomass and density, and fish biomass treatment, time, and treatment-time interaction F and probability values for the control and the 200 $\mu\text{g/L}$ treatments without and with the covariate adjustment are listed in Table 4.

Phytoplankton Primary Production

Control and 200 $\mu\text{g/L}$ treatment primary production means adjusted by the covariate are plotted over time in Figure 2. The only significant differences ($P \leq 0.05$) observed occurred between some sample date means (time) (Table 4). Time was expected to show significant differences between some sample date means due to colonization and succession. Not only were treatment means (i.e., means within sample dates and means over time) not found to be different; the probability that chance alone was responsible for differences in the control and 200 $\mu\text{g/L}$ over time treatment means doubled when the covariate was included. This implies that a large amount of the difference between the treatment means was a result of differences between microcosm pretreatment productivity states.

Table 3. Primary production, chironomid biomass and density, and fish biomass values of six tebuthiuron treatment levels analyzed via a microcosm arrangement. Values are covariate-adjusted means over time (there is no covariate adjustment for fish).

<u>Covariate Adjusted Means Over Time</u>					
Treatment	Microcosms/ treatment	Primary production (O ₂ mg/L/hr)	Chironomid density (numbers/m ²)	Chironomid biomass (g/m ²)	Fish biomass (g/microcosm)
0	3	0.28	10,162	0.9671	5.6263
200	3	0.14	7,069	0.5957	4.9607
10	1	0.19	9,288	0.8587	
70	1	0.21	9,161	1.6149	
500	1	0.03	4,688	0.2880	
1000	1	0.09	6,052	0.5001	

Table 4. Analysis of variance table with and without covariate adjustment of primary production and selected secondary producer variables of the control and the 200 $\mu\text{g/L}$ treatments. The covariate for each microcosm was the average of three pretreatment samples (primary production) or the value of one pretreatment sample (chironomid density or biomass standing crop) for that microcosm. The control and the 200 $\mu\text{g/L}$ treatment effects were each analyzed in three microcosms ($n = 3$).

<u>Without Covariate Adjustment</u>			<u>With Covariate Adjustment</u>		
Source of	F	Probability	Source of	F	Probability
Variation	Value	F Exceeded	Variation	Value	F Exceeded
<u>Primary Production</u>					
Treatment	1.717	0.260	Treatment	0.498	0.531
Time	3.655	0.001	Time	4.230	0.000
Treatment X	0.698	0.733	Treatment X	0.693	0.73
Time			Time		
			Covariate	0.199	0.686
 <u>Chironomid Density</u>					
Treatment	20.737	0.010	Treatment	10.564	0.047
Time	188.634	0.000	Time	107.796	0.000
Treatment X	9.755	0.007	Treatment X	5.675	0.029
Time			Time		
			Covariate	1.335	0.332

Table 4. Continued.

<u>Without Covariate Adjustment</u>			<u>With Covariate Adjustment</u>		
Source of	F	Probability	Source of	F	Probability
Variation	Value	F Exceeded	Variation	Value	F Exceeded
<u>Chironomid Biomass</u>					
Treatment	2.580	0.183	Treatment	1.287	0.339
Time	30.961	0.000	Time	15.406	0.002
Treatment X	2.055	0.190	Treatment X	0.906	0.442
Time			Time		
			Covariate	0.332	0.605
 <u>Fish Biomass</u>					
Treatment	0.080	0.800	Treatment	--	--
Time	18.500	0.000	Time	--	--
Treatment X	0.080	0.994	Treatment X	--	--
Time			Time		
			Covariate	--	--

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Other investigators have observed similar algal productivity results. In some instances, toxicants have been shown to have no long-term inhibitory effect on production in mixed-species (community level) cultures of algae (Ferens and Beyers 1972; Couch and Nelson 1982; Maly and Ruber 1983). Instead, the toxicant stress may result in a shift in community composition (Ryther 1970).

Figure 2 illustrates the apparent consistent depression of primary production in the 200 $\mu\text{g/L}$ tebuthiuron treatment as compared to the control. This experiment-long depression results in a control mean (across sample dates) that is two times larger than the 200 $\mu\text{g/L}$ treatment mean (Table 3). These apparent differences between treatment means were not great enough to overcome the extremely high variation between experimental units within a treatment. This large within treatment variation, as evidenced by large coefficient of variation values (over 100%), prevented satisfactory replication.

Albeit the use of microcosms is expounded as a benefit because of replicability (Beyers 1964; Draggan and Reisa 1980; Ferens and Beyers 1972), sufficient replicability is often not the case (Taub 1976). Taub (1976) found that variation between microcosm replicates was a problem in statistical demonstration of toxic effects, if they occur. The coefficient of variation was often greater than 100%. Coefficients of variation of approximately 10-15% have been considered to represent reasonable reproducibility (Abbott 1966). If all microcosms are complex as those used in the

present study, the stochastic behavior of nature can intrude and microcosms may not replicate well (Pilson and Nixon 1980; Bowling et al. 1980). Even though microcosms of a particular treatment level may behave similarly, small variations in timing of productivity peaks can cause difficulties in the statistical analysis (Pilson et al. 1980; Taub 1976).

Other sources of variation that may have contributed to obscuring possible treatment differences included that variation inherent in taking rather "instantaneous" measurements of primary production (Steward et al. 1971) and variation in the continuous-type response of the equipment used to make the "instantaneous" measurement.

Chironomid Density

Control and 200 $\mu\text{g/L}$ treatment chironomid density means adjusted by the covariate are plotted over time in Figure 3.

Chironomid density was depressed ($P = 0.047$) in the 200 $\mu\text{g/L}$ treatment. This was the only investigated microcosm component impacted by this treatment level.

Also, of noteworthy interest was the implication of using the covariate. The 200 $\mu\text{g/L}$ treatment chironomid density mean was significantly less ($P \leq .010$) than the control before covariate adjustment. When the covariate was taken into account, differences between treatments were all but removed ($P = .047$). Thus, a large portion of the difference between treatment means was accounted for

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by the dissimilarity in the pretreatment state between treatment microcosms.

This phenomenon has implications for similar microcosm studies. Investigators may draw erroneous conclusions if the pretreatment status of each microcosm is not taken into account. Consequently, the pretreatment state of all experimental units should be thoroughly assessed before treatment.

Additionally, a treatment-time interaction occurred. The interaction developed because the 200 $\mu\text{g/L}$ treatment density was depressed ($P \leq 0.050$) from the control density at only one (64 DAT) of the three sample dates. This date represented peak density for both treatments. This finding is similar to that found in a cadmium toxicology study (Bowling et al. 1980). Only during peak macroinvertebrate density of the control systems were differences between treatment densities apparent. Since macroinvertebrate density differences between treatments can be time dependent, researchers should observe experimental systems over an extended period. This will help assure the detection of treatment effects if they occur.

Since primary productivity of both treatment levels was shown statistically to be the same, chironomid density reductions in the 200 $\mu\text{g/L}$ treatment may be a result of a direct toxic effect on the chironomids and/or a species shift in the algal community. A shift in algal community composition to less palatable species may have made less energy available to the chironomid population (Petzel

1975). This situation could have caused lowered density via lowered carrying capacity and/or a concomitant species shift in the chironomid assemblage.

Sample date means (time) were found to be highly different with and without covariate adjustment. This result was expected because of colonization of the artificial substrate samplers over time and time-dependent production peaks.

Chironomid Biomass

Control and 200 $\mu\text{g/L}$ treatment chironomid biomass means adjusted by the covariate are plotted over time in Figure 4. Time was the only variable demonstrating mean differences ($P = 0.002$). Although the covariate adjusted 200 $\mu\text{g/L}$ treatment mean was only approximately 60% that of the control (Table 3), no statistical separation was determined. This finding is of particular importance since biomass is generally a better ecological descriptor than density (Odum 1971).

The probability that differences between the control and 200 $\mu\text{g/L}$ treatments were due to chance alone almost doubled with the inclusion of the covariate. Again, a large portion of the apparent differences between treatments can be attributed to the disparity that existed between the treatment microcosms before tebuthiuron inoculation.

DISCUSSION: The results of the study
have shown that the use of the
new system is a significant improvement
over the old system.

Fish Biomass

Fathead minnows were not impacted by a tebuthiuron concentration of 200 $\mu\text{g/L}$. Moreover, fish biomass was not correlated to primary production, chironomid density, or chironomid biomass.

These findings, nevertheless, should be viewed with caution. Bowling et al. (1980) determined that, in general, microcosms are not large enough to support sufficient macrosecondary consumers (e.g., fish) to allow periodic sampling of that component. One must have the organisms incorporated in ecologically meaningful quantities; that is, the organism biomass must approximate carrying capacity. Toxic effects at lower trophic levels are then given a greater opportunity to be reflected in the macrosecondary consumer population.

In this study, fathead minnow biomass was suspected to be well below carrying capacity. Total biomass that was periodically removed from the microcosms did not total one half of the possible "instantaneous" fathead standing crop biomass cited for ponds (adjusted to 157 g/microcosm, Clay 1975). This situation may have buffered the fish population from changes in productivity of its prey items.

Omnivory is another factor that may have helped protect the fish population from perturbations. A limited food habits study was performed on the test fish. Gut content remains included chironomid larvae, aquatic beetles, and algae. Fathead minnows are omnivorous

(Pflieger 1975), which is also a common feeding strategy in many western endemic fish species (Lee et al 1980). Omnivory is advantageous because it allows the animal a much larger food base as compared to more specific feeders. Hence, omnivorous populations may be less susceptible to selected changes in energy availability. This can result in greater population stability (Murdoch et al. 1975).

In other microcosm work, Goodyear et al. (1972) found a significant relationship between primary production (gross photosynthesis) and production of an omnivorous fish species. McConnell (1965) likewise observed significant correlations between gross photosynthesis and growth of a herbivorous fish species. Their microcosms, however, did not contain a sediment component. This probably precluded meaningful benthic macroinvertebrate production, possibly forcing the fish to narrow their food base. Any changes in phytoplankton production would essentially be a change in the overall food base. Hence, there is a greater chance for alterations in primary production to be reflected in the fish population. Most importantly, both studies allowed the test fish to reproduce. This method assumedly allowed the fish population in each microcosm to approach carrying capacity and thus be more susceptible to changes in lower trophic levels.

Hall et al. (1970) studied microcosms containing both water and sediment (similar to microcosms used in this investigation). They noted no relationship of fish production to benthic production, a

result analogous to that found in the present study. This conclusion was made despite observation of the omnivorous test fish feeding largely on benthic macroinvertebrates. The microcosm's broader food base, which may be a more accurate reflection of the energy capabilities of natural systems, might be responsible for this lack of correlation. Likewise, the broad food base contained in the microcosms used in this study may have prevented quantifiable interrelationships between fish biomass/primary production and fish biomass/chironomid standing crop. Accordingly, omnivorous fish species that are at population densities below carrying capacity may not be affected by tebuthiuron concentrations ranging up to 200 $\mu\text{g/L}$. Tebuthiuron effects to insectivorous fish species, however, should be investigated since depressions of an important macroinvertebrate group occurred at 200 $\mu\text{g/L}$.

CHAPTER IV

CONCLUSIONS AND SUMMARY

The following conclusions were drawn from this study:

- 1) tebuthiuron concentration declined over 106 days in the water columns of all microcosms. This loss was best described by exponential mathematical expressions of decay;
- 2) except in the two lowest doses, tebuthiuron was found in the sediments of all tanks; dynamics of tebuthiuron in sediment was described by logarithmic mathematical models;
- 3) the adsorption of tebuthiuron on sediments contained in the ten microcosms was described by the Freundlich equation, $x/m = 3.24[C^{0.68}]$;
- 4) of the system variables measured, only chironomid larvae density exhibited effects at the 200 $\mu\text{g/L}$ treatment level; a treatment-time interaction occurred because reductions in chironomid density were evident only at the 64 DAT sampling date; that is, density differences between treatments were time dependent;
- 5) although the 200 $\mu\text{g/L}$ primary production and chironomid biomass covariate adjusted means over time were only 50% and 62% respectively of the control, no statistical differences were found; poor replicability as evidenced by high coefficients of variation may have precluded observing significant differences.

The pathway by which chironomid density depressions occurred is unknown. Possible explanations include a direct toxic effect on the

chironomids and/or a shift in the algal community resulting in a greater percentage of "unpalatable" biomass. Moreover, the most reliable analysis was likely performed on this microcosm component (due to the nature of the sampling techniques and the fact that the macroinvertebrates were allowed to self-colonize). Although no management recommendations concerning tebuthiuron can be made at this point, the changes in chironomid density suggest that effects may occur at the 200 $\mu\text{g/L}$ treatment level. Additional microcosm research that intensively investigates system responses to tebuthiuron in a dose range bracketing 200 $\mu\text{g/L}$ is needed.

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