



**US Army Corps  
of Engineers**  
Waterways Experiment  
Station

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*Aquatic Plant Control Research Program*

# **Laboratory and Mesocosm Evaluations of Controlled-Release Matrices as Potential Herbicide Delivery Systems**

*by Michael D. Netherland, R. Michael Stewart*

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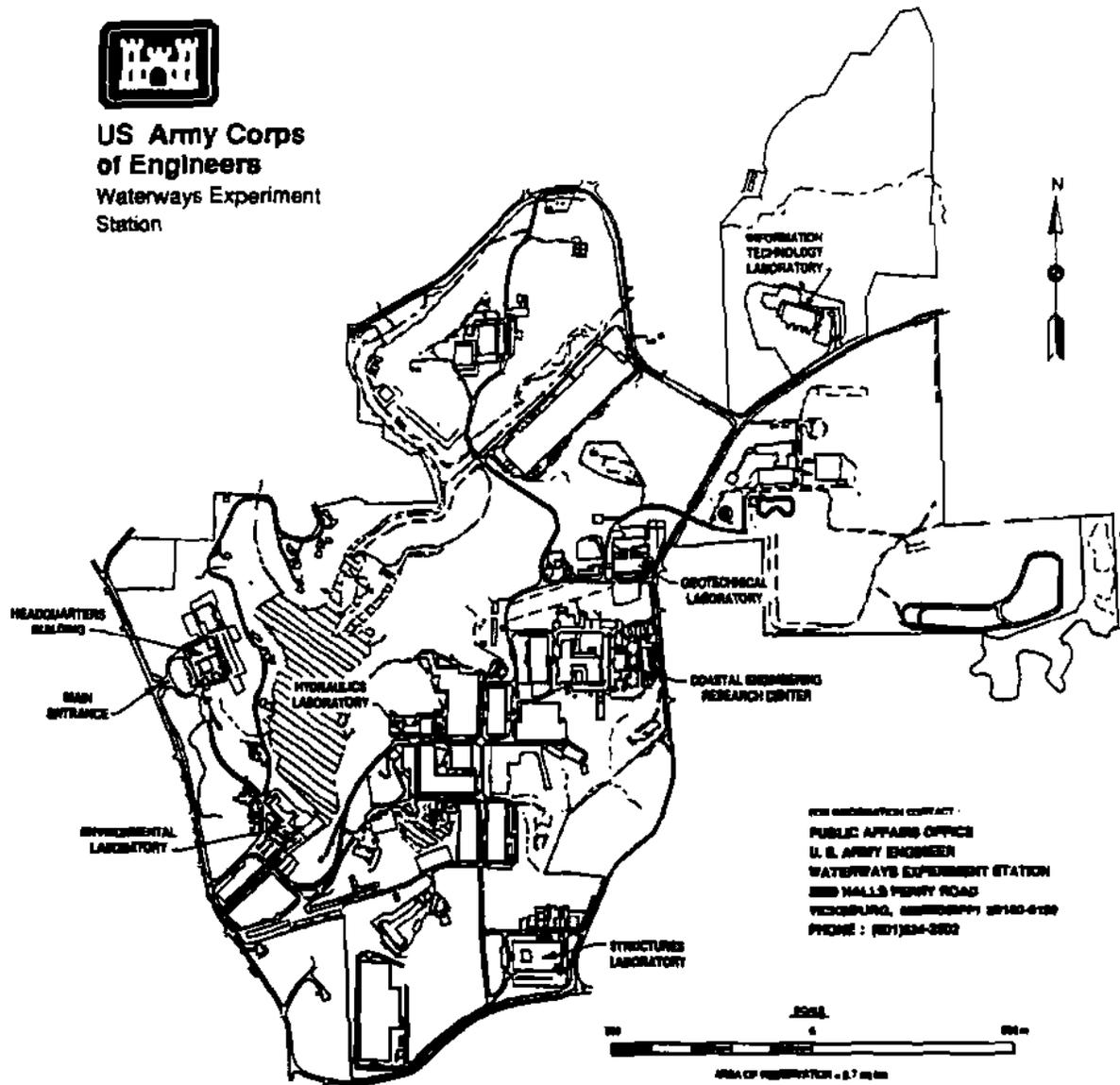
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# Preface

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The work reported herein was conducted as part of the Aquatic Plant Control Research Program (APCRP), Work Unit 32437. The APCRP is sponsored by the Headquarters, U.S. Army Corps of Engineers (HQUSACE), and is assigned to the U.S. Army Engineer Waterways Experiment Station (WES) under the purview of the Environmental Laboratory (EL). Funding was provided under Department of the Army Appropriation No. 96X3122, Construction General. The APCRP is managed under the Environmental Resources Research and Assistance Programs (ERRAP), Mr. J. L. Decell, Manager. Mr. Robert C. Gunkel was Assistant Manager, ERRAP, for the APCRP. Technical Monitor during this study was Ms. Denise White, HQUSACE.

The principal investigator for the study was Dr. Kurt D. Getsinger, Ecosystem Processes and Effects Branch (EPEB), Environmental Processes and Effects Division (EPED), EL, WES. The study was conducted and the report prepared by Mr. Michael D. Netherland and R. Michael Stewart, EPEB, David Sisneros, Bureau of Reclamation, Denver, CO, and E. Glenn Turner, ASci Corp.

Technical reviews of this report were provided by Dr. Getsinger and Ms. Linda Nelson, EPEB. Technical assistance was provided by Mr. Charles Mayfield and Mr. Michael Bull, EPEB, and Mr. Carl Wilmer, Mr. Rick Johnson, Dr. Earl Burns, and Dr. Buff Crosby, Tennessee Valley Authority (TVA). The cooperation of DowElanco and Elf Atochem for providing some of the herbicide formulations and residue analyses for this study is appreciated. In addition, the cooperation of Ms. Jennifer Moses and Mr. Dan Haraway, TVA-Aquatic Research Laboratory, is greatly appreciated.

This investigation was performed under the general supervision of Dr. John Harrison, Director, EL; Mr. Donald L. Robey, Chief, EPED; and Dr. Richard E. Price, Acting Chief, EPEB.

At the time of publication this report, Director of WES was Dr. Robert W. Whalin. Commander was COL Bruce K. Howard, EN.

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# 1 Introduction

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Plant physiologists contend that a minimum amount of herbicide must be absorbed into living plant tissues before specific biochemical processes can be sufficiently disrupted to cause plant death (Ashton and Crafts 1981; Ross and Lembi 1985; Devine, Duke, and Fedtke 1993). This minimum herbicide level is often referred to as the critical tissue burden. The amount of chemical absorbed by a plant is a function of both concentration and contact time. Since the amount of chemical that can be applied to a given area is limited by product labels, it is essential to maintain an adequate exposure period. This is true for both terrestrial and aquatic plants. However, maintaining sufficient contact time in aquatic systems (primarily for submersed vegetation) poses a much greater challenge. In terrestrial systems the herbicide can be applied directly to the plants. In contrast, treatment of submersed vegetation requires herbicide application to the water column surrounding the plants. Thus the duration of herbicide exposure in an aquatic system can be significantly reduced by flow-, thermal-, tidal-, and wind-generated water circulation patterns (Fox, Haller, and Getsinger 1990; Getsinger, Green, and Westerdahl 1990; Getsinger, Haller, and Fox 1990; Fox, Haller, and Getsinger 1991). Furthermore, each aquatic site has its own unique water exchange patterns. As a result, widespread inconsistencies in submersed plant control can often be attributed to the high degree of variability in herbicide dispersion and dilution following conventional and liquid applications. Recognition of this problem has stimulated research into various methods of extending herbicide contact time in the water column, especially in high water-exchange environments. One approach for extending herbicide contact time is to develop a controlled-release (CR) carrier or matrix as a delivery system.

A CR matrix combines a pesticide with an inert carrier such as polymers, lignins, or clays. Such a system is designed to deliver the active ingredient to the target organism at a controlled rate for a specified period of time. Potential advantages of CR technology for managing aquatic plants include: (a) minimizing the aqueous concentration of herbicide in the water column at any point in time after application, (b) increasing potential for long-term control of target vegetation, (c) minimizing impacts on nontarget vegetation and organisms (through placement or selectivity due to lower aqueous concentrations), and (d) expanding the present uses of existing registered aquatic herbicides. Only six active ingredients are currently labeled for submersed aquatic plant control.

## History and Development of CR Matrices for Aquatic Herbicide Applications

The concept of using CR technology to chemically control aquatic nuisance vegetation is not new. CR matrices have been developed and evaluated from materials such as rubber, plastics, lignins, clays, polyvinyl chloride (PVC), polymers, and alginates during the past two decades (Steward and Nelson 1972; Harris, Norris, and Post 1973; Cardarelli and Raddick 1983; Connick et al. 1984; Van and Steward 1982, 1983, and 1985; Harris 1984, and Dunn et al. 1988). A review of these and several other matrices that have been tested for the controlled release of aquatic herbicides is provided by Riggle and Penner (1990). Difficulties encountered with the development of CR systems were: production problems, field application difficulties, buoyancy (off-target movement), sinking (bound in sediment) problems, and inconsistent release profiles. Also, a lack of understanding the effects of water exchange and optimal release rates slowed further research in this area.

The goal of much of the early research was to produce a CR matrix that would release sustained, very low concentrations of herbicide over long periods of time (>20 days). To achieve this goal, matrices were initially tested in the laboratory for CR properties and then further developed to meet release criteria. Following laboratory development, CR matrices were further evaluated in the field.

As part of this early research, a work unit was initiated within the Aquatic Plant Control Research Program (APCRP) in the late 1970s to develop and evaluate CR formulations for controlling hydrilla (*Hydrilla verticillata* (L.f.) Royle) and Eurasian watermilfoil (*Myriophyllum spicatum* L.), hereafter called milfoil. Two CR formulations of 2,4-D were tested in the laboratory and in the field. These formulations consisted of: (a) 2,4-D acid ((2,4-dichlorophenoxy) acetic acid) in Kraft lignin pellets (Westvaco, Inc.), and (b) an acrylic polymer, glycidyl methacrylate (Poly GMA), plus 2,4-D impregnated in clay pellets (Wright State University). These CR matrices were developed to release 2,4-D based on preliminary threshold concentrations developed by Westerdahl and Hall (1983).

Laboratory tests revealed that herbicide release rates of Poly GMA formulations provided a slow release of 2,4-D under static conditions for several months; however, in flowing water tests, herbicide washout occurred between 7 and 14 days (Van and Steward 1982). The Kraft lignin formulation provided 2,4-D release for approximately 2 months under static conditions (Van and Steward 1982, 1983). When field tested against milfoil in Lake Seminole, Georgia, both matrices provided poor to average short-term milfoil control. As a result of the water sampling protocol used in these studies, it was difficult to determine the release performance of the matrices as well as the relationship of aqueous water concentrations to plant control. The authors attributed the failure to obtain milfoil control to: (a) higher water exchange rates in the enclosed embayments of the lake than had been previously anticipated, and

(b) limited herbicide release to the water column caused by the pellets sinking into the sediment (Hoeppel and Westerdahl 1982). These assumptions are supported by the fact that sediment levels of 2,4-D remained unexpectedly high several months following the disappearance of 2,4-D from the water column. As a result of poor field performance and problems encountered with scale-up procedures, these matrices were not further developed.

A third formulation, 14-ACE-B, a natural rubber elastomer combined with 2,4-D (Creative Biological Laboratory, Inc.) was field tested in Lake Seminole in 1983 (Getsinger and Westerdahl 1984). This matrix did not perform to specification and released most of the herbicide into the water during the first few days; therefore, posttreatment was no longer pursued.

In the mid 1980s a fibrous CR matrix consisting of polycaprolactone (PCL) was developed at the Southern Research Institute, Birmingham, Alabama, for delivering the herbicides diquat (6,7-dihydrodipyrido[1,2- $\alpha$ :2',1'-c]pyrazinedium ion) and fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1*H*)-pyridinone) (Dunn et al 1988). Small-scale outdoor studies by Van and Steward (1985) showed that the PCL fluridone fibers effectively controlled hydrilla in tanks adjusted to provide one water volume exchange every 24 hr. Under the same conditions, the liquid formulation of fluridone was ineffective, and a commercially available pellet formulation provided only marginal control. Based on laboratory release rate profiles and small-scale efficacy trials, it was determined that a fluridone/PCL matrix should be further evaluated in the field. The fluridone fibers were applied to stands of hydrilla (Toledo Bend Reservoir, Texas, and Lateral 28 Drainage Canal, Florida) and milfoil (Pend Oreille River, Washington) but resulted in only fair to poor control of target plants (Westerdahl, Getsinger and Hall 1984). Residue analyses indicated that poor control was achieved in high water-exchange areas (fluridone was nondetectable 3 to 14 days posttreatment); whereas improved control was observed in areas where fluridone was detectable (3 to 20  $\mu\text{g/L}$ ) for a 21-day posttreatment period. Further development of these fibers was not pursued because of application difficulties, failure to provide adequate plant control, and a high degree of matrix buoyancy which resulted in rapid off-target movement of the matrix.

In summary, scale-up operations from the laboratory to the field presented several problems. The major problem proved to be the high variability in water exchange rates at different sites, which, in combination with the long-term release criteria, greatly impacted aqueous herbicide concentrations over time, and therefore efficacy. In addition, the lack of understanding the correlation between concentration and exposure time (CET) requirements and efficacy often resulted in applications that had no chance of successfully controlling the target plants. Either minimum critical water concentrations were never achieved, or exposure periods were not sufficient to control target plants. As mentioned previously, other problems included formulations that sank to the bottom and became covered by silt (greatly reducing release rates), buoyant fibers that moved off-target with water flow, and application difficulties that likely resulted in nonuniform distribution of the matrices. Since CR

technology has demonstrated limited success at controlling plants, potential advantages over conventional application methods have not been realized. In response, industry has remained reluctant to research and develop experimental CR formulations.

## **Relationships of CET Requirements and Water Exchange to the Success of CR Matrices**

Information generated from laboratory studies and cooperative field studies by the U.S. Army Engineer Waterways Experiment Station (WES), the U.S. Army Engineer District, Jacksonville, and the University of Florida in flowing-water systems has sparked a renewed interest in developing and applying new herbicide delivery systems (including CR). This work includes studies designed to determine concentration and exposure time requirements for each herbicide and target species and field studies with the fluorescent tracer dye, rhodamine WT (often co-injected with herbicides) to determine the effects of water movement on herbicide dissipation and distribution.

Field studies with rhodamine WT have focused on the effect of water movement in hydrodynamic environments on herbicide dispersion and dilution. Good correlations in rate of dissipation have been shown between rhodamine WT and the aquatic herbicides endothall (7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid)(Fox, Haller, and Getsinger 1993), fluridone (Fox, Haller, and Shilling 1991), bensulfuron methyl (methyl 2-[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]methyl]benzoate)(Fox, Haller, and Getsinger 1992), and triclopyr [3,5,6-trichloro-2-pyridinyl]oxy]acetic acid)(Getsinger, Turner, and Madsen 1993). These studies have demonstrated that, in large systems following dye/herbicide treatment, rapid dispersion of residues can occur (Fox, Haller, and Getsinger 1990; Getsinger, Green, and Westerdahl 1990). Moreover, dye studies in plant stands in quiescent waters indicate that thermal stratification may present a physical barrier that prevents thorough mixing of liquid herbicides throughout the water column (Getsinger, Haller, and Fox 1990). The warm, herbicide-laden upper layers of water are susceptible to off-target movement under windy conditions. Spot treatments of small plant infestations along lake and reservoir shorelines are also subject to rapid herbicide dispersion. These factors (rapid dispersion, off-target movement, and poor distribution), acting either alone or in concert, can result in herbicide contact times that are insufficient to provide adequate plant control.

To better understand the effects of herbicide dispersion rates on plant control, CET relationships have been developed in the laboratory for diquat, endothall, and fluridone versus hydrilla (Hall, Westerdahl, and Stewart 1984; Van and Conant 1988; Netherland, Green, and Getsinger 1991; and Netherland, Getsinger, and Turner 1993), and 2,4-D, bensulfuron methyl (BSM), endothall, and triclopyr versus milfoil (Green and Westerdahl 1990; Netherland, Green, and Getsinger 1991; Netherland and Getsinger 1992; and Nelson and Netherland 1993). These results indicated that compounds such as 2,4-D, endothall,

and triclopyr at concentrations from 1/4 to 1/10 the maximum label rate are extremely effective given an adequate exposure period (2 to 7 days). This suggests that instead of designing carriers to deliver these herbicides at minimum threshold concentrations for long periods of time (several weeks), a more favorable design may be one in which low to moderate herbicide concentrations are released over considerably shortened periods. To date, the most effective means of increasing contact time and distribution with a carrier (matrix) has been with 2,4-D Butoxy ethyl ester (BEE) and endothall granules. These formulations help to slow rapid dispersion rates, improve placement, and penetrate thermal barriers. While these granular applications remain superior to liquids in some situations, they still have limited utility in many high water-exchange environments because of rapid release rates. The development of a matrix with short- to medium-term release profiles could improve plant control in these situations.

This shift in emphasis from long-term threshold concentration release to short-term low concentration release represents a deviation in the approach to controlled release of aquatic herbicides. Review of previous work indicated that despite extensive laboratory testing of CR matrices, long-term field release of herbicides was rarely achieved and residues were highly site-specific depending on water exchange. This combination of factors usually led to poor efficacy. While the theory of maintaining low threshold concentrations for plant control remains valid, the technical complications of achieving this in the field have led to a different approach in sustaining release of herbicides that require short to moderate contact times.

CET studies have determined that, regardless of the formulation, the herbicides fluridone and bensulfuron methyl will require long exposure periods (30 to 90 days) at high or low concentrations (10 to 100  $\mu\text{g/L}$ ) to effectively control the target plants. Developing CR matrices for these compounds will dictate that threshold concentrations (5 to 20  $\mu\text{g/L}$ ) be maintained for long exposures.

The current lack of a long-term CR matrix for fluridone has led to innovative application techniques designed to maintain low concentrations for extended periods of time. One technique has been to employ multiple treatments over time to maintain threshold water concentrations in flowing systems (Fox and Haller 1993, Getsinger 1993). The use of mechanical metering devices (solar-powered turkey feeders) for application to canals and drains has also proven to be effective. Both of these techniques have the capability of providing low herbicide concentrations over long periods of time and of responding to changes in flow rate by adjusting the amount of chemical applied, neither of which has been accomplished utilizing CR formulations. In addition, the unique flow characteristics of each water body make it unlikely that one CR formulation would be effective in all situations.

In summary, the coupling of information from water-exchange studies and herbicide CET work indicates that a lack of chemical contact time is likely responsible for the failure of many herbicide treatments. Moreover,

information gained from these studies is essential to the design of more effective delivery systems.

## **Relationship of Critical Tissue Burden to Herbicide CR Matrices**

In aquatic systems, increased herbicide contact time should maintain or increase internal herbicide concentrations in the plant and thus result in better plant control. Previous research has shown that, with many aquatic herbicides, a relatively small proportion of the herbicide in the water is taken up by the plant (Haller and Sutton 1973; Van and Steward 1985; Reinert et al. 1985; Cassidy and Rodgers 1989). Depending on plant biomass, internal tissue values tend to range from 1 to 6 percent of the total amount of herbicide applied. Although plant uptake accounts for only a small portion of herbicide removal from the water column, residue analyses show that tissue concentrations exceed aqueous concentrations by 2 to 50 times. However, evidence suggests that only a fraction of the herbicide applied to a treatment area is actually required to provide effective control. Although treatment areas have been "overdosed" in a physiological sense using conventional application techniques and formulations, this "overdosing" has been required to produce acceptable efficacy. Developing methods to deliver herbicides to submersed plants in a more efficient manner offers the potential of using less active ingredients and, at the same time, achieves equal or greater efficacy. This process ultimately translates into lower treatment costs and better environmental compatibility.

The dynamics of aquatic herbicide uptake and depuration are not completely understood, and further development of this concept should lead to more efficacious use of herbicides (especially with CR matrices) in aquatic systems. Differences in plant uptake and depuration patterns between high and low concentrations and short- and long-term exposures will also give information on design criteria for CR matrices. Studies conducted by Van and Conant (1988) on  $^{14}\text{C}$  uptake of diquat and endothall indicated that tissue concentrations increased as herbicide treatment rates increased; however, bioconcentration factors (BCF) (ratio of herbicide concentration in plant tissue divided by concentration in water) remained equal as concentrations were increased up to ten-fold. This concept is important to CR technology because it shows that, although aquatic plants accumulate herbicide over time, tissue concentrations are directly correlated with aqueous concentrations. This helps explain the failure to control plants when high treatment rates are followed by short contact times, and this may also explain the failure of some low initial treatment concentrations followed by long exposure periods.

## Research Approach and Objectives

Previous attempts at developing CR technology for aquatic use placed most of the emphasis on formulating and testing matrices in the laboratory. At that time, the U.S. Environmental Protection Agency (EPA) did not require a separate registration for each formulation; therefore, it was advantageous to screen a large number of compounds for field testing. The objective was to develop an end-use CR product, and the initial laboratory phase was costly and time-consuming. Once a matrix met laboratory release criteria (low-rate/long-term release), it was taken directly to the field and tested for efficacy.

Today, EPA requires a separate registration for each CR formulation developed, as well as approval to field test these products on areas larger than 0.4 ha (1 acre). Given the regulatory climate and industry's reluctance to pursue new formulations, it was decided that it would be unwise to pursue a large-scale screening program of potential CR matrices. Therefore, plans were modified to identify environmentally compatible existing CR matrices (preferably with prior EPA approval for use with other pesticide active ingredients), to test them for compatibility with aquatic herbicides, and to continue evaluating those with the most promising herbicide release profiles.

Two matrices were identified as potential candidates for preliminary release rate testing in the laboratory. These matrices included a gypsum-based ( $\text{CaSO}_4$ ) and protein colloid matrix manufactured by Controlled Release Systems Research, Inc. (CRSR). These matrices were chosen because of their proven ability to slow-release insecticides in an aquatic environment. In addition, the EPA has reviewed and approved this gypsum-based matrix for use with the larvicide methoprene. This product is currently licensed by the Zoccon Corporation. Herbicide release rates of these formulations were compared with release rates of conventional granular carriers (if available), and experimental formulations were developed by industry to determine if further testing was warranted.

In contrast to previous CR work, matrices that looked promising in the laboratory were not taken directly to the field; instead, they were tested in large hydraulic flumes (flowing-water mesocosms) with a known turnover rate. The defined water-exchange characteristics allowed evaluation of the CR formulation without the high degree of variable water exchange previously experienced in the field. The flume system also helped to determine the consistency of the matrix release profile and allowed for the comparison of mesocosm/field data versus laboratory data.

Herbicide residues were determined for water, plants, and sediment. Water analyses were used to determine the consistency of matrix release and to validate laboratory studies which defined concentration and exposure time requirements. Plant tissue analyses were used to define uptake and depuration patterns and critical tissue burden levels for optimal plant control. Sediment residues were taken to determine if sediment partitioning was occurring.

Specific objectives in this study were to (a) identify potential CR matrices that were compatible with aquatic herbicides, (b) determine if these matrices could maintain consistent herbicide release profiles over time in the laboratory and in a large flowing outdoor system (mesocosm), (c) determine the efficacy of matrices on milfoil in a flowing water environment, and (d) determine if CR technology remains a viable concept for controlling submersed aquatic plants.

## 2 Materials and Methods

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### Laboratory Herbicide Release Rate Evaluations

Gypsum- and protein-based matrices were formulated as 2 percent active ingredient (ai) granules with the herbicides fluridone and BSM and as 2 percent and 15 percent acid equivalent (ae) granules with 2,4-D and triclopyr. Fluridone and BSM were added to 55-L aquarium to achieve a total of 400 µg ai/L; whereas, triclopyr and 2,4-D were added to achieve a total of 4,000 µg ae/L and 3,000 µg ae/L, respectively. Gypsum pellets measured approximately 5 mm in diameter, whereas the protein matrices measured approximately 2 mm in diameter. Conventional commercial granular formulations of fluridone (Sonar SRP<sup>®</sup> as a 5-percent ai pellet was added to achieve 400 µg ai/L) and 2,4-D (Aquakleen<sup>®</sup> as a 19-percent ae pellet was added to achieve 2,000 µg ae/L) were also used as a comparison for release rate testing. In addition, the herbicide endothall was formulated by the manufacturer (Elf Atochem North America Inc.) as a 27-percent ai clay granule and a 45-percent ai superabsorbing polymer (potassium polyacrylate polyacrylamide copolymer). These matrices were compared with release rates of the conventional Aquathol (10.1 percent ai) granular formulation that is commercially available. All endothall matrices were added to test aquaria to achieve a total of 6,000 µg/L.

Laboratory evaluations of herbicide release rates were conducted in controlled-environment chambers at WES (Netherland, Green, and Getsinger 1991). Since the main objective of the initial laboratory evaluations was to determine the consistency of matrix release properties, studies were conducted in the absence of plant material. Fifty-five-liter aquaria, each capable of independent drain and fill, were filled to the 50-L mark with a water culture solution described by Smart and Barko (1984), and air was lightly bubbled in to produce a nominal amount of water circulation. Water temperature was maintained at 22 ± 2°C during the course of the study. Measured concentrations of herbicide were added to each individual aquarium, and each treatment was replicated three times.

Following addition of the matrices to the aquaria, water samples were collected at 2-, 12- and 24-hr posttreatment. The 2-hr samples were taken to determine if a spike release of the herbicide occurred after initial addition of the CR matrix. Following the 24-hr water sample, each aquarium was drained

and refilled twice to remove aqueous herbicide residues. A fine mesh screen (0.5 mm<sup>2</sup>) was placed over the drain intake to prevent removal of the granules from the system.

An identical protocol was followed on posttreatment days 2 through 7, with the exception of eliminating 2-hr water samples after 3 days. Water samples were collected in 500-ml polyethylene bottles and frozen until analysis. Fluridone (detection limit 5 µg/L), 2,4-D, and triclopyr (detection limit 10 µg/L) were analyzed by the Tennessee Valley Authority (TVA) Environmental Chemistry Laboratory, Chattanooga, Tennessee, using high performance liquid chromatography (HPLC) methods. BSM (detection limit 10 µg/L) was analyzed by El duPont de Nemours Inc., Wilmington, Delaware, using an HPLC method, and endothall (detection limit 1 µg/L) was analyzed by Columbia Laboratories, Corbett, Oregon, using a gas chromatography (GC) method. Recoveries for BSM, fluridone, 2,4-D, and triclopyr ranged from 88 to 103 percent, and for endothall, recoveries ranged from 76 to 112 percent.

## Mesocosm Herbicide Release Rate and Efficacy Evaluations

Matrix release rate and efficacy evaluation were conducted in flow-through concrete flumes located at the TVA Aquatic Research Laboratory at Brown's Ferry, Alabama. Each flume measured 112 m in length and 4.3 m in width, and the bottom was lined with approximately 50 cm of sediment (Figure 1). Water was drawn from nearby Wheeler Reservoir, and water depths from 0.6 to 1.2 m could be maintained using a slat-board control structure at the downstream end of each flume. Metal weirs located at the outflow of the flumes allowed for determination of water discharge rates. At maximum depth, flume volume was approximately 570,000 L. Flow rates ranged from 0.021 to 0.025 cu m/sec or 1.8 ml to 2.1 ml per day. Therefore, complete water volume exchange occurred approximately 3.2 to 3.7 times per day in each flume.

In May of 1992 and 1993, milfoil stands were established in each flume. Plants were obtained from Guntersville Reservoir, Alabama, and three to five apical tips were planted 0.3 m apart to provide two plant stands measuring 4.3 × 12 m each. Plant stand 1 was centered 65 m below the inlet of the flume and stand 2 was centered 103 m below the inlet of the flume (Figure 1). In addition to milfoil, mixed stands of naiads (*Najas guadalupensis* and *N. minor*), pondweeds (*Potamogeton crispus* and *P. nodosus*), coontail (*Ceratophyllum demersum*), and muskgrass (*Chara* spp.) were present throughout the flumes. These species were especially prevalent in areas where milfoil was not planted.

Plants were given a 6-week pretreatment growth period, which allowed for thick canopy formation and root development. Milfoil pretreatment biomass estimates were obtained by sampling with a 0.25-m<sup>2</sup> frame placed randomly

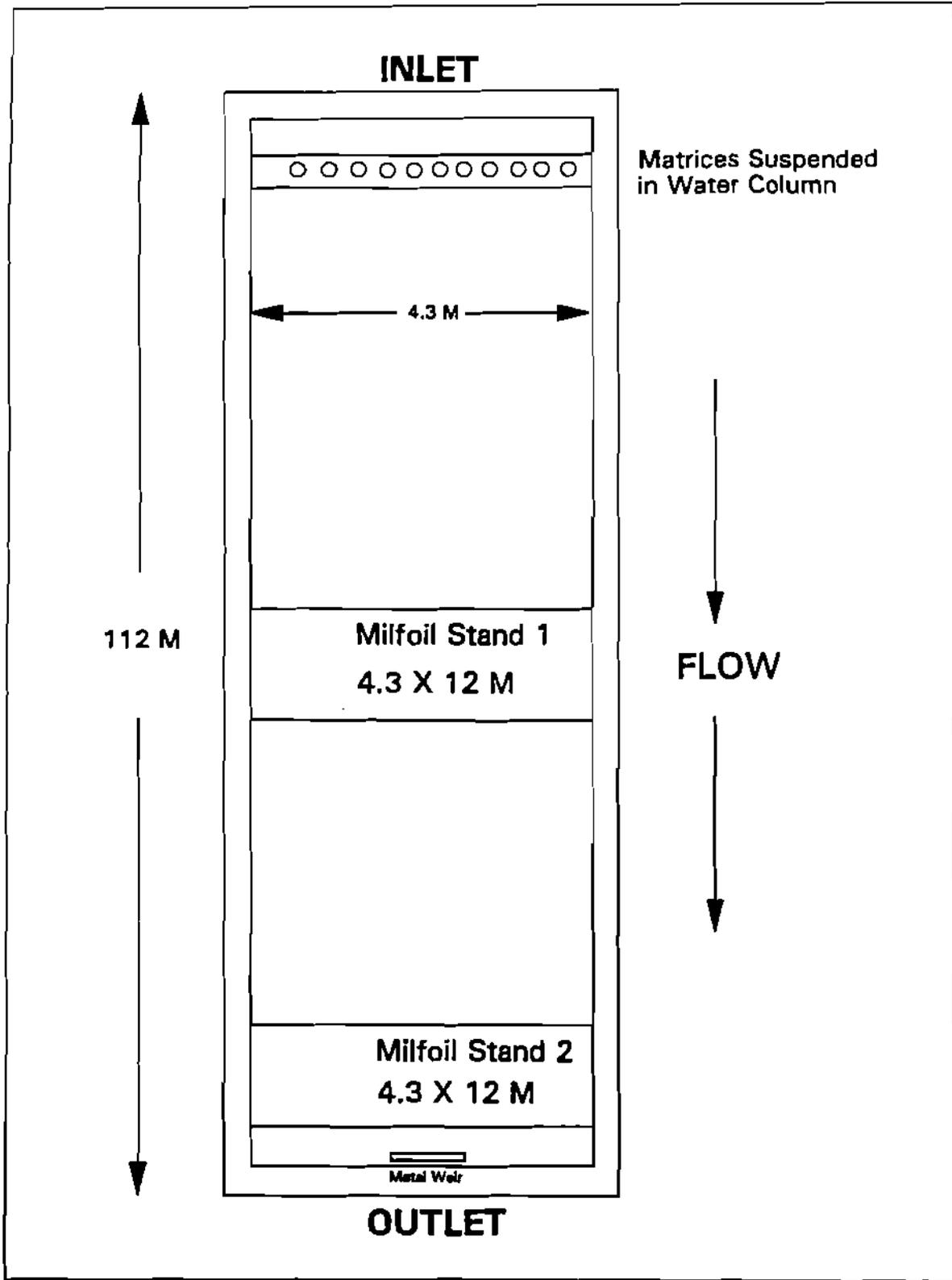


Figure 1. Overhead view showing flume characteristics and dimensions, location of plant stands, and the section of the flume where matrices were deployed

within the milfoil stands. Four samples were obtained from each stand and fresh and dry weights (oven dried at 70 °C for 48 hr) were recorded.

Triclopyr was chosen for evaluation in the TVA flume system because of its high degree of selectivity for milfoil (Getsinger, Turner, and Madsen 1993) and its ability to control milfoil in the laboratory at very low rates and moderate contact times (Netherland and Getsinger 1992). In addition, the Experimental Use Permit (EUP) status of the compound makes it a good candidate for further field efficacy and dissipation testing.

The gypsum matrix was chosen for further evaluation for several reasons including: (a) consistent release profiles obtained in the laboratory, (b) previous use of the matrix in the Pend Oreille River showed that consistent release of rhodamine WT dye was achieved (Sisneros 1991), and (c) matrix components (principally CaSO<sub>4</sub>) are inert and environmentally compatible.

The density of the gypsum matrix led to concern that granules would rapidly sink into the soft sediments thereby preventing aqueous release. The tendency of granules (matrices) to sink into the sediment has been mentioned in previous CR studies as the causal agent for failure to obtain desired aqueous herbicide concentrations. It was decided that for initial release rate testing in the flumes, a modified matrix design would be used to suspend the matrices in the water column to prevent sinking.

The matrix design for flume evaluations was based on a prototype used by Sisneros (1991) for dye release studies in the Pend Oreille River. These matrices consisted of the herbicide formulation Garlon 3A (triclopyr) incorporated into the gypsum matrix, which was then encased within a 4-cm-thick PVC housing and covered with hardware cloth (Figure 2). Matrices were formulated by Accugran, Inc, Minneapolis, Minnesota.

## 1992 CR Matrix Evaluations

Two formulations were evaluated: (a) one was formulated to deliver 100 µg/L triclopyr over a period of 6 days, and (b) and one was designed to deliver 300 µg/L over a period of 6 days. Matrices formulated for 100 µg/L weighed  $2.2 \pm 0.23$  kg and consisted of 7 percent triclopyr, whereas matrices formulated for 300 µg/L weighed  $4.3 \pm 0.38$  kg and consisted of 12 percent triclopyr.

On June 23, 1992, matrices were suspended near the inlet end of two flumes at mid-depth in the water column by tying nylon cord to an eye bolt on the PVC housing and attaching this to metal catwalks extending across the length of the flume. Flume 1 was targeted at 100 µg/L for 6 days with six matrices deployed, while flume 2 was targeted at 300 µg/L for 6 days with 11 matrices deployed. Matrices were removed from the water at 6 days posttreatment.

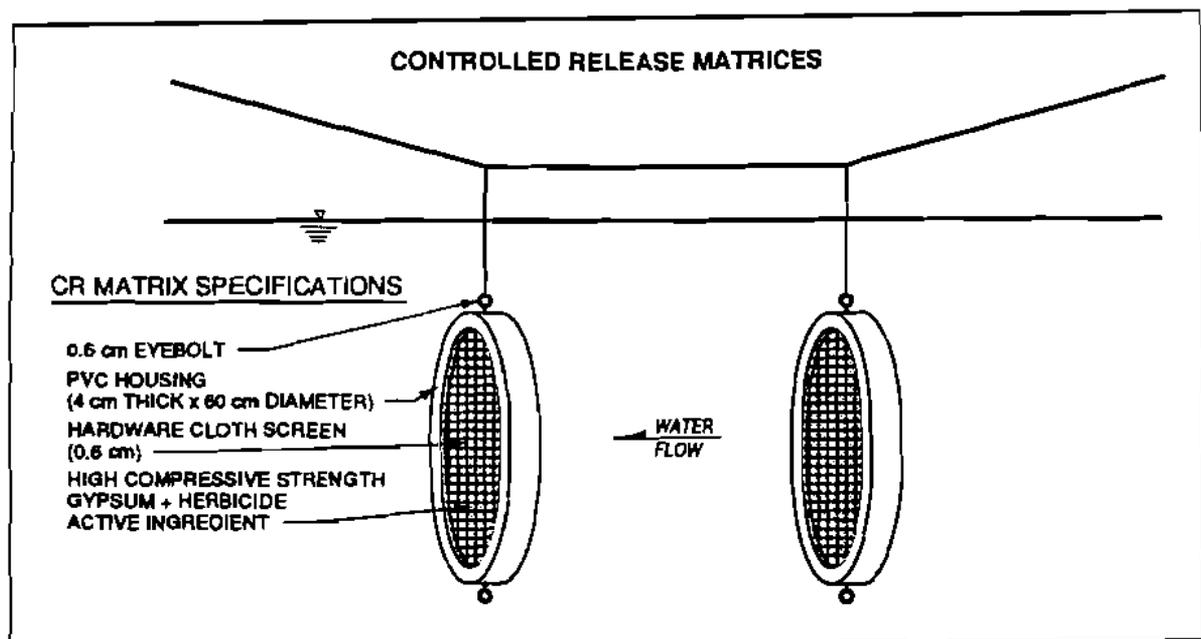


Figure 2. Diagram of prototype CR matrix used for flume testing (To convert inches to centimeters, multiply by 2.54)

Following addition of the matrices, water samples were collected at 6- and 12-hr posttreatment. Thereafter, water samples were collected at 12-hr intervals for the next 6 days. Water samples were collected from mid-depth at 40 m (open water), 65 m (plant stand 1), 85 m (open water), and 105 m (plant stand 2) downstream from the inlet end of each flume. After collection, samples were placed in a freezer and remained frozen until analysis. Water samples were analyzed for triclopyr by A&L Great Lakes Laboratories, Fort Wayne, Indiana, using a GC method (detection limit 1  $\mu\text{g/L}$ ) and by U.S. Bureau of Reclamation (BOR) laboratory, Denver, Colorado, using an HPLC method (detection limit 10  $\mu\text{g/L}$ ). Thirty duplicate samples were taken and analyzed by both laboratories, and results showed very little average difference ( $\approx 5$  percent) between the analyses. Furthermore, matrices were examined for conformation and consistency (i.e. cracks, dissolution) throughout the exposure period.

Following deployment of the matrices, visual observations of plant injury were recorded daily for 8 days posttreatment. At 6 weeks posttreatment, plant biomass was sampled as described for pretreatment biomass to determine treatment efficacy.

In addition to CR matrix treatments, other flume treatments with triclopyr included maximum rate (2.5 mg/L) liquid applications to flowing water, sequential 1.0-mg/L liquid treatments to flowing water, metering pumps used to deliver low rates (250  $\mu\text{g/L}$ ) to simulate CR matrix release rates, and an untreated reference. Although these methods and results will not be discussed in detail in this report, some mention of efficacy will be used for comparative purposes.

## 1993 CR Matrix Evaluations

Results from the 1992 evaluations resulted in modifications in matrix loading rates, target aqueous concentrations, and analytical protocol. Matrix design was essentially unchanged from the 1992 evaluations; however, triclopyr target rates were increased to achieve 300 and 500 µg/L in the water column. Average matrix weight was  $9.4 \pm 0.4$  kg and loading rates were 12 percent for the 300-µg/L matrix and 18.5 percent for the 500-µg/L matrix. In addition, the herbicide Aquathol K<sup>R</sup> (endothall) was incorporated into the gypsum matrix and was designed to release 500 µg/L in the water column. The active ingredient and loading rate (14 percent) were the only notable differences between the triclopyr and endothall matrices.

On June 27, 1993, 11 matrices were suspended near the inlet of each of five flumes at mid-depth in the water column. Flumes 1 and 2 were targeted to achieve 300 µg/L triclopyr for 5 days, flumes 4 and 7 were targeted for 500 µg/L triclopyr for 5 days and flume 6 was targeted to achieve 500 µg/L endothall for 5 days. At 84-hr posttreatment, the catwalks to which matrices were attached were moved below plant stand 1. Therefore, milfoil in stand 1 received only an 84-hr exposure, while plants in stand 2 remained exposed to triclopyr.

Following initial addition of the matrices, water samples were collected at 12-hr intervals for 6 days. Samples were collected from mid-depth at the front and back of milfoil stands 1 and 2 in each flume. After collection, triclopyr samples were immediately placed in a refrigerator and analyzed within 12 hrs. Endothall samples were placed in the freezer and remained frozen until analysis. Triclopyr residues (detection limit 10 µg/L) were analyzed onsite using an HPLC method, and selected duplicate samples were frozen and shipped to the TVA Environmental Chemistry Laboratory using an HPLC analytical method (detection limit 1 µg/L). Endothall samples (detection limit 10 µg/L) were analyzed by AS Environmental, Reading, Pennsylvania, using a GC method.

Visual observations of plant injury were recorded for 7 days posttreatment. At 8 weeks posttreatment, plant biomass was sampled (as previously described) and dried at 70 °C for 48 hr to determine treatment efficacy.

## Internal Herbicide Tissue Burden Levels

Following treatment with CR matrices and liquid applications, milfoil biomass was sampled to determine how herbicide tissue loading and depuration compared with aqueous triclopyr concentrations. Tissue samples were pulled from a designated area of the plant stand to prevent interference with biomass and efficacy assessments. Stem and shoot tissue was collected using a garden rake, and plants were immediately placed on a mesh screen and thoroughly washed to remove particulate material and filamentous algae (mainly *Hydrodictyon* spp. and *Cladophora* spp.). Washed plants were placed in large mesh

bags and vigorously slung to remove excess water. A fresh weight was recorded, and each sample was double wrapped in aluminum foil and then placed in a labeled plastic zip-lock bag. Samples were placed in the freezer and remained frozen until analysis.

Milfoil tissues were analyzed for triclopyr content by A&L Great Lakes Laboratories using a GC method with a detection limit of 50 µg/L. In addition, moisture content was determined for each sample. Triclopyr tissue concentrations are reported as milligrams per kilogram (parts per million) dry weight (DWT).

Following triclopyr treatment in 1992, tissue samples were taken at 0, 24, 48, 72, 96, and 120 hr posttreatment. Nine replicate samples weighing between 400 and 800 g fresh weight were collected at each time period. Flumes sampled included the gypsum treatments (100 and 300 µg/L for 6 days) and a metered treatment calculated to achieve 250 µg/L for 4 days.

Sediment samples were also collected in these flumes to determine if triclopyr was partitioning into the sediment over the long exposure periods. Sediment collected from Brown's Lake, Vicksburg, Mississippi, was placed into 22.5- by 30-cm aluminum pans to a depth of 2.5 cm. Sediment pans were placed near the outflow of each flume sampled. Sediment samples were collected at 0, 24, 72, and 120 hr posttreatment. Eight replicate samples weighing between 350 and 400 g fresh weight were collected at each time period. Sediment analyses were conducted by A&L Great Lakes Laboratories using a GC method with a detection limit of 10 µg/L. Results are presented on a milligrams per kilogram dry weight basis.

Following treatment in 1993, tissue samples were taken at pretreatment, 2, 6, 12, 24, 48, 72, and 120 hr posttreatment. Four replicate samples weighing between 120 and 150 g fresh weight were collected at each time period. Flumes sampled included a gypsum treatment (500 µg/L for 5 days) and 48-hr static liquid treatments of 1,500 and 3,000 µg/L. Furthermore, tissue samples were collected from one flume which was left flowing and treated at 3,000 µg/L.

## 3 Results and Discussion

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### Laboratory Release Rate Evaluations

Visual inspection showed matrices were dust free and showed no signs of breakage. All matrices were negatively buoyant. The gypsum and protein matrices generally maintained their integrity during the course of the study; whereas, conventional clay granules (endothall and 2,4-D) tended to break apart and dissolve during the course of the 7-day sample period. Prior to aqueous application, the supersorbent endothall polymer was a small (4 mm long) granule; however, immediately following contact with the water, granules changed form to a much larger (15 mm long) clear gelatinous matrix.

Initial laboratory evaluations were conducted to determine if the matrices had the potential for controlled release of several aquatic herbicides. Therefore, initial studies were conducted for a short duration (7 days) to identify matrices that warranted further evaluation. Moreover, matrices that did not show potential CR properties were not evaluated past this initial stage.

Laboratory release rate results for fluridone and BSM are presented in Figures 3 and 4. The gypsum matrices showed a consistent release of fluridone and BSM over the 7-day sample period. At the end of 7 days, 91 percent of the fluridone and 75 percent of the BSM were calculated to remain within the matrices, indicating the potential for an extended period of release. Calculations of the amount of herbicide remaining in the matrices were based on the total amount of ai applied, divided by the total amount of herbicide released over the 7-day treatment.

The protein matrices did not show the consistency of the gypsum compound (Figures 3 and 4). Fluridone release remained consistent for 5 days; however, residues were nondetectable at 6 and 7 days posttreatment. Greater than 90 percent of the fluridone was calculated to remain bound within the matrix. The fact that fluridone release levels on days 1 through 5 were just above the analytical detection limit (5 µg/L) suggests that release may have continued to occur on days 6 and 7, but fluridone release was not detectable. Release of BSM from the protein matrix was inconsistent with a spike release occurring on day 1 followed by a steady decline. BSM was nondetectable at days 6 and 7 even though calculations showed approximately 35 percent of the

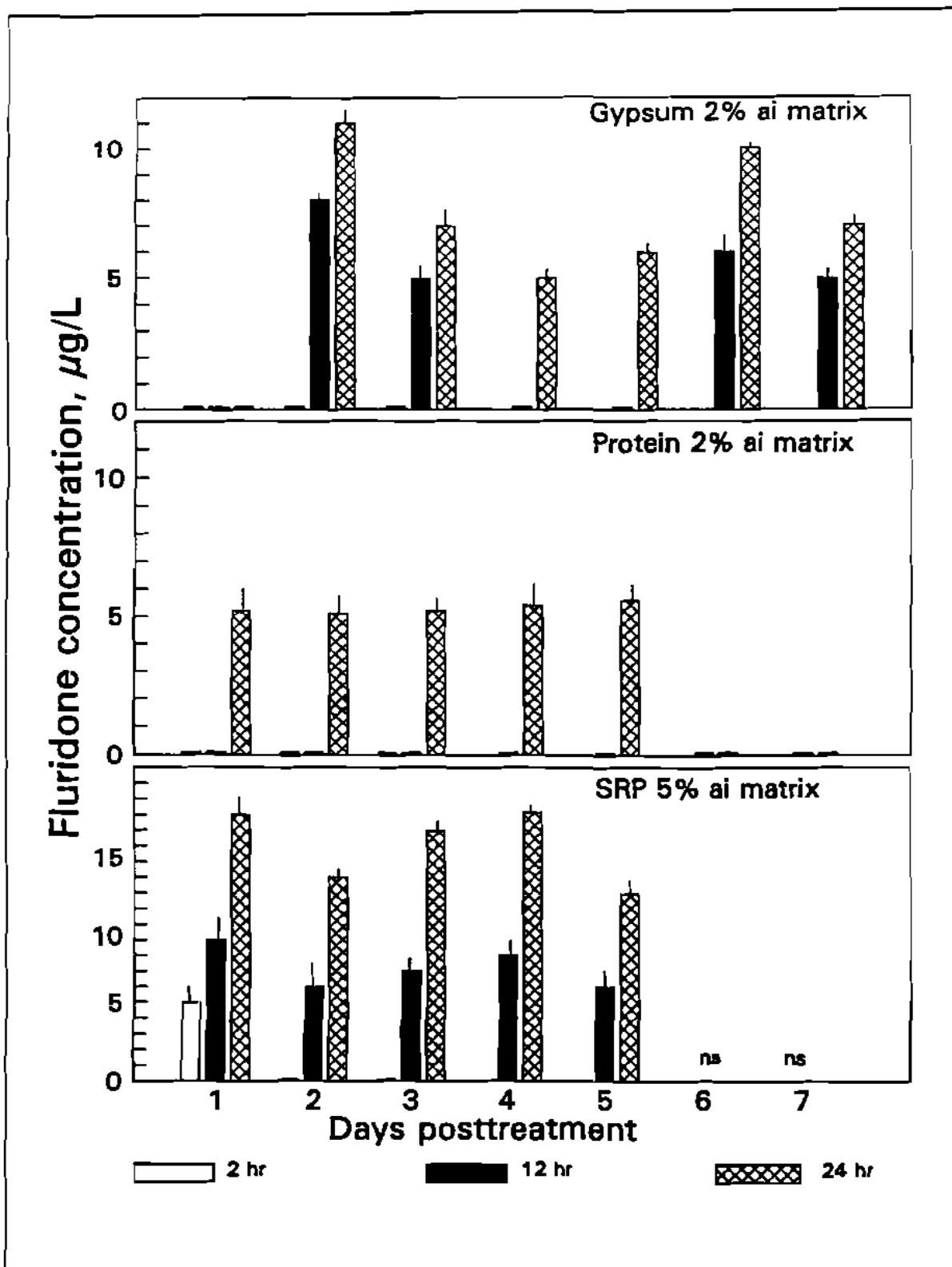


Figure 3. Fluridone release rates from 2-percent ai gypsum and protein matrices and a conventional 5-percent ai Sonar<sup>®</sup> SRP matrix over a 7-day period. Analytical limit of detection was 5 µg/L. Each bar is the average of three replicates, and vertical lines represent one standard error of the mean

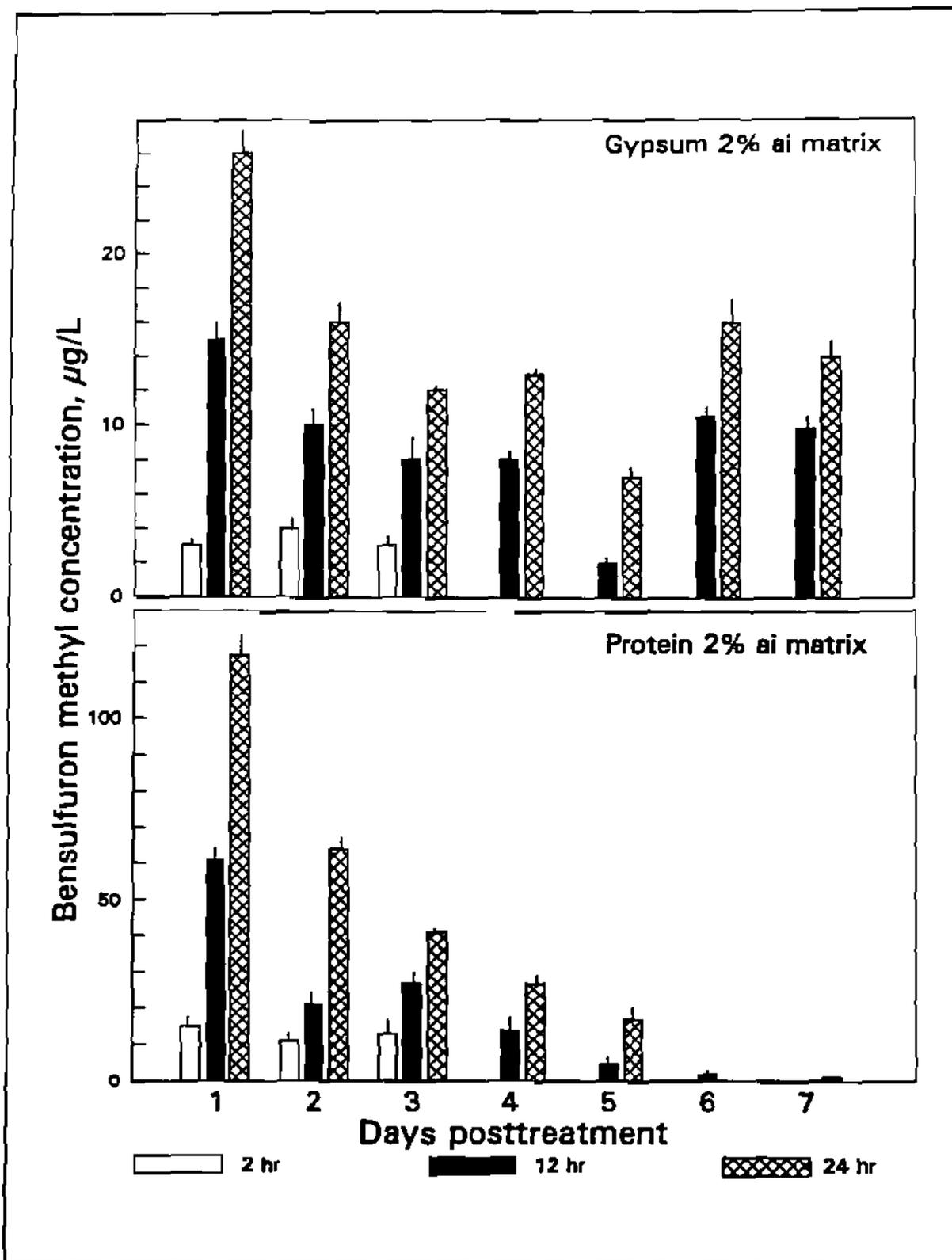


Figure 4. Bensulfuron methyl release from 2-percent ai gypsum and protein matrices over a 7-day period. Analytical limit of detection was 1 µg/L. Each bar is the average of three replicates, and vertical lines represent one standard error of the mean

BSM remained within the matrix. The lack of significant release at days 6 and 7 suggests the protein matrix may have limited potential as a long-term sustained release carrier.

The Sonar<sup>®</sup> SRP pellet exhibited steady release properties over 5 days of sampling. Approximately 77 percent of the fluridone remained within the matrix, indicating the potential for a much longer period of sustained release.

All of these matrices released herbicide at approximately 5 to 15 µg/L per day, which suggests that, in areas of high water exchange, aqueous concentrations would be much lower due to dispersion and dilution. Increasing treatment rates would result in higher aqueous concentrations; however, this approach could require application rates which exceed conventional application rates (on a total kilograms per hectare basis). In addition, off-target movement would also have to be considered as herbicide release continued to occur over several weeks. Technical difficulties and problems with variable water exchange have not been sufficiently overcome to produce a reliable long-term sustained release matrix. Therefore, although the release profile of the gypsum matrix compared favorably with the SRP pellet, it was decided that CR studies involving herbicides such as fluridone and BSM (which require long exposure periods) would not be pursued in the laboratory or field at this time.

Release rates for triclopyr and 2,4-D are presented in Figures 5 and 6. Both the 2- and 15-percent gypsum matrices exhibited consistent release profiles of both triclopyr and 2,4-D over the 7-day period. Release rates from the 15-percent matrices generally resulted in higher aqueous concentrations than release from the 2-percent matrices. Calculations (total amount applied/total amount released) at 7 days indicated that 48 to 51 percent of the triclopyr remained within the two formulations and 61 and 40 percent of the 2,4-D remained available for release from the matrices.

The protein matrices released inconsistently as both the 2- and 15-percent formulations of triclopyr and 2,4-D performed poorly. Both formulations resulted in a spike release the day of treatment, followed by a rapid decline in residues by day 2 (the 2-percent 2,4-D protein matrix declined more gradually). This rapid decline in release occurred despite calculations which indicated that between 35 and 60 percent of the herbicide remained available for release within the matrices.

The conventional Aquakleen<sup>®</sup> granule released the majority of active ingredient on days 1 and 2, at which point, release rates dropped considerably (Figure 5). Although these granules had significantly dissolved by 4 days posttreatment, approximately 20 percent of the 2,4-D still remained unaccounted for following the 5-day sample.

The results of 2,4-D and triclopyr release profiles from the gypsum matrices demonstrated sustained release with herbicides requiring short to moderate exposure periods. By comparison, the Aquakleen<sup>®</sup> granule did not exhibit similar sustained release properties; however, the ability of this matrix to

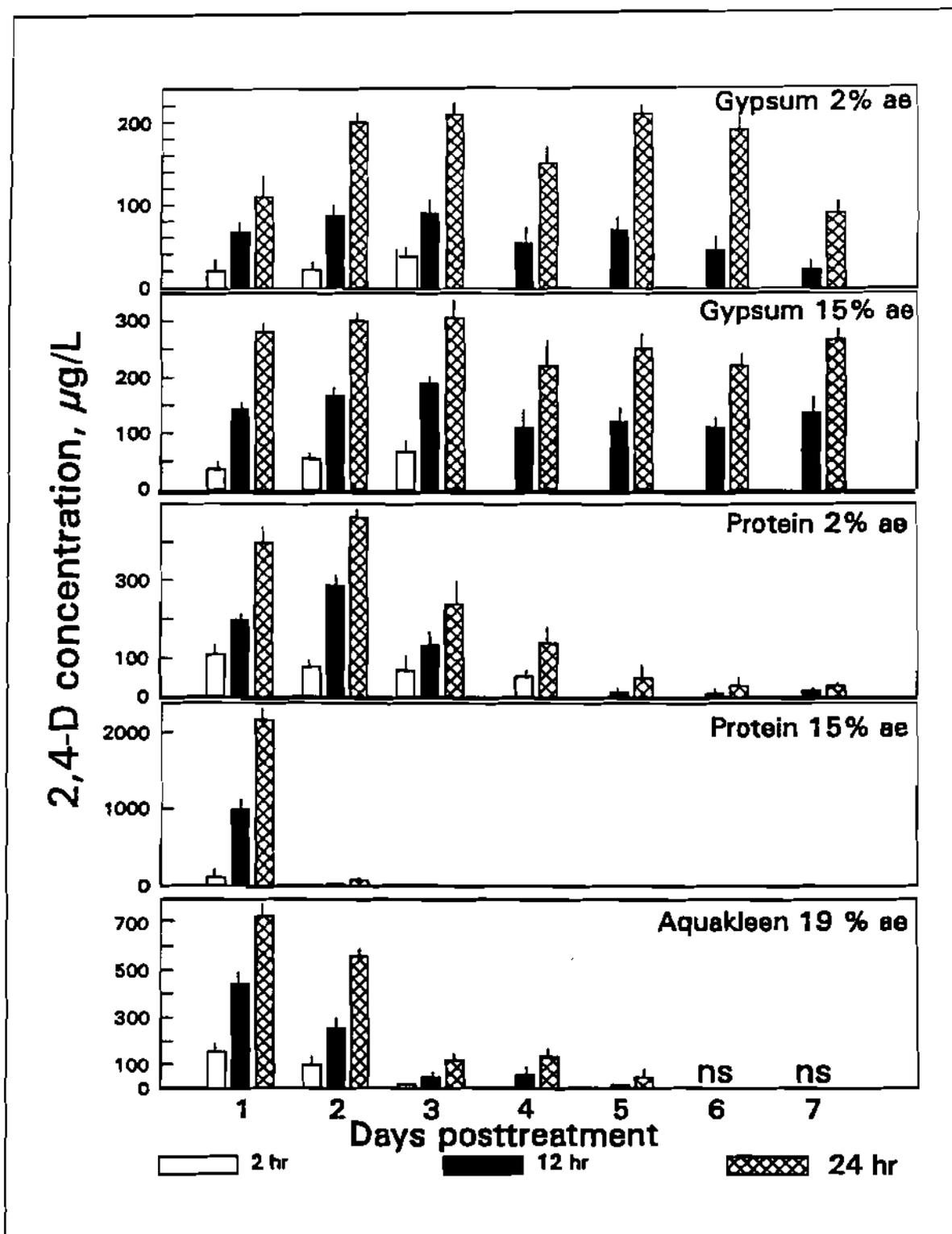


Figure 5. 2,4-D release rates from 2- and 15-percent ae gypsum and protein matrices and a conventional Aquakleen<sup>®</sup> 19-percent ae matrix over a 7-day period. Analytical limit of detection was 10 µg/L. Each bar is the average of three replicates, and vertical lines represent one standard error of the mean

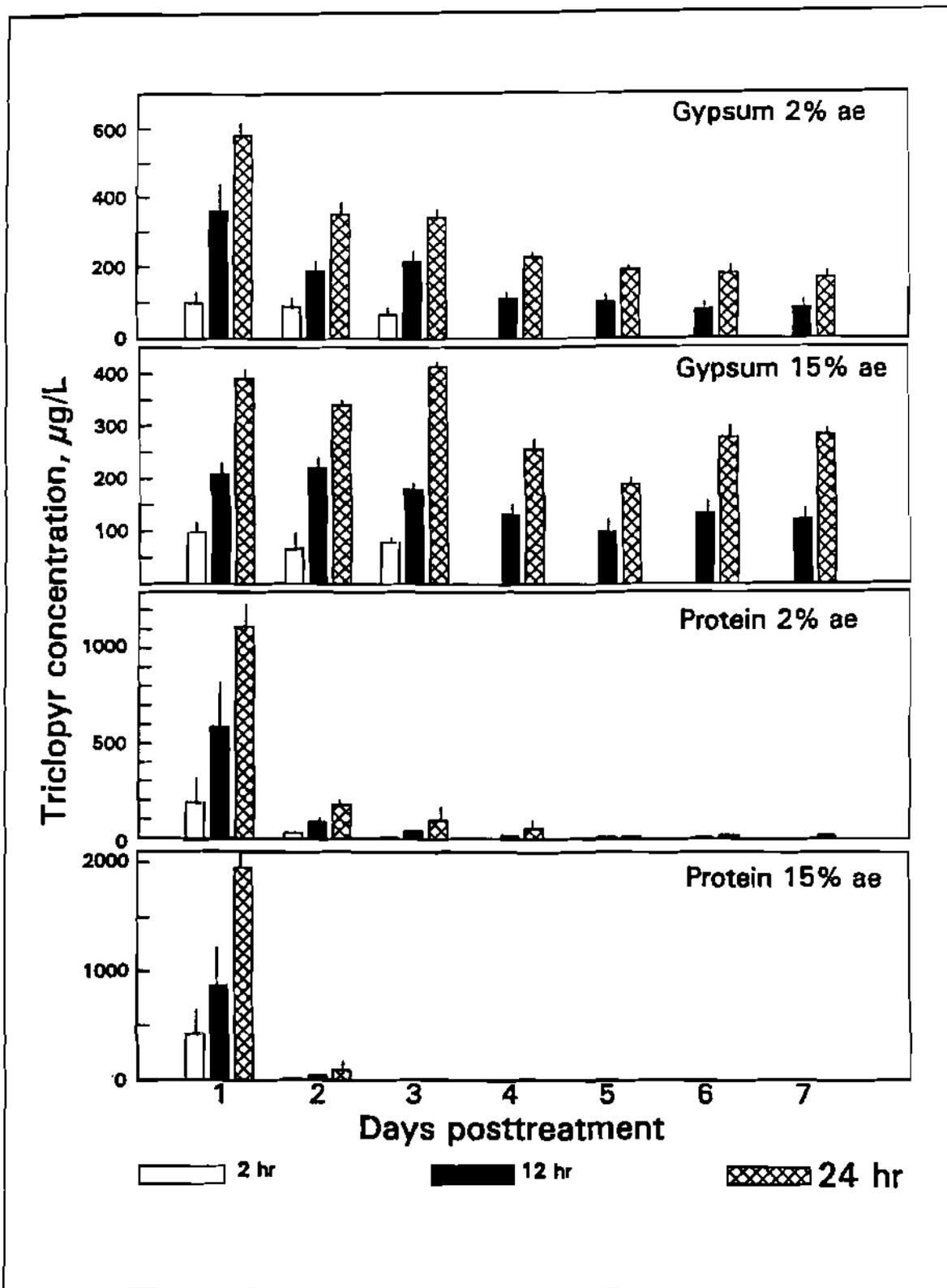


Figure 6. Triclopyr release rates from 2- and 15-percent ae gypsum and protein matrices over a 7-day period. Analytical limit of detection was 10 µg/L. Each bar is the average of three replicates, and vertical lines represent one standard error of the mean

release significant levels of 2,4-D over a 2-day period may help explain the success of this product in certain hydrodynamic environments.

Release rates for the endothall matrices are presented in Figure 7. Results indicated that all matrices released greater than 90 percent of the endothall load within 2 hr posttreatment. This resulted in minimal release occurring between 12 and 72 hr posttreatment. Reinert et al (1985) also noted greater than 90 percent release of endothall from the Aquathol<sup>®</sup> (10.1 percent ai) pellet within 24 hr posttreatment in a static shake flask test. These authors also showed the highest level of endothall release occurred between 0 and 2 hr. Results from this study indicate that none of the endothall matrices tested exhibited sustained release properties, and they are likely to be more effective for placement and distribution in the water column (rather than as CR matrices).

Field efficacy trials comparing the Aquathol<sup>®</sup> and 27-percent ai endothall granules indicated the 27-percent granule was not as effective. It has been suggested that spatial distribution in the water column may not be as thorough with the 27-percent matrix due to fewer granules (rapidly releasing endothall) per area.<sup>1</sup> The potential for aqueous endothall distribution to decrease as loading rates increase deserves further evaluation. The superabsorbing polymer formulation (45 percent ai) would make a good candidate for testing this hypothesis. Furthermore, the reduced bulk (due to higher percent ai loading), lack of dusting, and ability to increase surface area on contact with water warrant field evaluation of this product.

Based on laboratory studies, it was decided that the gypsum matrix in combination with 2,4-D or triclopyr would make good candidates for further evaluation. Triclopyr was chosen over 2,4-D because no granular formulation of triclopyr currently exists, and triclopyr is an EUP product with limited efficacy, dissipation, and degradation information in aquatic systems.

## 1992 Mesocosm Release Rate Evaluations

Release rates of triclopyr from the gypsum matrices are shown in Figure 8. Results showed that the target rates of 100 and 300 µg/L were not achieved at any point during the 6-day exposure period. However, residue analyses indicated that release profiles were quite consistent from both of the matrices tested. The average daily concentration for the treatment targeted for 100-µg/L was  $39 \pm 7.8$  µg/L, and  $82 \pm 23$  µg/L for the treatment targeted for 300-µg/L. Overall matrix weights were reduced 45 percent following the 100-µg/L treatment and 19.5 percent following the 300-µg/L treatment. Although aqueous residues reached only 39 and 22 percent of the respective target rates, these

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<sup>1</sup> Personal Communication, August 1993, Dr. Kurt D. Geisinger, Plant Physiologist, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

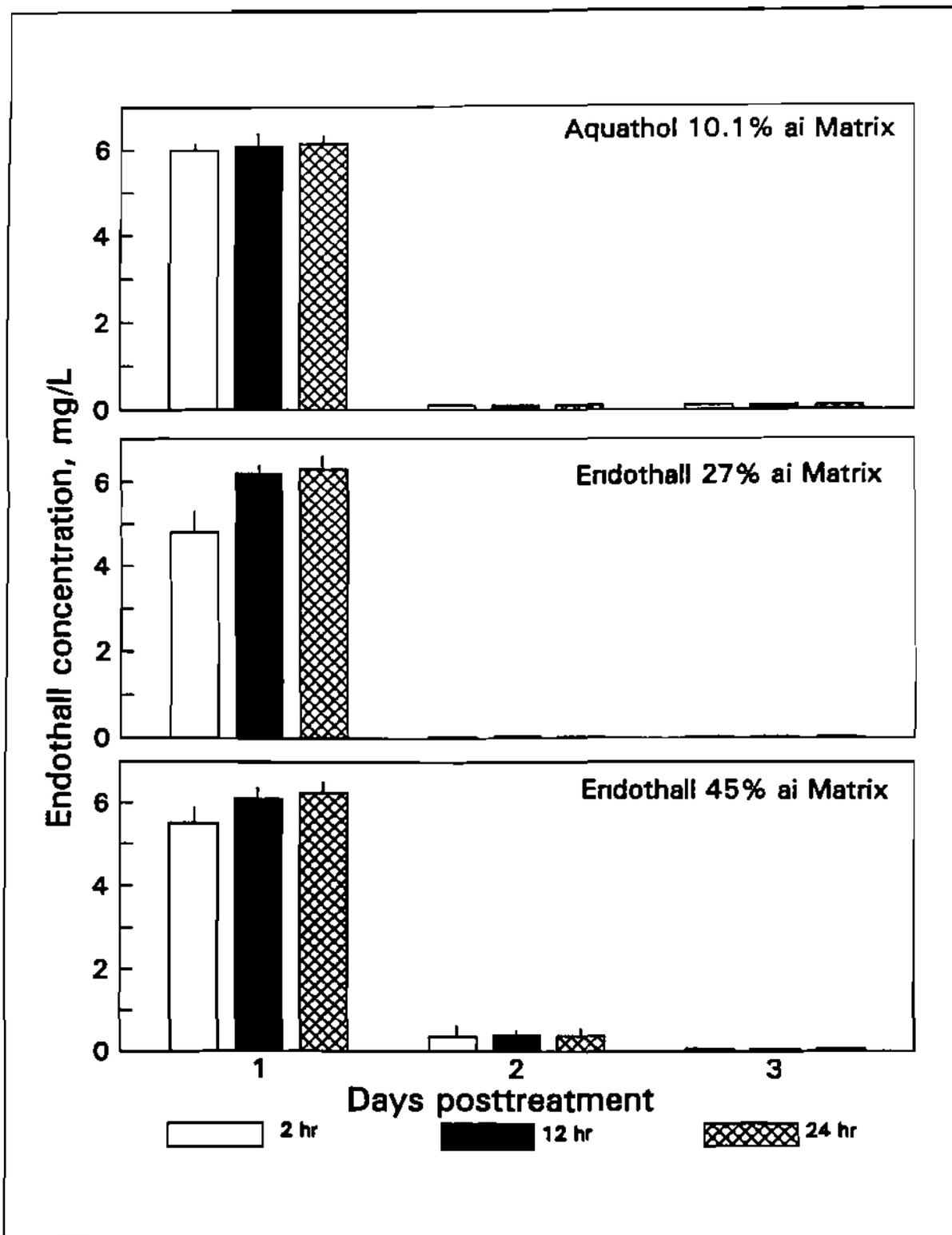
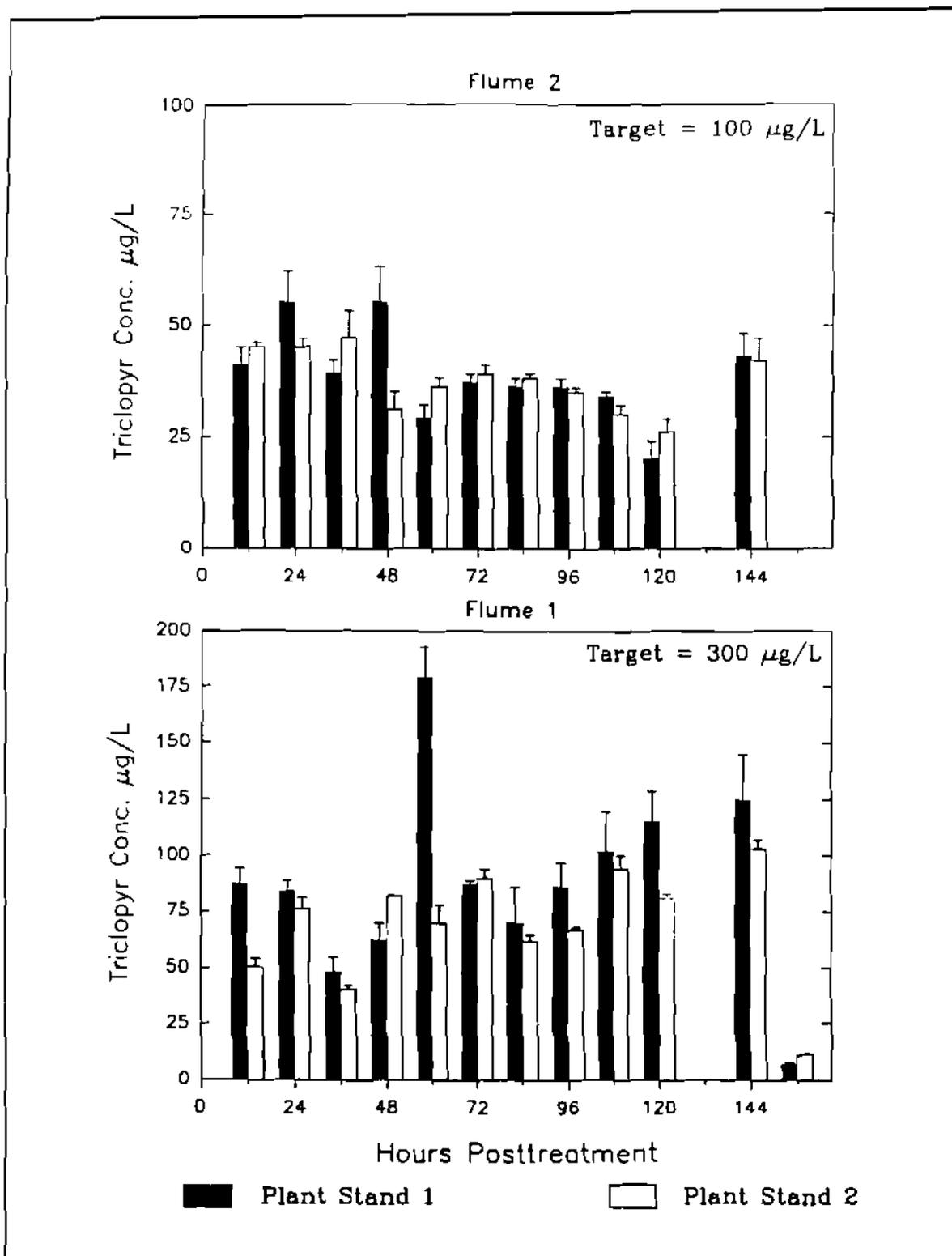


Figure 7. Endothall release rates from the 10.1-percent ai conventional Aquathol<sup>®</sup> matrix, a 27-percent ai clay granule, and a 45-percent ai superabsorbing polymer granule over a 3-day period. Analytical limit of detection was 10  $\mu\text{g/L}$ . Each bar is the average of three replicates, and vertical lines represent one standard error of the mean



**Figure 8.** Aqueous triclopyr concentrations in two hydraulic flumes following deployment of a 7-percent and 12-percent ae matrix targeted to achieve 100 and 300 µg/L, respectively, for a 6-day exposure. Bars represent the average of two replicates, and vertical bars represent one standard error of the mean

matrices provided sustained release of triclopyr over time. In contrast to the sustained release from the CR matrices, a liquid application of 2.5 mg/L had declined by approximately 90 percent within 8 hr posttreatment and was below the detection limit by 13 hr posttreatment (Turner et al. 1993).

Sediment herbicide residue levels are reported in Figure 9. Results showed that sediment levels generally mimicked aqueous concentrations during the course of the exposure period. This indicates that triclopyr has little affinity to accumulate in the sediment over time. Field studies with liquid applications have shown triclopyr does not accumulate in the sediment, and sediment residues were negligible following triclopyr dispersion from the water column (Westerdahl et al. 1989, Getsinger and Westerdahl 1984).

Within 6 hr following deployment of the matrices, milfoil manifested triclopyr injury symptoms. Apical tips became epinastic (twisting and bending), and some epidermal rupture was apparent. These symptoms persisted for the length of the exposure. At 1 week posttreatment, all treatments (CR matrices, 1,000 µg/L, 2,500 µg/L, and a metered treatment of 250 µg/L) were equally effective. Symptoms of milfoil treated with CR matrices were not distinguishable from the liquid treatments of 1,000 and 2,500 µg/L (even though these concentrations were 25 to 60 greater than CR results).

Milfoil biomass at the 6-week posttreatment harvest is shown in Figure 10. Results indicate that no milfoil control was achieved following treatment with the CR matrices. Moreover, milfoil biomass increased three to five times over the pretreatment levels in just 5 weeks. Data analysis indicated that no significant difference ( $p = 0.27$ ) in biomass existed between the untreated reference plants and the treated plants at the 6-week harvest.

The metered application which was targeted to deliver 250 µg/L for 84 hr resulted in 100-percent milfoil control (Turner et al. 1993). However, plants exposed 250 µg/L for 24 and 48 hr resulted in 0 and 60 percent control respectively. Moreover, the liquid application of 2,500 µg/L was very ineffective (0 percent control) in plant stand 1 where herbicide was rapidly dispersed, but resulted in 100-percent control in plant stand 3 which received a longer exposure period (Turner et al. 1993). In addition, it is likely that triclopyr was more thoroughly mixed in the water column by the time it had reached plant stand three. In the areas where milfoil control occurred, dense stands of *Najas* spp. and *Chara* spp. were noted.

The failure of the CR matrices to control milfoil can be directly attributed to the failure to achieve the target aqueous concentrations of 100 and 300 µg/L. Results showed that 120 hr of exposure to rates ranging between 30 and 80 µg/L (CR treatments) were completely ineffective for controlling milfoil; whereas, 84 hr of exposure to rates ranging between 165 and 315 µg/L (metered treatment) provided 100-percent milfoil control. Aqueous concentrations achieved with the CR matrices were closer to earlier CR testing strategies which targeted minimum threshold levels. These release rates would have required a much longer exposure period to provide milfoil control. Although

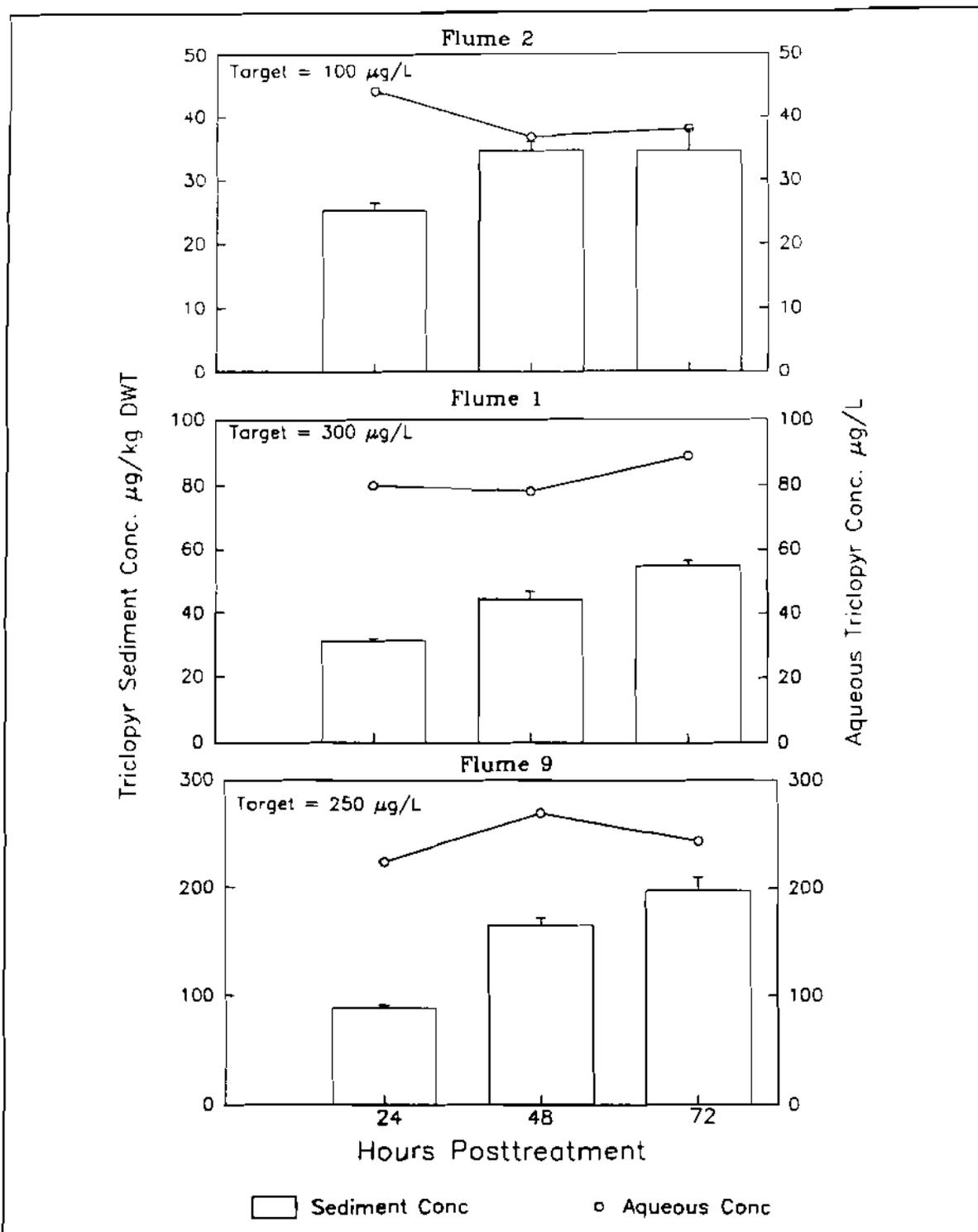


Figure 9. Sediment concentrations of triclopyr in three hydraulic flumes following deployment of a 7-percent and 12-percent ae matrix targeted to achieve aqueous concentrations of 100, 300, and 250  $\mu\text{g}/\text{L}$ , respectively, for a 6-day exposure. Bars represent the average of eight replicates, and vertical lines represent one standard error of the mean. In addition, data are presented for a metered liquid application targeted to achieve 250  $\mu\text{g}/\text{L}$  for 4 days

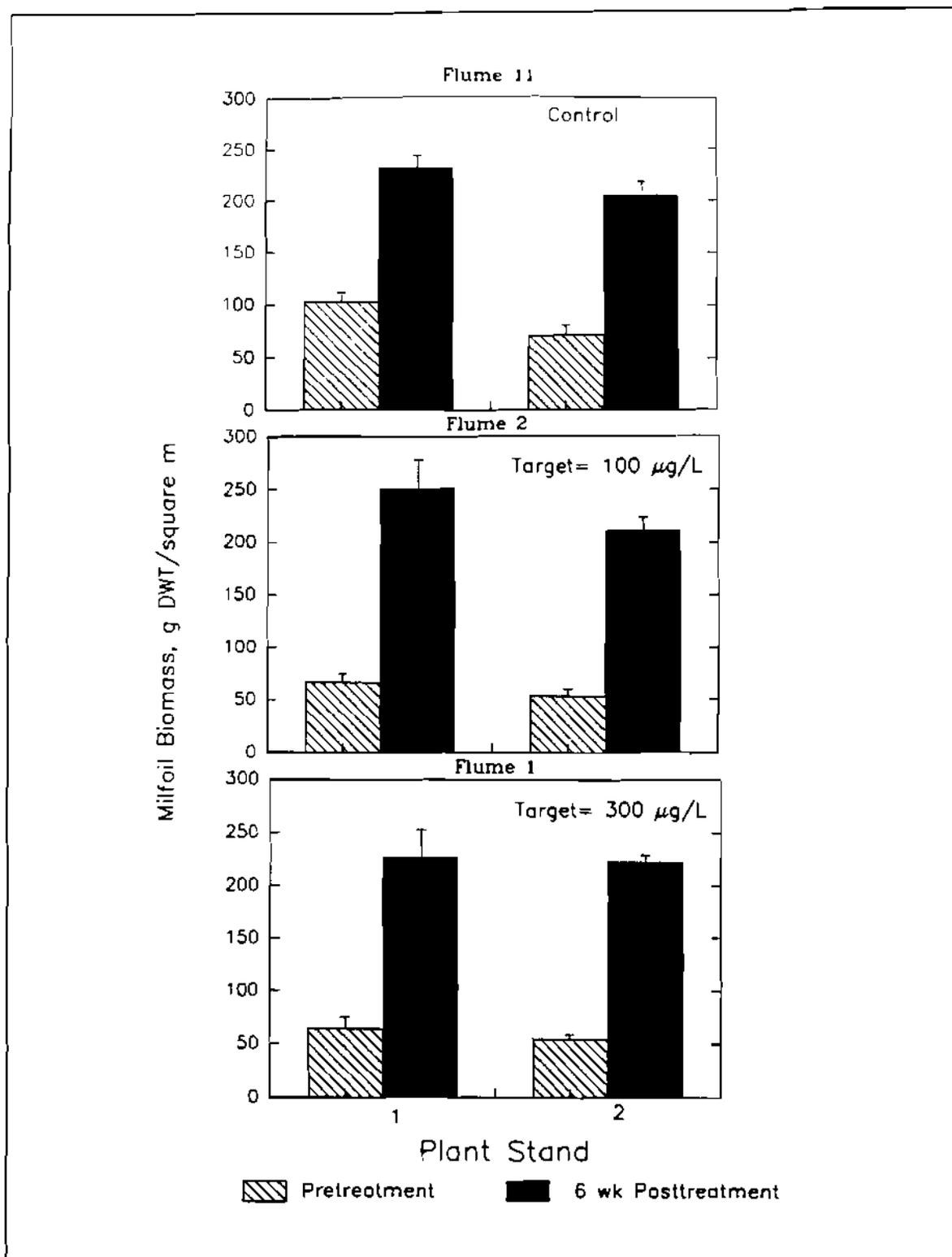


Figure 10. Pretreatment and 6 week posttreatment Eurasian watermilfoil biomass in two plant stands following treatment with two CR matrices formulated to deliver concentrations of 100 and 300 µg triclopyr/L for a 6-day exposure period. An untreated control stand is included for comparative purposes. Bars represent the average of four replicates, and vertical lines represent one standard error of the mean

milfoil showed an injury response when exposed to these low levels of triclopyr, the ability of these concentrations (even at longer exposure periods) to control milfoil remains uncertain.

## 1992 Milfoil Herbicide Tissue Burden

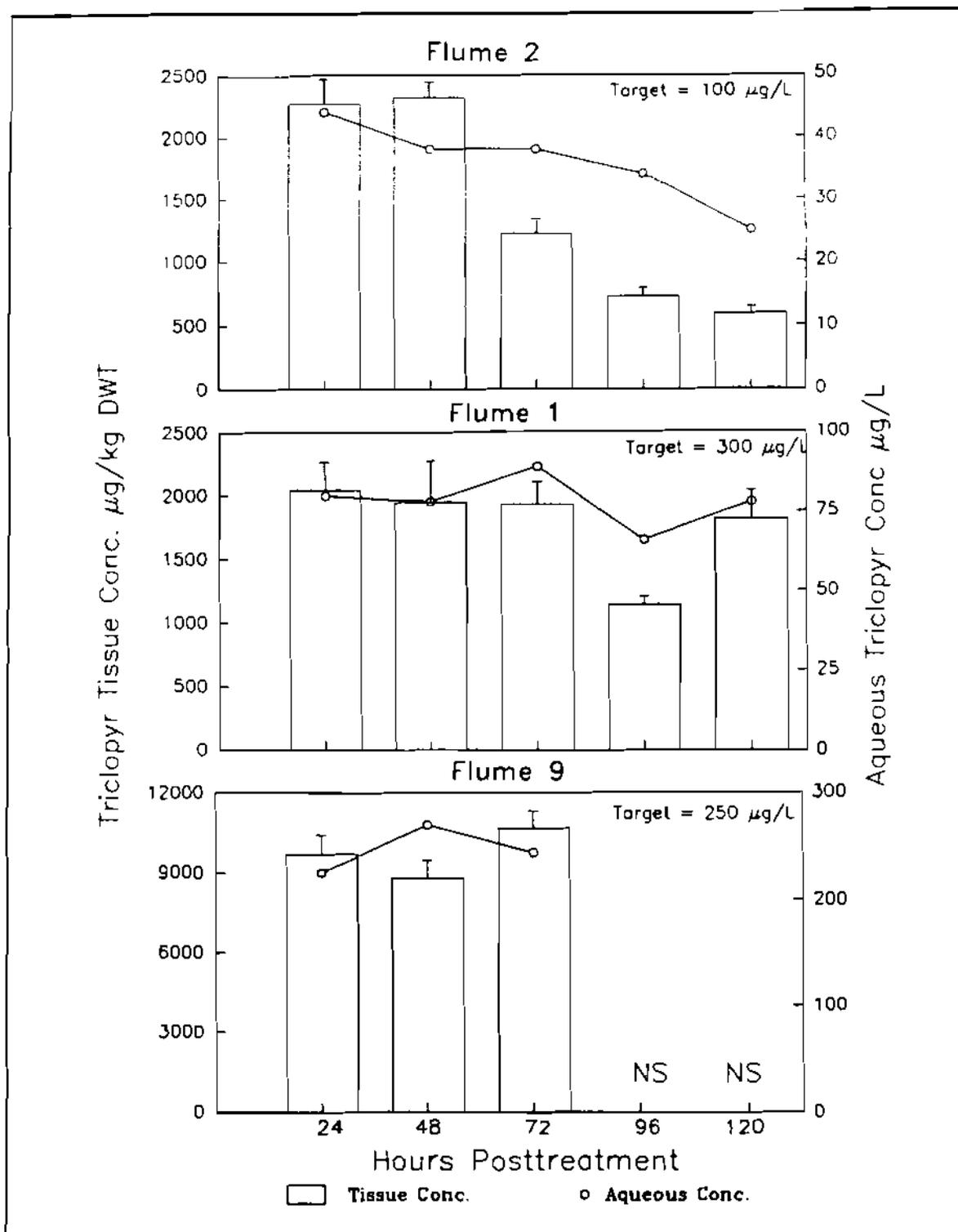
The uptake of triclopyr by milfoil occurred rapidly, and near maximal tissue levels were achieved by 24 hr posttreatment (Figure 11). Results show that the majority of the uptake occurred within 24 hr and then remained quite steady in conjunction with aqueous concentrations. It is uncertain if uptake ceased at 24 hr, or if uptake and depuration rates were equivalent, resulting in little net change of triclopyr tissue concentrations.

The 100- $\mu\text{g/L}$  treatment produced tissue levels (on a microgram per kilogram dry weight basis) that were greatest on days 1 and 2 posttreatment, followed by a significant decline ( $p = 0.02$ ) on days 3, 4, and 5 (Figure 11). A general decline was also noted in aqueous concentrations at days 3, 4, and 5. Bioconcentration factors (BCFs) ranged from 24 to 56 over the 5-day sample period.

Internal tissue and aqueous concentrations remained quite constant following the 24-hr sample in the 300- $\mu\text{g/L}$  treatment (Figure 11). The 4-day tissue sample was the only treatment that was significantly different from the others. BCFs ranged from 20 to 26 over the sample period.

Following the metered treatment targeted to deliver 250  $\mu\text{g/L}$ , internal tissue levels rapidly elevated within 24 hr and remained constant over the 3-day sampling period. The higher tissue levels in comparison to the CR treatments were correlated with higher aqueous concentrations achieved in the metered treatment. BCFs ranged from 34 to 42 over the 3-day sample period. It is notable that the BCFs for all three triclopyr treatments were similar; whereas, the aqueous concentrations increased up to ten fold between treatments. Van and Conant (1988) noted that tissue concentrations of diquat and endothall increased as treatment rates increased; consequently, within a given exposure period, the BCFs remained similar as concentrations increased.

Studies by Haller and Sutton (1973), Cassidy and Rodgers (1989), and Van and Conant (1988), with diquat and endothall, showed uptake of these compounds was generally linear for periods up to 4 days. However, these studies also showed that lower initial concentrations of diquat or endothall resulted in decreased uptake and accumulation of these herbicides. In comparison, the majority of triclopyr uptake in this study occurred within 24 hr and did not increase over time. Although, it is possible that initial aqueous triclopyr concentrations were too low to observe significant increases in plant uptake over time.



**Figure 11.** Triclopyr concentrations in Eurasian watermilfoil tissue and water following deployment of two CR matrices formulated to deliver concentrations of 100 and 300 µg/L for a 6-day period. In addition data are presented for a metered liquid application targeted to achieve 250 µg/L for a 4-day exposure period. Bars represent the average of nine replicates, and vertical lines represent one standard error of the mean. NS denotes that samples were not taken

Preliminary results from this study suggest that tissue concentrations below 2,500 µg/kg dry weight (although maintained for at least 5 days) may be an indicator of poor milfoil control. It remains unclear if longer term exposure to these internal concentrations would increase efficacy. However, it is possible that the critical threshold concentration was not reached, and this made the exposure period irrelevant.

Tissue concentrations following the 250-µg/L treatment approached 9,350 µg/kg DWT within 24 hr and remained constant over the next 48 hr. Although tissues loaded rapidly, milfoil control was not achieved if aqueous concentrations were not maintained for greater than 48 hr. This indicates that use of a single internal tissue level at a given time (especially early) may be an unreliable indicator of plant control. In addition, these results suggest that although tissues load very rapidly with triclopyr, they still require a certain length of aqueous exposure to maintain a lethal internal concentration.

Although initial uptake results provided interesting information, interpretation of the results remains difficult because of the limited sampling protocol. More tissue sampling, prior to 24 hr and following herbicide dispersion from the water column, would provide valuable information concerning uptake and depuration.

## 1993 Mesocosm Release Rate Evaluations

Triclopyr release rates of the two gypsum CR formulations are shown in Figures 12 and 13. Results showed that release rates were in the range of the target concentrations (300 and 500 µg/L) throughout most of the 84- and 120-hr exposure periods. Although some residue variability was noted over time and between plant stands within sample times, overall matrix performance was consistent. Some of the variability observed within and between plant stands was likely caused by the limited number of samples analyzed and channeling and pooling of water as it flowed through the flumes (plant stands). Uneven distribution of residues in the flumes has also been observed following liquid applications of triclopyr and rhodamine WT.

Following matrix deployment, little variability was noted until the 60-hr samples were analyzed. A spike release (approximately double the target rates) occurred in three of the four treated flumes. It is possible that during the 54-hr posttreatment inspection of the matrices for integrity and cracks, agitation (lifting and placing the matrix back in the water) may have enhanced release. Furthermore, cracks were noted in some of the matrices, and this condition could have contributed to increased release rates.

The large pumps that supply water and flow to the flumes failed at 115 hr posttreatment. In response, matrices were removed (at 117 hr) to prevent accumulation of triclopyr in the flumes. In spite of rapid removal of the matrices, residue analyses showed that triclopyr residues moved back into plant

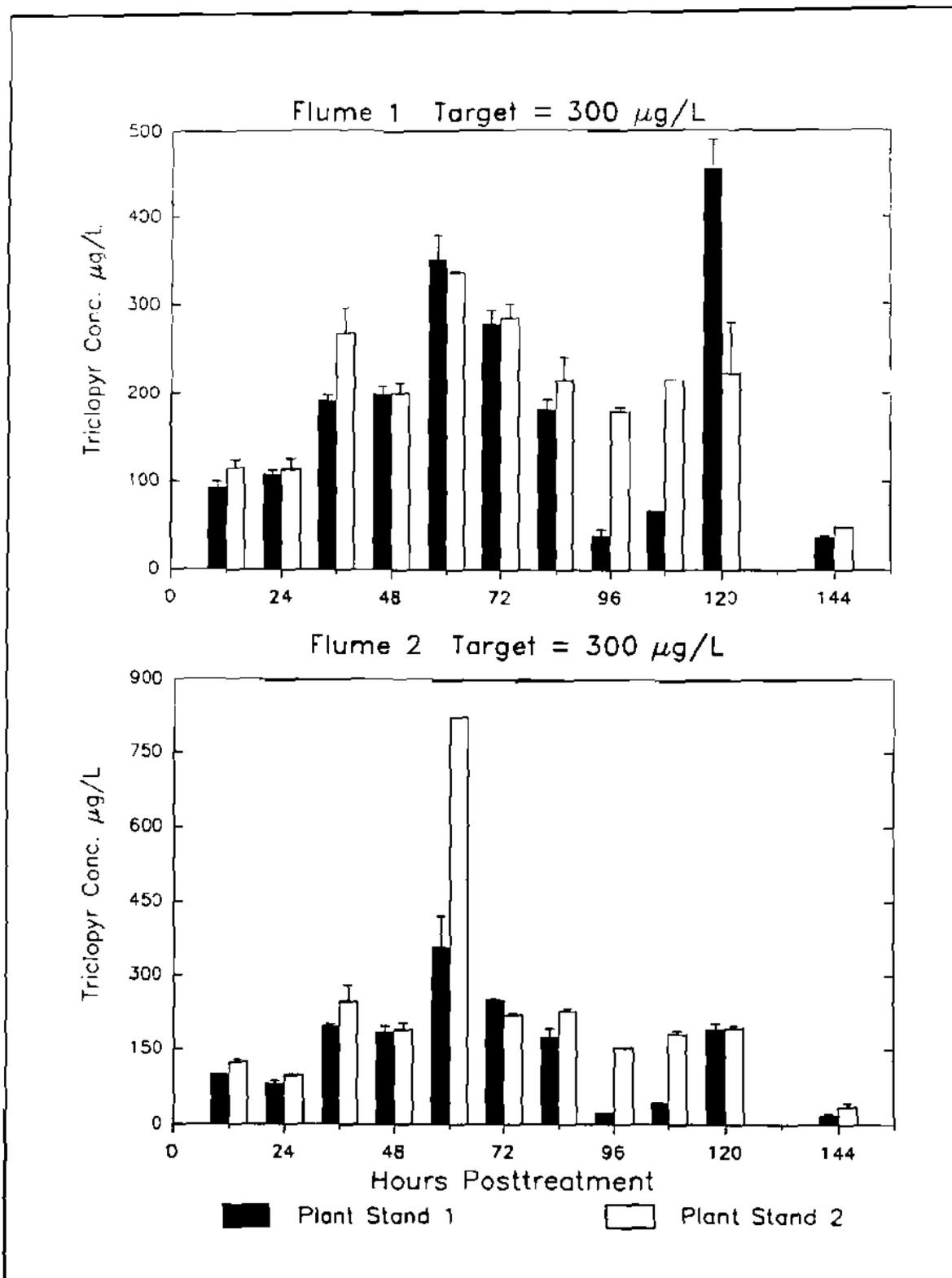
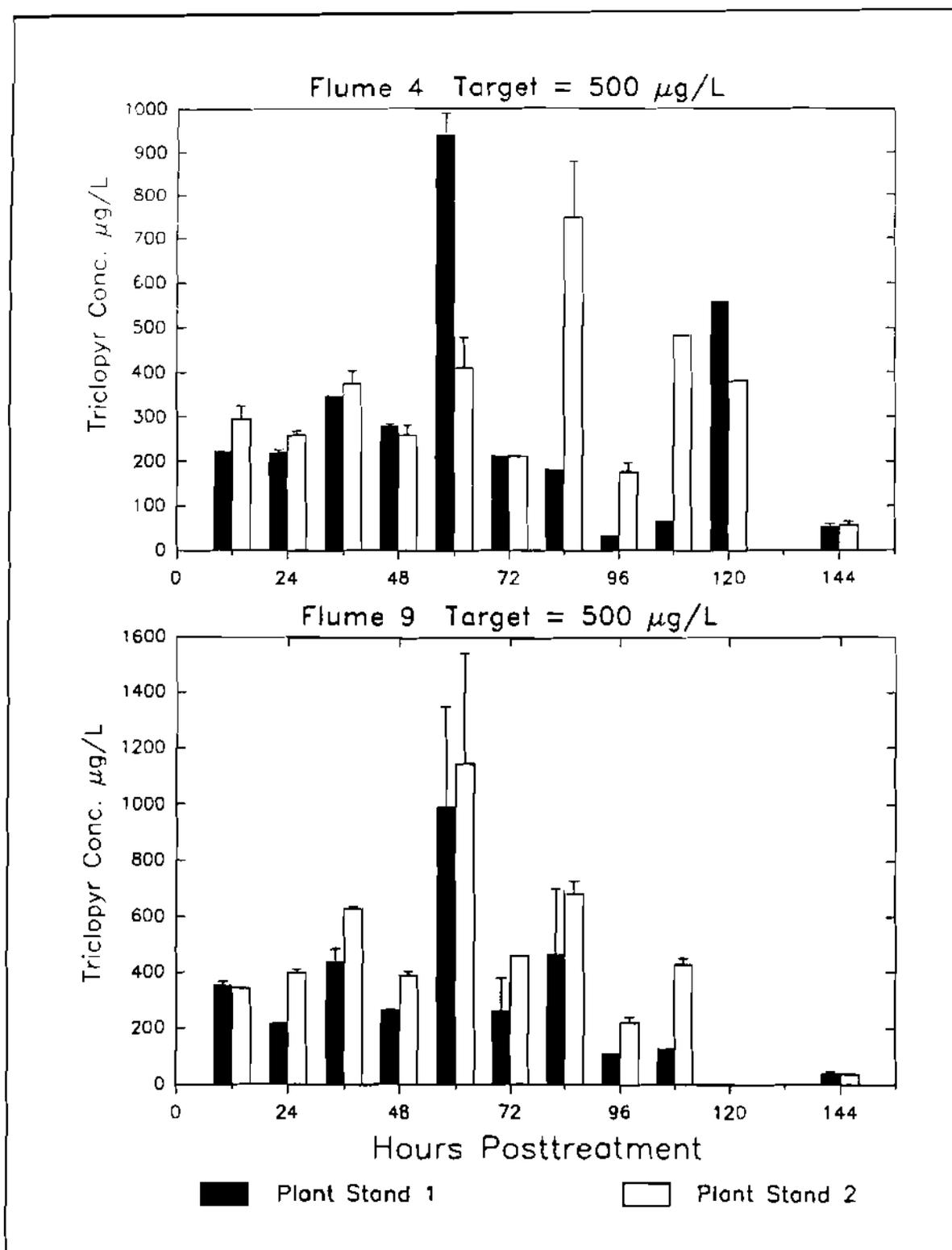


Figure 12. Aqueous triclopyr concentrations in two hydraulic flumes following deployment of a 12-percent as matrix targeted to deliver 300  $\mu\text{g/L}$  for a 5-day exposure period. Bars represent the average of two replicates, and vertical lines represent one standard error of the mean



**Figure 13.** Aqueous triclopyr concentrations in two hydraulic flumes following deployment of a 17-percent ae matrix targeted to achieve 500 µg/L for a 5-day exposure period. Bars represent the average of two replicates, and vertical lines represent one standard error of the mean

stand 1 by 120 hr (stand 1 concentrations were very low at 96 and 108 hr) resulting in further exposure of the milfoil. However, analyses showed that residues had significantly dropped from 120- to 144 hr posttreatment (Figures 12 and 13). Samples collected at 120 hr were taken just 5 hr following pump failure, and it is probable that limited dilution (much of the flume contained no triclopyr residues) had occurred during this time. In addition, Woodburn et al. (1993) reported that triclopyr has a photolytic half-life in river water of 38 hr at 25 °C. Therefore, the rapid residue decline was likely influenced by a combination of dilution and photolysis.

Complete matrix dissolution occurred in four of the eleven 500- $\mu\text{g/L}$  matrices (and two of the eleven 300- $\mu\text{g/L}$  matrices). Matrices in which cracks had been detected were also the same matrices which completely dissolved. Cracks in the matrices likely increased the surface area which led to enhanced dissolution of the matrix. The higher triclopyr loading rates of the 500- $\mu\text{g/L}$  matrices may have resulted in some of the cracking problems. It should be noted that the majority of the matrices remained intact with no cracking or uneven dissolution. Posttreatment weights of several of the matrices indicated that both formulations were reduced by 49 to 65 percent during the exposure period.

Following matrix deployment, triclopyr injury symptoms were noted within 6 hr. Characteristic epinastic symptoms persisted and progressed over the next 7 days. No visual symptoms were noted for the naiads, chara, or pondweeds. Visual differences in milfoil injury were not noted; however, tissue samples began to indicate some qualitative differences existed between treatments by 72 hr posttreatment. Milfoil treated with liquid applications at rates of 1,500 and 3,000  $\mu\text{g/L}$  for 48 hr became extremely brittle by 72 hr posttreatment. Following CR treatments, milfoil tissue was still pliable and generally remained intact at 72 hr. However, by 5 days posttreatment, all tissue samples from CR treatments had become brittle.

Pretreatment and posttreatment biomass results are presented in Figures 14 and 15. In addition to milfoil, *Najas* spp. biomass was included as an indicator of triclopyr selectivity. Data showed that both matrix treatments resulted in near 100-percent milfoil control in plant stands 1 and 2 at 8 weeks posttreatment. Although triclopyr exposure was targeted at 84 hr in plant stand 1, previously mentioned failure of the pumps resulted in reexposure of the milfoil. As a result, no significant differences in efficacy based on exposure period were noted.

Triclopyr remained very selective following the CR treatments, and naiads, chara, and pondweeds were abundant in plant stands formerly dominated by milfoil (Figures 14 and 15). A few milfoil stems were detected in the upper plant stands of the matrix treatments, but no milfoil was collected in the random biomass samples.

The failure of the pumping system, which left water static over several days, could have enhanced the overall efficacy results. However, based on

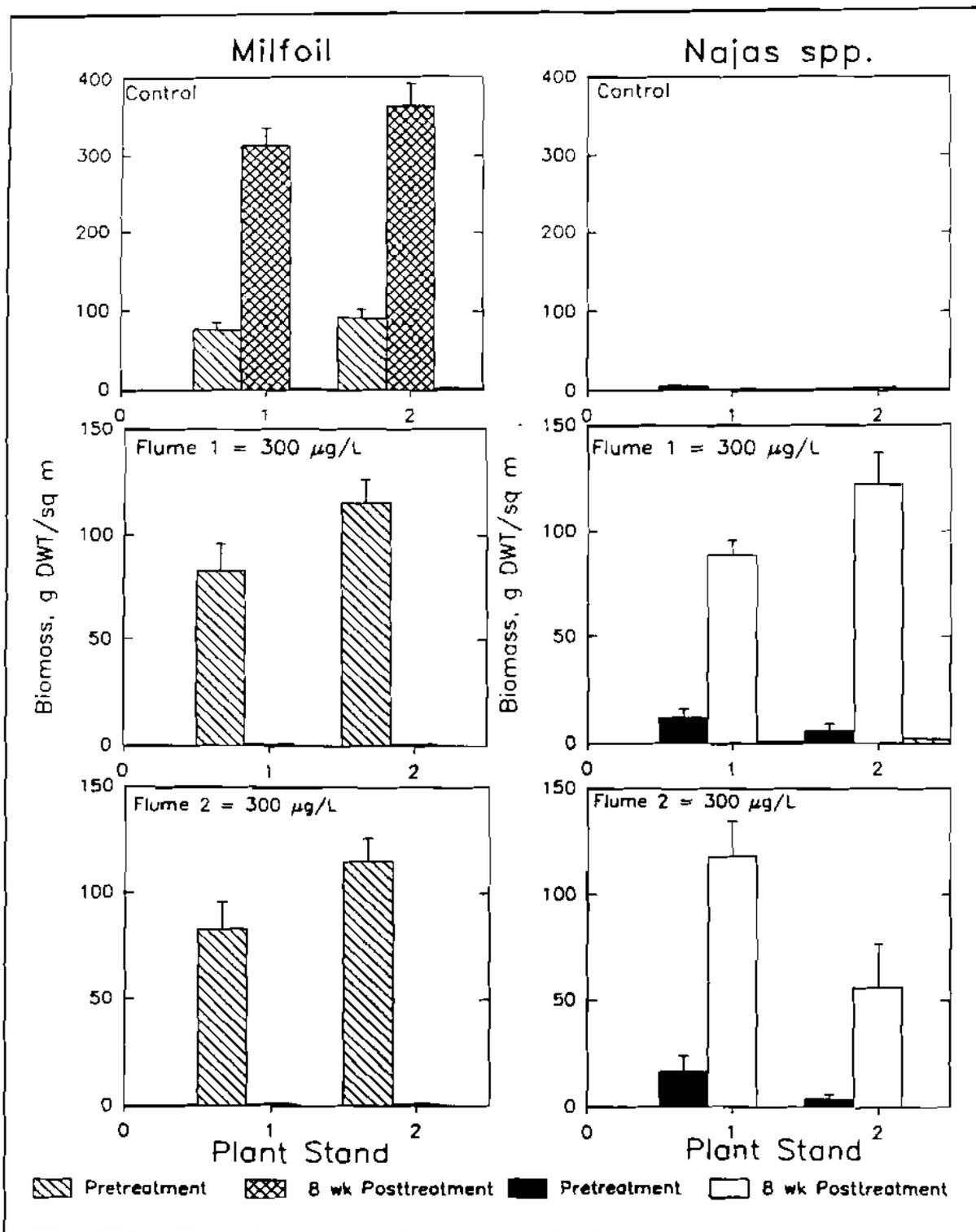


Figure 14. Pretreatment and posttreatment Eurasian watermilfoil and *Najas guadalupensis* biomass in two plant stands following treatment with a CR matrix formulated to deliver 300 µg triclopyr/L for a 3.5- and 5-day exposure period. An untreated control stand is included for comparative purposes. Bars represent the average of four replicates, and vertical lines represent one standard error of the mean

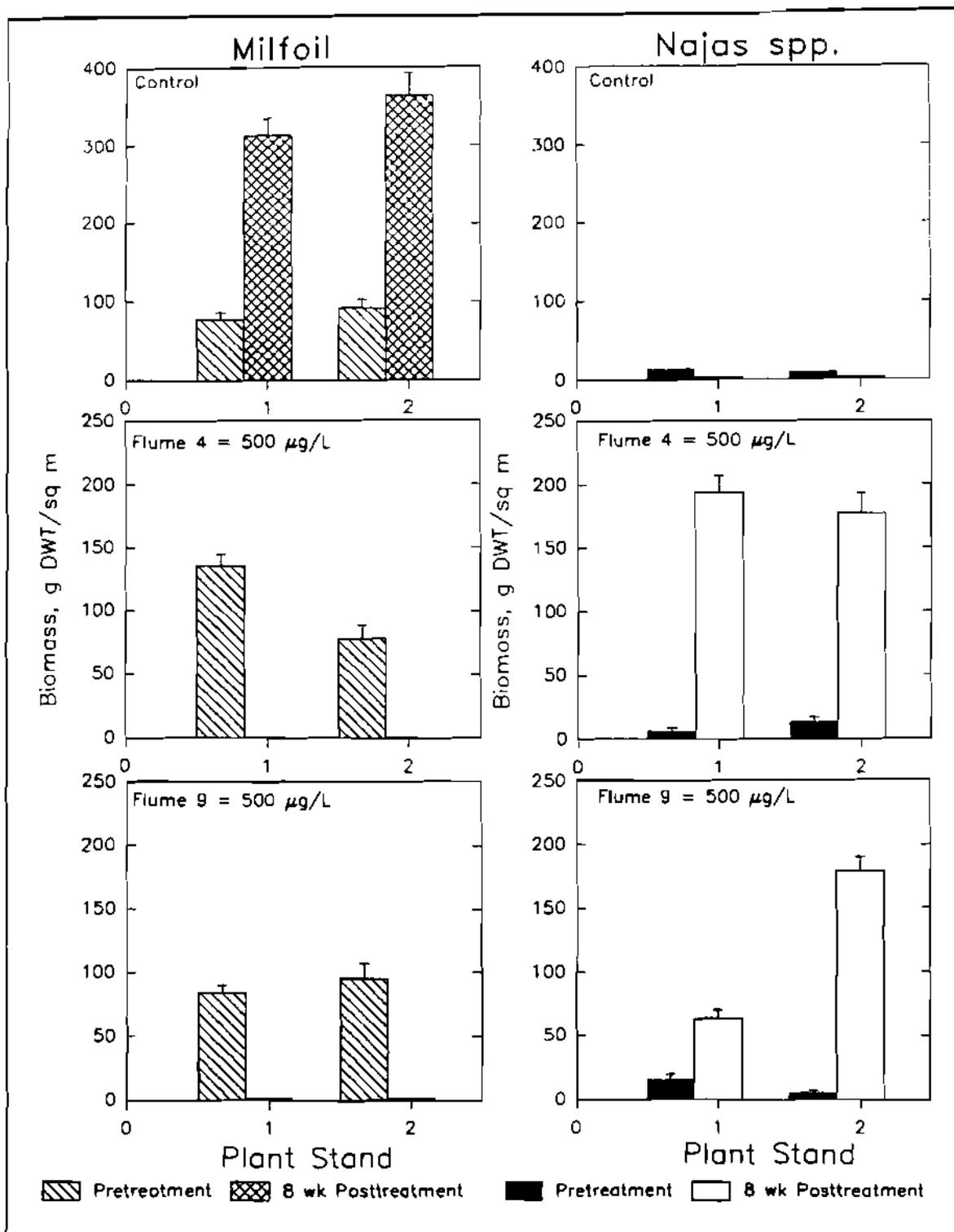


Figure 15. Pretreatment and posttreatment Eurasian watermilfoil and *N. guadalupensis* (80 percent) and *N. minor* (20 percent) biomass in two plant stands following treatment with a CR matrix formulated to deliver 500 µg triclopyr/L for a 5-day exposure period. An untreated control stand is included for comparative purposes. Bars represent the average of four replicates, and vertical lines represent one standard error of the mean

previous flume studies, it is likely that an 84-hr exposure to the triclopyr levels achieved during both matrix treatments would have provided excellent milfoil control. Water analyses indicated that average triclopyr residue levels were below 60 µg/L by 6 days posttreatment and below detection following a 10-day posttreatment sample.

Calculations indicated that liquid static treatments of 1,500 and 3,000 µg/L required a total of 0.85 and 1.43 kg of triclopyr per flume; whereas, CR target rates of 300 and 500 µg/L were determined to require 0.6 and 1.0 kg of triclopyr per flume per day. Consequently, a total of 3.0 and 5.0 kg of triclopyr were required over the 5-day exposure period. Although it appears that these CR treatments resulted in greater herbicide use, one must consider the potential area of treatment. For example, if the length of the flumes were increased from 100 to 1,000 m, the static treatments would also need to increase tenfold from 0.85 and 1.43 to 8.5 and 14.3 kg of triclopyr per flume. CR treatments were based on flow-rates; therefore, although the treatment area increased tenfold, the total amount of triclopyr required (3.0 and 5.0 kg) remained constant over the 5-day exposure period.

The flumes demonstrate that spot treatment of small areas in which plants are growing in high-flow environments could require more herbicide loading (kg/ha) for a CR matrix than for a conventional application. In addition, treatment of small areas with CR formulations (especially long-term release matrices) could result in significant off-target movement and non-target injury. Treatment sites will need to be carefully picked to avoid these potential problems.

In addition to the triclopyr matrices, one endothall formulation targeted to achieve 500 µg/L for a 4-day exposure was evaluated. Results of endothall release rates are presented in Figure 16. Data showed that endothall release was close to target concentrations and remained quite consistent over the 96-hr exposure period. Following matrix removal from the water, visual inspection indicated that four of eleven matrices had completely dissolved. Although some quality control problems existed with these matrices, release rates achieved were consistent and reliable.

Following matrix deployment, damage was noted at the apical tips of the milfoil by 36 hr posttreatment. Initial injury symptoms were not as obvious as triclopyr symptoms; however, by 7 days posttreatment, both milfoil and naiad stems were discolored and waterlogged.

Biomass results of the endothall matrix treatment are presented in Figure 17. Results are reported for plant stand 1 only, as milfoil never became established in plant stand 2. In addition, unlike triclopyr, endothall is considered to be a nonselective herbicide; therefore, control of all submersed species would be expected. Results show that endothall was highly effective at controlling milfoil and naiads following 96 hr of exposure. Complete submersed plant control was observed at 8 weeks posttreatment within the plant stand and throughout the flume. Because endothall matrices were removed from the

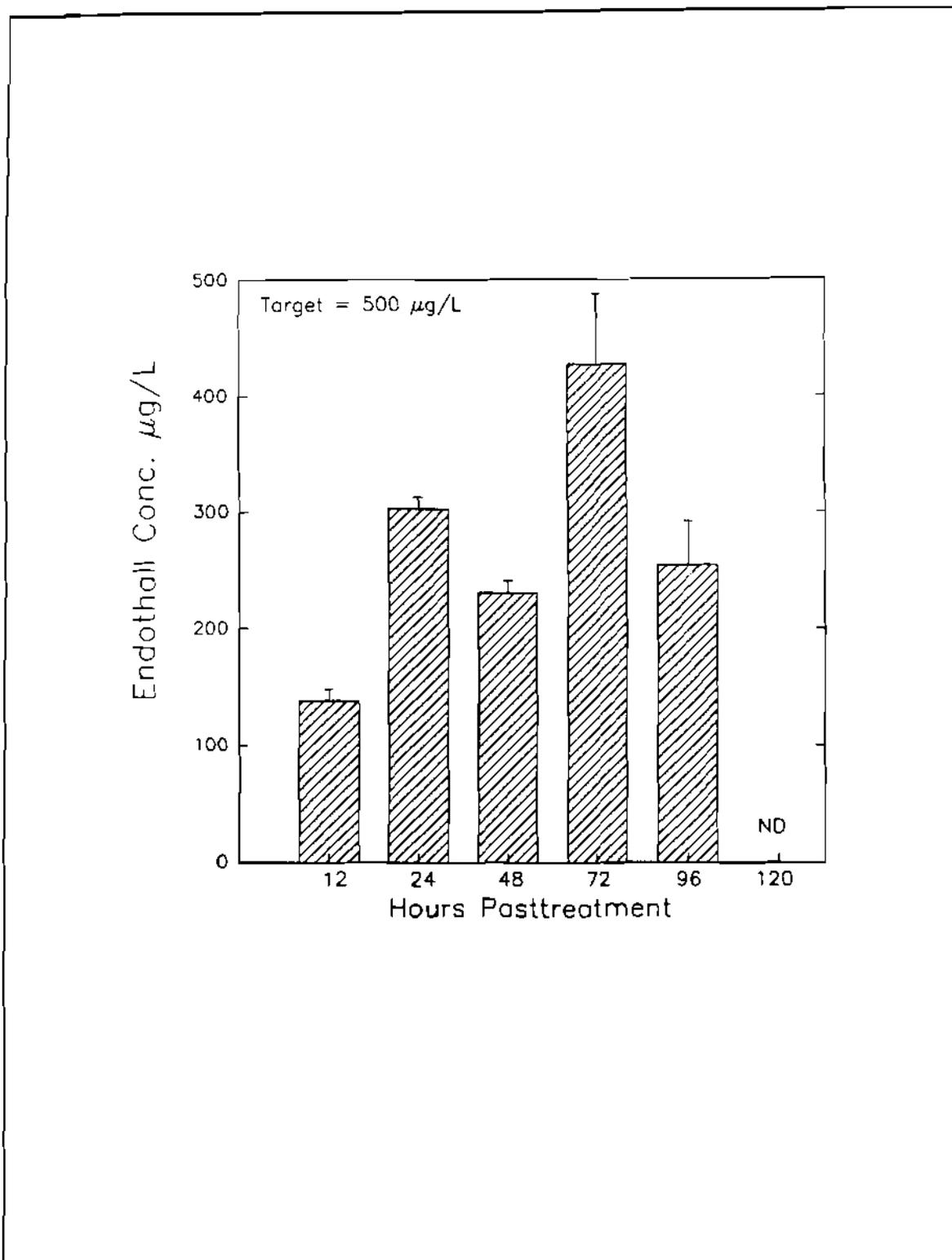
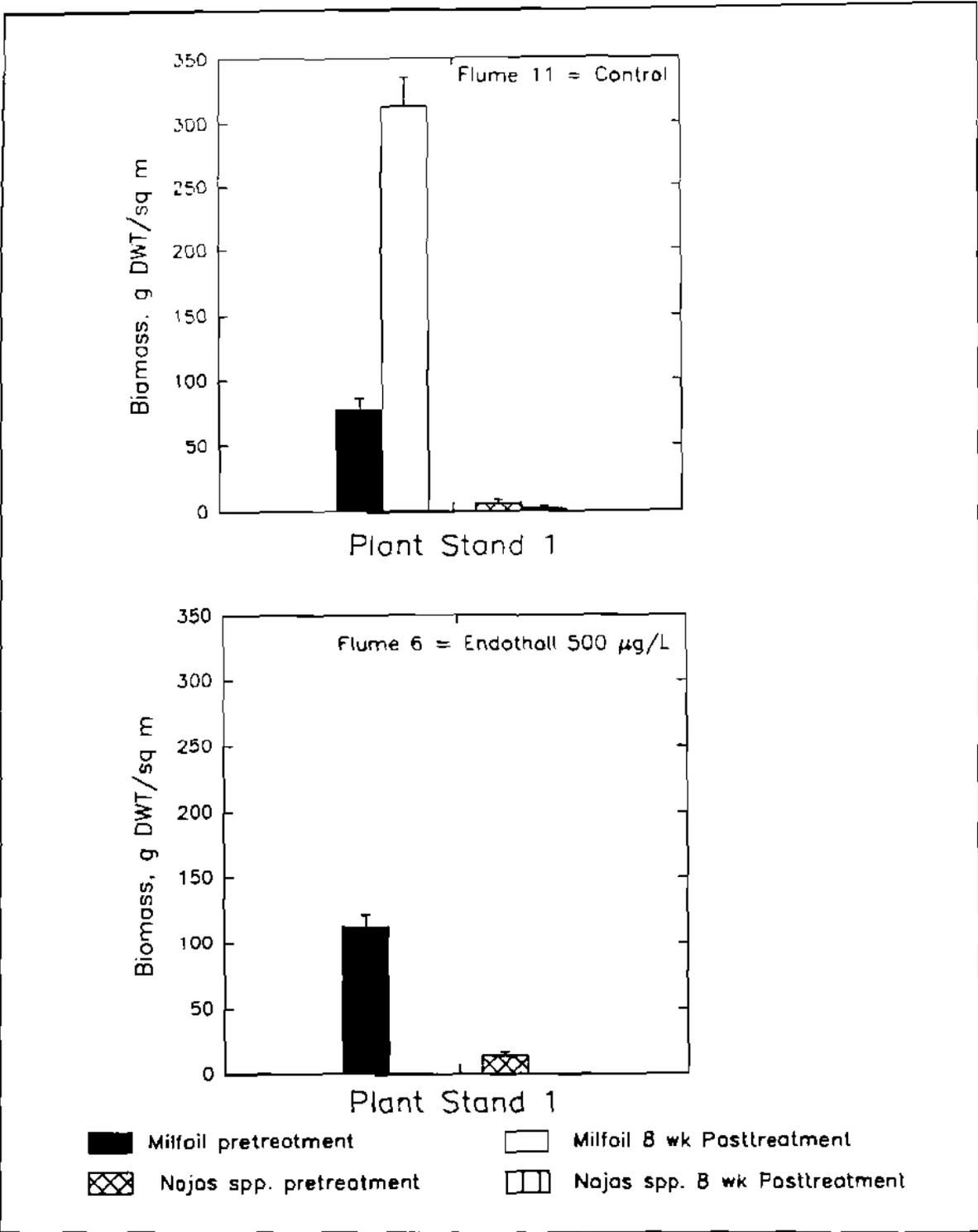


Figure 16. Aqueous endothall concentrations in a hydraulic flume following deployment of a 14 percent ae matrix targeted to achieve 500 µg/L for a 4-day exposure period. Bars represent the average of two replicates, and vertical lines represent one standard error of the mean. ND denotes endothall was not detectable at the sample time



**Figure 17.** Pretreatment and posttreatment Eurasian watermilfoil and *N. guadalupensis* (75 percent) and *N. minor* (25 percent) biomass in plant stand 1 following treatment with a CR matrix formulated to deliver 500 µg endothal/L for a 5-day exposure period. An untreated control stand is included for comparative purposes. Bars represent the average of four replicates, and vertical lines represent one standard error of the mean

flume at 96 hr posttreatment, residues had significantly declined prior to the pump failure at 115 hr.

Water quality parameters were measured daily in each flume during the treatment period. Water temperatures ranged from 24.2 to 33.2 °C, pH ranged from 7.9 to 9.9, dissolved oxygen ranged from 7.7 to 13.8 mg/L, and conductivity ranged from 151 to 172 µS. Although values fluctuated, no differences were noted between the treated and untreated reference flumes.

## 1993 Milfoil Herbicide Tissue Burden

Milfoil uptake of triclopyr following a 48-hr static exposure to concentrations of 1,500 and 3,000 µg/L is presented in Figure 18. Results show that uptake occurred rapidly (within 2 hr), and maximum concentrations were achieved by 6 hr posttreatment. Following the 6-hr peak concentrations, tissue concentrations declined but remained fairly stable from 12 to 48 hr posttreatment. Aqueous triclopyr concentrations in these flumes showed a significant decrease from 24 to 48 hr posttreatment (Figure 18). Triclopyr degradation in the water column is likely due to photolysis as exposures remained static for 48 hr. Following removal of triclopyr-treated water at 48 hr, tissue concentrations showed a significant decline ( $p = 0.01$ ) by 72 hr. Results indicate that although triclopyr was rapidly depurated from plant tissue following removal of aqueous concentrations, decreased but significant levels of triclopyr remained within milfoil tissue at 120 hr following treatment.

It is possible that adsorption to milfoil tissue constitutes a significant fraction of the triclopyr measured. A rapid adsorption phase followed by release may help to explain the significant drop in tissue levels at 12 hr posttreatment. Uptake results compare favorably with the 1992 matrix data because maximum tissue concentrations were achieved within 24 hr posttreatment. However, BCFs in the range of 5 to 14 for these static treatments are considerably less than values observed following the 1992 treatments. As expected, following the 48-hr static exposures, 100-percent milfoil control was achieved. *Najas guadalupensis* thrived following exposure to these treatments.

Triclopyr uptake results from a flowing-water flume treated at 3,000 µg/L are presented in Figure 19. Data show that aqueous concentrations dispersed rapidly as residues were below 100 µg/L in plant stand 1 and below 700 µg/L in plant stand 2 by 12 hr posttreatment. Residues in both plant stands were below detection by 24 hr posttreatment. Although maximum tissue concentrations were achieved within 2 to 6 hr in both plant stands, concentrations in plant stand 1 had significantly declined ( $p = 0.03$ ) by 12 hr posttreatment in conjunction with aqueous concentrations. Tissue concentrations in plant stand 2 remained significantly higher ( $p = 0.04$ ) than tissue levels in stand 1 from 12 through 48 hr posttreatment. This was likely influenced by aqueous concentrations remaining significantly higher in stand 2 following treatment. Results showed that triclopyr depuration in plant tissue initially mimicked aqueous degradation. Aqueous concentrations were nondetectable by 24 hr

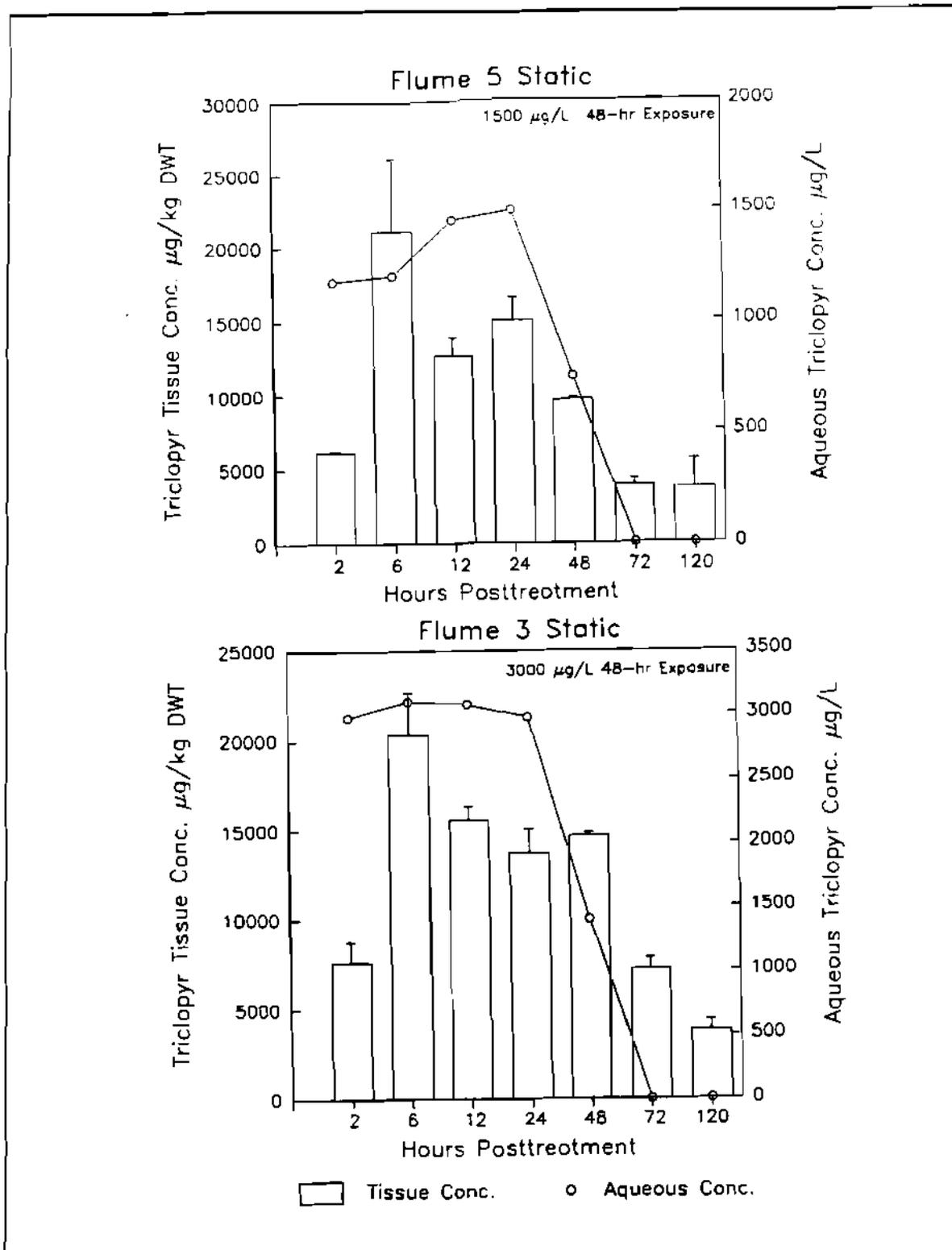


Figure 18. Triclopyr concentrations in Eurasian watermilfoil tissue and water following liquid applications targeted to deliver 1,500 and 3,000 µg/L for a 48 hr static exposure period. Bars represent the average of four replicates, and vertical lines represent one standard error of the mean

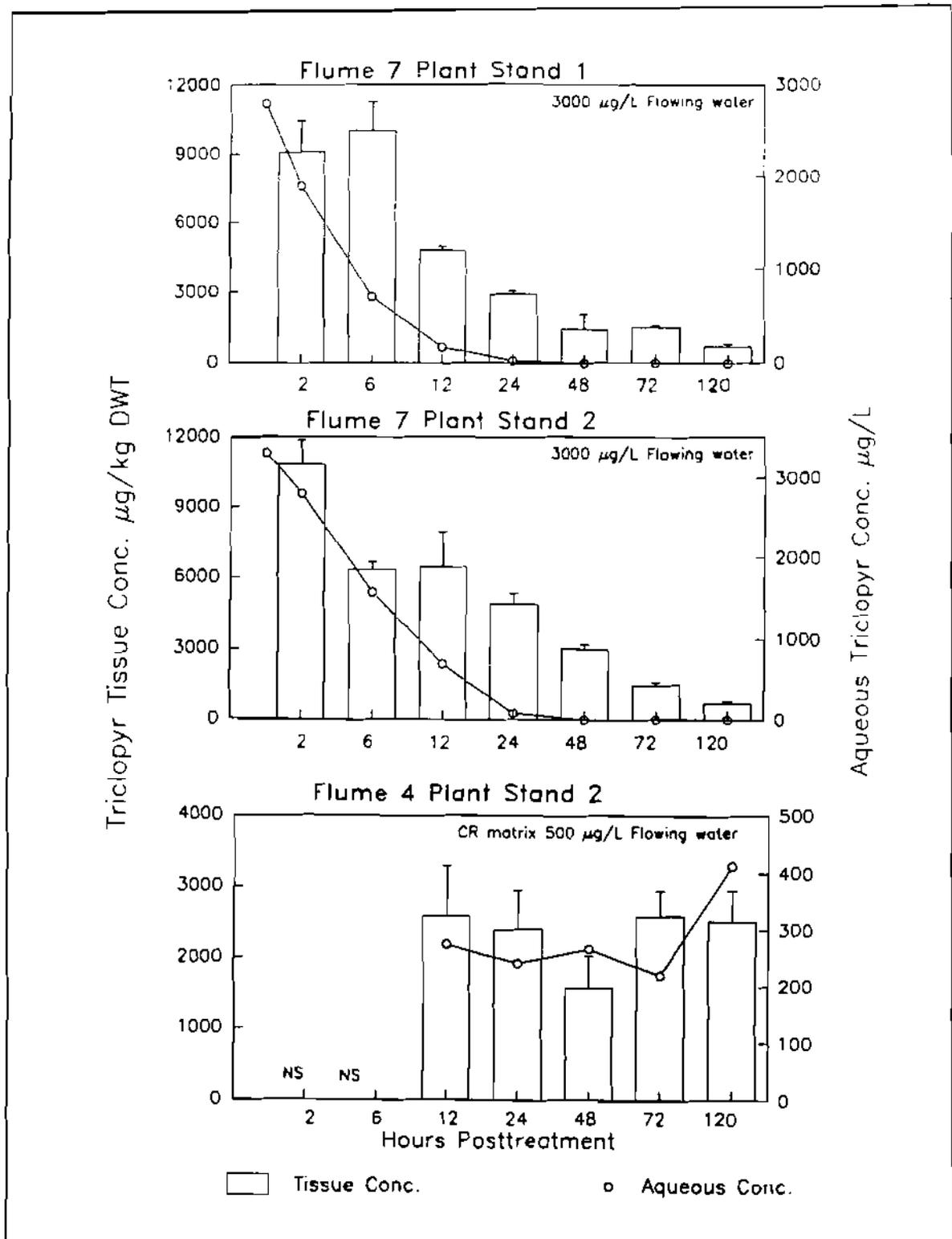


Figure 19. Triclopyr concentrations in Eurasian watermilfoil tissue and water following a liquid application to flowing water targeted to deliver an initial concentration of 3,000  $\mu\text{g}/\text{L}$  and a CR matrix application targeted to deliver 500  $\mu\text{g}/\text{L}$  for a 5 days exposure period. Bars represent the average of 4 replicates and vertical lines represent one standard error of the mean. NS denotes no sample was taken

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posttreatment, whereas, triclopyr tissue residues remained detectable at 120 hr posttreatment. BCFs were highly variable following this treatment, ranging from 5 to 50 at the 6- and 12-hr samples.

Biomass results at 8 weeks posttreatment showed that 70-percent milfoil control was achieved in plant stand 1; whereas, 100-percent control was achieved in plant stand 2. These results suggest that the increased aqueous exposure period and tissue loading enhanced the efficacy achieved in plant stand 2. *Najas minor* was abundant at the 8-week harvest.

Milfoil tissue loading following exposure to a CR matrix treatment targeted to achieve 500 µg/L is presented in Figure 19. Results show that maximal loading occurred within 24 hr posttreatment, and tissue concentrations correlated with aqueous concentrations over time. Although tissue concentrations were 10 times less than maximal tissue levels following liquid static applications, these tissues remained loaded at a constant rate for a 120-hr period. This long-term loading resulted in the CR treatment and achieved 100-percent milfoil control. Milfoil tissue burden data for all CR treated flumes is currently being analyzed and will be reported in a future report.

Data from 1992 and 1993 matrix treatments are similar because the majority of the uptake occurred within 24 hr and remained constant throughout the exposure period. However, 1993 BCF values (7 to 13) are approximately one-third of those reported for 1992. Moreover, 1992 data show that at aqueous concentrations of approximately 250 µg/L tissue concentrations reached levels near 10,000 µg/kg dry weight; whereas, 1993 data indicate that at similar aqueous concentrations tissue levels ranged from 1,700 to 2,500 µg/kg dry weight. In addition, 1992 data showed that tissue levels ranging from 1,200 to 2,000 µg/kg dry weight for 5 days resulted in no milfoil control; whereas data from 1993 show tissue concentrations in this range for 5 days resulted in 100-percent milfoil control. These differences in apparent uptake and efficacy are not easily explained at this time. Explanations may include differences in adsorption or field handling of tissue samples. In addition, tissue concentrations were only measured for 5 days, leaving open the possibility that duration of exposure may have been different between 1992 and 1993.

The apparent rapid depuration of triclopyr from milfoil tissue in conjunction with aqueous dissipation is somewhat surprising. Most auxinlike active herbicides are generally accumulated and retained in the phloem (Devine, Duke, and Fedtke 1993). Further studies may be required to separate herbicide adsorption and desorption from uptake and depuration in the presence of aqueous concentrations.

In summary, internal herbicide tissue concentrations can give valuable insight to the general uptake and depuration patterns of triclopyr by milfoil. Results show that triclopyr uptake is rapid, and that the length of time a tissue remains loaded is critical to achieve efficacy. The internal concentration and length of exposure in milfoil tissue is dependent on the aqueous concentration and exposure period. Due to the unknown effect of adsorption, it is not

possible to provide a direct correlation between tissue concentration and efficacy at the present time. In addition, because time of tissue exposure is an important variable, use of a single internal tissue value to predict efficacy is unlikely. The use of an exposure unit similar to that proposed by Fox and Haller (1993b) for fluridone equivalent days (FEDS) in the water column may also be appropriate for determining internal tissue burden over time.

# 4 Conclusions and Recommendations

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## Conclusions

### Laboratory herbicide release rate evaluations

Based on knowledge of CET requirements and herbicide release profiles in the laboratory, the following conclusions can be drawn:

- a.* When combined with the herbicides triclopyr and 2,4-D, the gypsum matrix performed as a suitable CR formulation.
- b.* The protein matrix (when combined with all herbicides) and the 27-percent ai clay granule and 45-percent ai superabsorbing polymer endothall herbicide matrices performed poorly as CR formulations.
- c.* By simultaneously considering herbicide release rates, water column distribution of the matrix, and application techniques, mesocosm and field evaluations can provide a more effective assessment of CR formulations than laboratory evaluations can.

### Mesocosm release rate evaluations

Based on results of flume evaluations, the following conclusions can be drawn:

- a.* CR technology is a viable concept and can be effective in controlling Eurasian watermilfoil.
- b.* CR technology may provide acceptable Eurasian watermilfoil control while delivering herbicide rates below established potable water tolerance levels.
- c.* The use of long-term (>14 days) critical threshold concentration release may not be feasible in areas of high water exchange (<4 hr half-life) due to excessive herbicide loading rates. However, low

herbicide concentration release over several days could improve efficacy in areas where moderate water exchange (8 to 12 hr half-life) preclude the use of most conventional herbicide formulations.

### **Herbicide tissue burden**

Based on results of herbicide tissue evaluation, it can be concluded that a direct relationship seems to exist between internal tissue concentrations and external aqueous concentrations of triclopyr. However, at this time this information cannot be used to provide a reliable prediction of herbicide efficacy.

## **Recommendations**

Based on results of laboratory and flume/mesocosm studies the following recommendations are made:

- a.* The laboratory should continue to be used as an initial evaluation procedure for newly identified CR matrices with potential for aquatic use.
- b.* Development of the gypsum matrix formulation should continue in the laboratory to improve design specifications, evaluate loading rates, and improve overall quality control. These evaluations should be conducted with the herbicides triclopyr and endothall.
- c.* The roles of triclopyr adsorption and desorption and uptake and depuration in relation to values obtained for tissue concentrations are not clearly understood; therefore, laboratory studies should be conducted on uptake and depuration of triclopyr when applied to Eurasian watermilfoil.
- d.* Two application rates of the 45-percent ai endothall supersorbent polymer and two formulations of endothall gypsum matrices (250 and 500 µg/L) should be evaluated against conventional endothall formulations (Aquathol<sup>®</sup> and Aquathol K<sup>®</sup>) in flowing-water flumes for submersed plant control.
- e.* Additional tissue burden studies should be conducted to reduce variability and to build better correlations between internal tissue concentrations and plant control. In addition, the concept of a tissue exposure unit (µg/kg/day) should be evaluated as it relates to plant control.
- f.* CR matrices should be field-tested with triclopyr or endothall in flowing water systems such as canals, drains, or other linear flow systems.

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| 13. ABSTRACT (Maximum 200 words)<br>Formulations for the controlled release of aquatic herbicides were tested in the laboratory and in flowing water hydraulic flumes. Protein- and gypsum-based matrices were formulated with bensulfuron methyl (2 percent active ingredient (ai)), fluridone (2 percent ai), 2,4-D (2 and 15 percent acid equivalent (ae)), and triclopyr (2 and 15 percent ae). These formulations were tested in the laboratory for release properties over a 7-day period. In addition, conventional granular formulations of fluridone (Sonar <sup>®</sup> SRP 5 percent ai) and 2,4-D (AquaKleen <sup>®</sup> 19 percent ae) were tested to provide a basis for comparison of release rates. Results showed the protein matrices produced inconsistent release profiles; whereas, the gypsum matrices resulted in consistent release rates during the course of the study. Triclopyr and 2,4-D were identified as excellent candidates for hydraulic flume testing. Triclopyr was chosen due to its experimental status and the lack of field efficacy and dissipation information for this compound.<br>Three formulations of endothall were also tested for controlled-release properties and included the conventional granule (Aquatol <sup>®</sup> 10.1 percent ai), a 27-percent ai clay granule, and a 45-percent ai supersorbent polymer. Results showed all matrices released >90 percent of the active ingredient within 2 hr posttreatment. Although no controlled-release properties were demonstrated, the 45-percent ai polymer is a good candidate for field testing due to the high percent ai load and the lack of dusting.<br>(Continued) |  |                            |  |  |                            |
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Hydraulic flumes were planted with the exotic target species Eurasian watermilfoil 4 weeks prior to release rate testing. During the summer of 1992, release testing was conducted with gypsum/triclopyr matrices targeted to achieve 100 and 300 µg/L in two flumes for a 6-day exposure. Results showed that both loading rates delivered consistent amounts of triclopyr during the course of the study; however, release rates were only one-third to one-half of the target rates. As a result of failing to achieve target rates, milfoil control was very poor. Although injury symptoms were visible following these treatments, biomass increased twofold to fourfold during the 6-week posttreatment period.

Plant tissue was also sampled and analyzed for triclopyr content. Data showed that uptake of triclopyr was rapid as near maximal levels occurred within 24 hr posttreatment. Although aqueous concentrations remained constant, further tissue accumulation of triclopyr did not occur past 24 hr posttreatment. The rapid uptake and lack of triclopyr accumulation over time was unexpected based on other herbicide uptake studies. Efficacy data indicate that tissue levels in the range of 2,000 µg/kg DWT for 5 days provided poor milfoil control; whereas, tissue levels in the range of 9,000 µg/kg DWT for 3 days provided excellent milfoil control. Tissue levels in the range of 9,000 µg/kg DWT for 1 and 2 days resulted in 0- and 60-percent control. These results suggest that concentration and exposure interact to produce plant control.

In 1993, release testing was conducted with gypsum/triclopyr matrices targeted to achieve 300 and 500 µg/L in four flumes for a 5-day exposure. Furthermore, a gypsum/endothall matrix targeted to achieve 500 µg/L for a 4-day exposure was also tested. Results showed that matrices delivered consistent amounts of triclopyr (with some exceptions) and endothall during the course of the study. Some spike release was noted in both triclopyr treatments and was attributed to matrix agitation, higher loading rates and cracking. Failure of the pumps supplying water flow to the flumes (115 hr), resulted in static triclopyr exposures which forced removal of the matrices at 117 hr posttreatment. Residue analyses indicated that following loss of water flow, an increase in triclopyr residues was noted at 120 hr posttreatment; however, triclopyr levels had significantly dropped by 144 hr posttreatment.

The 8-week posttreatment harvest indicated that 100-percent milfoil control was achieved following all controlled release matrix treatments. Following triclopyr treatment, thick stands of naiads and pondweeds were abundant in areas once dominated by milfoil.

Plant tissue analyses following liquid static treatments (1,500 and 3,000 µg/L for 48 hr) and liquid flowing treatments (3,000 µg/L) again indicated that triclopyr loading was rapid as near maximal levels occurred within 6 hr posttreatment. No accumulation of triclopyr was noted past 6 hr posttreatment even though most aqueous exposures remained quite stable for much longer periods of time. Release of triclopyr from plant tissues was closely correlated to aqueous dissipation. Comparison of results from 2 years of sampling indicated variability existed in bioconcentration factors. Preliminary results indicate that use of tissue burden information for efficacy prediction will be difficult due to the interaction of tissue concentration, exposure period (increased number of samples required), and variability that is likely to exist when sampling plants in the field. Furthermore, the role of adsorption was not evaluated in these studies and will likely require laboratory testing to determine its significance.