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# The Use of Rhodamine Water Tracer (RWT) Dye to Improve Submersed Herbicide Applications

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Final Report



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# Abstract

The inert fluorescent dye rhodamine water tracer (RWT) has been widely used in freshwater aquatic systems for many years to quantify bulk water exchange patterns and as a tracer for submersed herbicide movement. The dye is well-suited for tracer work due to its high solubility and detectability in water (<0.01 µg/L). Federal guidelines limit the aqueous concentration of RWT to <10 µg/L at drinking water intakes. The dye has proven to be harmless to aquatic organisms and humans in low concentrations and is relatively inexpensive. Since 1991, RWT has been used by Engineer Research and Development Center (ERDC) researchers to simulate aqueous herbicide applications in large, hydrodynamic systems in over 12 states. Such simulations have improved the effectiveness of herbicide treatments by linking in situ water exchange processes with appropriate herbicide selection and application rates. Understanding these parameters can be critical for mitigating herbicide exposure in environmentally sensitive settings and around potable water and irrigation intakes. A data-based estimate of water exchange patterns usually results in successful submersed herbicide applications—both with target-plant efficacy and limited injury to nontarget vegetation. Using RWT dye to simulate submersed herbicide applications is an important predictive and real-time tool in both experimental and operational settings.

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# Preface

This study was conducted for the US Army Engineer Research and Development Center, Environmental Laboratory (ERDC-EL), under Account Code U4398375, AMSCO Code 075098. Mr. Michael J. Greer was program manager, Aquatic Plant Control Research Program (APCRP); and Dr. Jennifer Seiter-Moser was the technical director for the Civil Works Environmental Engineering and Sciences Office.

The work was performed by the Aquatic Ecology and Invasive Species Branch of the Ecosystem Evaluation and Engineering Division (EE), ERDC-EL. At the time of publication, Dr. Bradley T. Sartain was acting branch chief, and Mr. Mark D. Farr was division chief. The deputy director of ERDC-EL was Dr. Brandon J. Lafferty, and the director was Dr. Edmond J. Russo Jr.

COL Christian Patterson was commander of ERDC, and Dr. David W. Pittman was the director.

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# 1 Introduction

## 1.1 Purpose

This report outlines the chemical properties and general use of the inert fluorescent dye rhodamine water tracer (RWT) that has been widely used in aquatic systems to quantify bulk water exchange patterns and as a tracer for submersed herbicide movement.

## 1.2 Background

Fluorescent dyes have been used in the US for over a century to measure bulk water exchange patterns, including gravity-driven flows in surface water and groundwater and as atmospheric tracers for agricultural sprays (Dole 1906; Smart and Laidlaw 1977; Wilson et al. 1986; Trudgill 1987; Sabatini and Austin 1991; Getsinger et al. 1996; Cai and Stark 1997; Martin and McCutcheon 1999; Skjolding et al. 2021). The Aquatic Plant Management Team at the US Army Engineer Research and Development Center (ERDC) pioneered the use of the quantitative inert fluorescent dye RWT to mimic aquatic herbicide dispersion in the late 1980s (Fox et al. 1990), which has since been used across numerous reservoirs and rivers in the US (Table 1; Figure 1 and Figure 2). Most of these studies have been in cooperation or consultation with the US Environmental Protection Agency (USEPA), the US Fish and Wildlife Service, the Tennessee Valley Authority, the US Bureau of Reclamation, and other federal and state agencies. The most successful treatment strategies employed in these water bodies were developed using a systematic and tiered investigative approach. Small-scale mesocosm studies determined optimal aqueous herbicide concentration and exposure time (CET) relationships to control target plants. Results from small-scale studies were verified in larger outdoor mesocosm evaluations, which included nontarget native plants, to determine species-selective control. Finally, in situ bulk water exchange patterns using RWT were linked with results from the CET studies to design operational-scale herbicide applications to optimize species-selective efficacy.

Figure 1. Subsurface application of rhodamine water tracer (RWT) to an open-water stand of submersed plants to simulate an aquatic herbicide application, Lake Pend Oreille, Idaho.

Figure 2. Subsurface application of RWT to a cove containing submersed plants to simulate an aquatic herbicide application, Columbia River, Washington.

Table 1. Bulk water exchange studies conducted with RWT by the Engineer Research and Development Center (ERDC) Aquatic Plant Management Team to simulate aquatic applications in selected states of the US.

| State | Bulk Water Exchange Studies | | | | | |
|---|---|---|---|---|---|---|
| Alabama | Turner et al. (1995) | | | | | |
| Florida | Fox, Haller, and Shilling (1991) | Fox, Haller, and Getsinger. (1991) | Fox, Haller, Getsinger, and Green. (1991) | Fox et al. (1993) | Sabol et al. (2019) | |
| Georgia | Fox et al. (1992) | Getsinger et al. (1994) | | | | |
| Idaho | Getsinger et al., n.d. | | | | | |
| Michigan | Getsinger et al. (2002) | Poovey et al. (2004) | | | | |
| Minnesota | Haller et al. (2002) | Getsinger et al. (2000) | | | | |
| Mississippi | Sartain (2014) | | | | | |
| Montana | Getsinger et al. (2014) | Getsinger et al. (2013) | Wersal et al. (2022) | Getsinger et al. (2017) | Pennington et al. (2015) | Padkowka et al. (2019) |
| New York | Netherland and Greer (2014) | | | | | |
| North Carolina | Nawrocki (2016) | Sartain et al. (2023) | | | | |
| Virginia | Getsinger et al. (2011) | | | | | |
| Washington | Getsinger et al.(1996) | Getsinger et al. (1997) | Turner et al. (1994) | Turner et al. (1991) | Sartain et al. (2022) | Sartain et al., n.d. |

Several fluorescent dyes are commercially available, but relatively few are suitable for environmentally compatible water tracer studies (Wilson et al. 1986). Dyes that have been used in water tracer studies include fluorescein; lissamine FF; 1, 3, 6, 8-Pyrenetetrasulfonic acid (PTSA); rhodamine B; and RWT (Dole 1906; Smart and Laidlaw 1977; Trudgill 1987; Cai and Stark 1997; Skjolding et al. 2021). Of these products, the properties of RWT are well-suited for surface water exchange studies; therefore, it is commonly used as a water tracer (Martin and McCutcheon 1999). Wilson et al. (1986) outlined the following desirable properties of RWT for tracer studies: (1) high solubility in water; (2) easily detectable and quantifiable in situ using commercially available portable fluorometers; (3) fluorescent in a part of the visible spectrum not common to materials generally found in water, thereby reducing the problem of interfering background fluorescence; (4) harmless to aquatic organisms and humans in low concentrations; (5) inexpensive; and (6) reasonably stable in a normal water environment.

Health and safety are primary considerations for aquatic tracer dye use in public waters, including the potential toxic effects on lake biota and human health. In the presence of high nitrite concentrations (>1 mg/L), RWT has been found to form the carcinogen diethylnitrosamine (DENA) (Abidi 1982).* However, the potential for DENA formation is very low in surface water bodies because of the relatively low nitrite concentrations in these waters (Wetzel 2001). Biota that are affected by RWT require much higher concentrations than those used in tracer studies (Martin and McCutcheon 1999). For the purpose of determining water exchange patterns and simulating submersed herbicide applications, a nominal aqueous concentration of 10 µg/L (10 ppb) or less is targeted. The lethal concentration of RWT required to kill 50% of the test population ($LC_{50}$) under a 96 hr exposure was >320 mg/L and 170 mg/L for the rainbow trout (*Oncorhynchus mykiss*) and water flea (*Daphnia magna*), respectively (Upstate Freshwater Institute 2008), which are 1,700- and 3,300-fold above the maximum use rate of 0.01 mg/L used to monitor water exchange (Podkowka et al. 2019). The USEPA and the US Geological Survey (USGS) have adopted a policy that prohibits the injection of fluorescent dyes in quantities that would result in dye concentrations greater than 10 µg/L at drinking water intakes. (USEPA 1998). All ERDC field evaluations adhere to the USEPA and USGS guidance for limiting nominal application rates to 10 ug/L or less.

Hazardous Materials Identification System ratings are presented in the Safety Data Sheet (SDS) for health (moderate hazard), flammability (slight hazard), and reactivity (slight hazard) for concentrated RWT (Keystone Aniline Corporation 2001). According to the Environmental and Water Quality Operational Studies by the US Army Corps of Engineers, RWT has

* For a full list of the spelled-out forms of the units of measure used in this document and their conversions, please refer to *US Government Publishing Office Style Manual*, 31st ed. (Washington, DC: US Government Publishing Office, 2016), 248–52 and 345–47, https://www.govinfo.gov/content/pkg/GPO-STYLEMANUAL-2016/pdf/GPO-STYLEMANUAL-2016.pdf.

been chosen as the dye most suitable for use in inflow studies and poses no known environmental or health hazards when used in unpolluted waters.

The regulatory standards that apply to the use of RWT are as follows:

The standards established by the USEPA in the *Federal Register* (vol. 63, no. 40) state the maximum RWT concentrations to be 10 µg/L for water entering a drinking water plant (prior to treatment and distribution) and 0.1 µg/L in finished drinking water (USEPA 1998).

- The drinking water standard established by the National Sanitation Foundation (NSF) in the NSF Standard 60 states the maximum concentration of RWT to be 0.1 mg/L (100 µg/L) (USEPA 1998).

The chemical formula of RWT dye is $C_{29}H_{29}ClN_2Na_2O_5$, and the elemental composition is presented in Table 2 (National Center for Biotechnology Information, n.d.).* This compound is reportedly chemically inert and characterized by the presence of the xanthene nucleus ($C_{13}H_{10}O$) (Podkowka et al. 2019).

Table 2. Elemental composition of RWT dye.

| Element | Symbol | Atomic Mass | Number of Atoms | Mass % |
|---|---|---|---|---|
| Carbon | C | 12.0107 | 29 | 61.43% |
| Hydrogen | H | 1.0079 | 29 | 5.16% |
| Chlorine | Cl | 35.4532 | 1 | 6.25% |
| Nitrogen | N | 14.0067 | 2 | 4.94% |
| Sodium | Na | 22.9897 | 2 | 8.11% |
| Oxygen | O | 15.9994 | 5 | 14.11% |

Environmentally compatible fluorescent dye tracers usually do not require formal permits for use in a study (ASTM International 2014). Aqueous RWT dye concentrations of 5 to 10 µg/L are essentially undetectable to the human eye; thus, they require hand-held or deployable fluorometers to measure the very low levels (as low as 0.01 µg/L [0.00001 mg/L]) of RWT applied in field studies or operational use conditions (Xylem 2019). Data

* For a full list of the spelled-out forms of the chemical elements used in this document, please refer to *US Government Publishing Office Style Manual*, 31st ed. (Washington, DC: US Government Publishing Office, 2016), 265, https://www.govinfo.gov/content/pkg/GPO-STYLEMANUAL-2016/pdf/GPO-STYLEMANUAL-2016.pdf.

from numerous ERDC research projects indicate that the dye dissipates and degrades in the water column within a few days. Tai and Rathbun (1988) suggested that RWT has a half-life of 15 to 30 days depending on the time of year. In addition, the authors have never observed RWT discoloring or staining boat or structural surfaces when the dye has been applied in and around marina docks and occupied boat slips.

# 2 Using Rhodamine Water Tracer (RWT) to Simulate Submersed Herbicide Applications, Select Products, and Predict Efficacy

Several studies have shown significant correlations between the dissipation patterns of RWT dye and those of commonly used aquatic herbicides fluridone, endothall, and triclopyr (Fox, Haller, and Shilling 1991; Fox et al. 1992, 1993; Getsinger et al. 1996). Results from these studies indicated that aquatic herbicide dissipation can be predicted by monitoring dye movement and concentration. These dye data can be paramount to inform operational-scale herbicide treatments in areas where water exchange processes will likely impact herbicide-CET relationships—and ultimately product efficacy. In situ water exchange information can aid in determining whether using an herbicide is feasible, the best product for the situation, and the appropriate application rate. While RWT can determine bulk water exchange processes within treatment plots and estimate herbicide dissipation in and around treatment sites, correlations in dispersal patterns for any given herbicide, determined by discrete sampling and chemical analysis over time, provide the most accurate estimate for herbicide dissipation.

Finally, RWT water exchange studies determine location, depth, and frequency of sampling with respect to treated areas to adequately monitor aqueous herbicide concentrations during posttreatment time lines. Understanding these parameters can be critical for mitigating herbicide exposure in environmentally sensitive settings and in and around potable water and irrigation intakes. The weight of the evidence clearly indicates that a data-based estimate of bulk water exchange patterns usually results in a successful submersed herbicide application—both with target plant efficacy and limited injury to nontarget vegetation. Thus, the use of RWT dye to simulate submersed herbicide applications has been an important predictive and real-time tool in both experimental and operational settings.

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# Abbreviations

| | |
|---|---|
| CET | Concentration and exposure time |
| DENA | Diethylnitrosamine |
| ERDC | Engineer Research and Development Center |
| LC | Lethal concentration |
| NSF | National Sanitation Foundation |
| PTSA | Pyrenetetrasulfonic acid |
| RWT | Rhodamine water tracer |
| SDS | Safety Data Sheet |
| USEPA | US Environmental Protection Agency |
| USGS | US Geological Survey |

# REPORT DOCUMENTATION PAGE

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The Use of Rhodamine Water Tracer (RWT) Dye to Improve Submersed Herbicide Applications

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**14. ABSTRACT**
The inert fluorescent dye rhodamine water tracer (RWT) has been widely used in freshwater aquatic systems for many years to quantify bulk water exchange patterns and as a tracer for submersed herbicide movement. The dye is well-suited for tracer work due to its high solubility and detectability in water (<0.01 µg/L). Federal guidelines limit the aqueous concentration 0f RWT to <10 µg/L at drinking water intakes. The dye has proven to be harmless to aquatic organisms and humans in low concentrations and is relatively inexpensive. Since 1991, RWT has been used by Engineer Research and Development Center (ERDC) researchers to simulate aqueous herbicide applications in large, hydrodynamic systems in over 12 states. Such simulations have improved the effectiveness of herbicide treatments by linking in situ water exchange processes with appropriate herbicide selection and application rates. Understanding these parameters can be critical for mitigating herbicide exposure in environmentally sensitive settings and around potable water and irrigation intakes. A data-based estimate of water exchange patterns usually results in successful submersed herbicide applications—both with target-plant efficacy and limited injury to nontarget vegetation. Using RWT dye to simulate submersed herbicide applications is an important predictive and real-time tool in both experimental and operational settings.

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