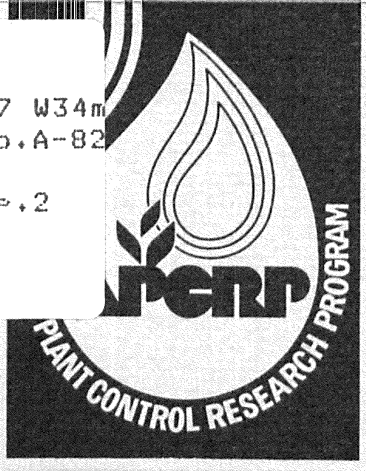

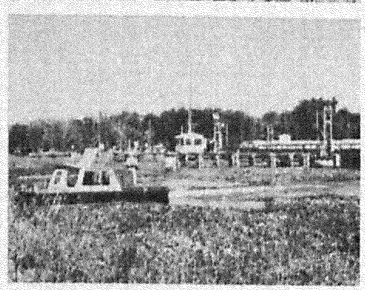

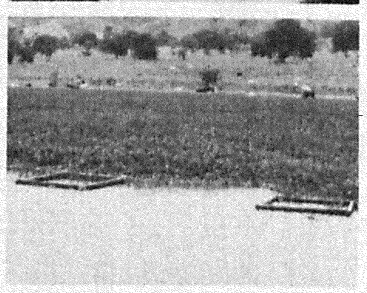

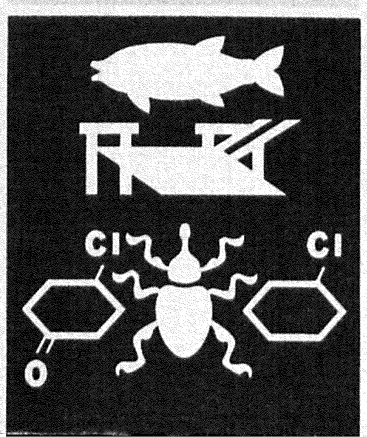

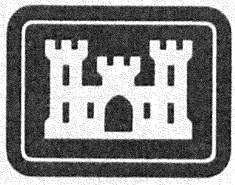

MISCELLANEOUS PAPER A-82-4

IMPROVING TECHNOLOGY FOR CHEMICAL CONTROL OF AQUATIC PLANTS

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20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

Because a need to modify existing aquatic herbicide evaluation techniques was recognized, it was decided during an interagency workshop in November 1979 that uniform procedures should be established to facilitate interlaboratory comparisons among Federal agencies. A protocol describing procedures for evaluating conventional and controlled-release herbicides was developed and tested during FY 1980. Progress on the implementation of the protocol and the results of the conventional herbicide evaluation program are discussed in this report.

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20. ABSTRACT (Continued).

During FY 1980 the following compounds were evaluated in the laboratory: three controlled-release formulations, one coded-confidential compound, one growth retardant, and six conventional herbicide formulations. Moreover, iron chelates were evaluated for enhancing efficacy of diquat and potassium endothall against hydrilla.

The release of 2,4-D from the MOE/GMA copolymer in a flowing water environment following an initial application rate of 0.01, 0.025, 0.05, 0.10, and 0.25 mg/ℓ was linear and predictable over a 70-day period. Moreover, when 2,4-D was applied at the rate of 1.2 mg per gram of polymer per day to Eurasian watermilfoil, essentially 100 percent control was observed on two of the four replicate treatments by 8 weeks posttreatment. This compound was found to be efficacious in consistency of release and in producing phytotoxicity over an approximate 4-month period.

Dichlobenil release from a beeswax controlled-release system was not constant. Microscopic examination of the pellets revealed an accumulation of the crystalline dichlobenil near the surface, which may account for the rapid and inconsistent release of the dichlobenil.

Two of three controlled-release fenac formulations were effective against hydrilla at a 1.0-mg/ℓ treatment rate in 10 weeks and against Southern naiad at a 1.0-mg/ℓ treatment rate in about 8 weeks. No control using the fenac formulation was apparent against cabomba. Eurasian watermilfoil control was similar to that of conventional fenac formulations, i.e. 16 weeks at 0.5-mg/ℓ treatment rates.

The coded compound (RO 3-7042) from MAAG Agrochemicals was effective against hydrilla over 16 weeks at a 1.0-mg/ℓ treatment rate and against Southern naiad in about 8 weeks at a 5.0-mg/ℓ treatment rate. No control was observed against cabomba. Eurasian watermilfoil control was observed after 16 weeks at a 0.5-mg/ℓ treatment rate. This coded compound was not effective against duckweed; marginally effective against waterhyacinth; but very effective against waterlettuce at a 1.0-kg/ha treatment rate.

A growth retardant (EL-507) from Elanco Research Laboratories was found effective against waterhyacinth at a 1.0-kg/ha treatment rate. Additional studies on its effects on other aquatic plants appear warranted.

PREFACE

This report presents the results for FY 1980 of an ongoing evaluation program to test new chemical formulations for their potential as aquatic plant herbicides. The program is being conducted for the Aquatic Plant Control Research Program (APCRP) by the U. S. Department of Agriculture (USDA), Science and Education Administration, Aquatic Plant Management Laboratory, Fort Lauderdale, Florida. Funds for this effort are provided by the Office, Chief of Engineers, U. S. Army, under Appropriation No. 96X3122, Construction General, and CWIS No. 31548 through the APCRP at the U. S. Army Engineer Waterways Experiment Station (WES).

The principal investigator for the work was Dr. Kerry K. Steward, USDA, who prepared this report.

The work was monitored at WES by Dr. Howard E. Westerdahl of the Environmental Laboratory (EL) Chemical Control Technology Team, under the direct supervision of Dr. R. M. Engler, Chief, Ecological Effects and Regulatory Criteria Group, and Mr. D. L. Robey, Chief, Ecosystem Research and Simulation Division. Mr. J. L. Decell was Program Manager, APCRP, and Dr. John Harrison was Chief, EL, during this study.

Commanders and Directors of WES during the conduct of the study and the preparation and publication of this report were COL Nelson P. Conover, CE, and COL Tilford C. Creel, CE. Technical Director was Mr. F. R. Brown.

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IMPROVING TECHNOLOGY FOR THE CHEMICAL CONTROL OF AQUATIC PLANTS

PART I: INTRODUCTION

Background

1. The impact of aquatic plants on utilization of water resources is well documented in both the scientific and popular literature. There is worldwide consensus among those who manage or maintain these resources that nearly every conceivable water use can be prevented or at least curtailed by unmanaged growths of these plants.

2. Of the few options available for management of aquatic plant growth, the use of herbicides is the most widespread. Since 1968, however, the number of chemicals registered nationally for aquatic use and available to the water manager has decreased approximately 72 percent, from 38 to fewer than 10. The reduction in the number of available chemicals is due to the loss of registration of older chemicals, usually because of adverse environmental impact, and to the reduction in the number of new chemicals being developed by industry. Safer, more effective, more economical herbicides and growth regulators need to be developed for selective removal and for regulation of growth of the more noxious species of aquatic plants. Formulation techniques that will reduce environmental impact, as well as increase efficacy of chemicals to be used in water, also need to be investigated.

3. With the assistance of government, industry, and university laboratories, the search for new chemicals and new technology should be expanded. The first step in a search for new chemical tools is synthesis. The next step is an evaluation process using plant species for which control is desired. This evaluation procedure involves culture of various target plants in artificial environments to produce optimum growth. Once the efficacy of a chemical against a particular species has been determined, attempts to improve performance or safety can be initiated through innovative formulation techniques.

4. Several recently developed techniques of formulating effective chemicals, such as 2,4-D, within various polymers or matrix structures to provide controlled release (CR) over time appear to hold great promise for maintaining control of regrowth of susceptible species. Evaluation systems for conventional aquatic herbicide formulations were designed to investigate herbicide effectiveness at fixed concentrations under static water conditions. New evaluation techniques will need to be developed for CR formulations since these formulations will be designed to deliver chemicals over extended periods of time at predetermined rates.

Purpose

5. Because the need to modify existing aquatic herbicide evaluation techniques was recognized, a workshop was held 7-8 November 1979 at Fort Lauderdale, Florida, to develop procedures for evaluating CR herbicides. It was agreed that uniform procedures should be established to facilitate interlaboratory comparisons. It was also agreed that uniform plant culture conditions should be established where possible or be characterized when not possible, for example, characterization of quality parameters of local natural water supplies.

6. The draft protocol described procedures for initial investigation in the laboratory with followup investigation of a more advanced nature in outside aquaria for formulations that appeared promising in laboratory investigations. A consensus was reached among workshop participants that controlled-release herbicide formulations (CRHF) should control plant regrowth after initial control has been attained by conventional means. As a consequence, it was also recognized that evaluation of CRHF's should be conducted under conditions of precisely regulated water flow so that reliability and consistency of herbicide release could be determined.

Scope

7. Progress on the implementation of the protocol and the results of the conventional program will be discussed in this report. The

details of methods development for herbicide analyses and details of analytical procedures are given in Appendix A.

8. Aquatic plants treated in FY 1980 are listed below:

Alligatorweed	*Alternanthera philoxeroides* (Mart.) Griseb.
Cabomba	*Cabomba caroliniana* Gray
Chara	*Chara* spp.
Duckweed	*Lemna* spp.
Hydrilla	*Hydrilla verticillata* Royle
Paragrass	*Brachiaria mutica* (Forsk.) Stapf
Southern naiad	*Najas guadalupensis* (Spreng.) Mangus
Torpedograss	*Panicum repens* L.
Waterhyacinth	*Eichhornia crassipes* (Mart.) Solms
Waterlettuce	*Pistia stratiotes* L.
Watermilfoil	*Myriophyllum spicatum* L.

The names and sources of chemical compounds evaluated in 1980 are listed in Table 1.

PART II: MATERIALS AND METHODS FOR EVALUATION OF CONTROLLED-RELEASE AND CONVENTIONAL FORMULATIONS

Controlled-Release Formulations

Release of 2,4-D from
MOE 2,4-D/GMA Copolymer

9. On 17 March approximately 24 g of MOE 2,4-D/GMA Copolymer (2-methacryloyloxyethyl 2,4-dichlorophenoxyacetate glycerylmethacrylate, Figure 1) containing 50 percent 2,4-D a.e. was received from Dr. Frank Harris at Wright State University, Ohio. The release rate was specified as 1.2 mg 2,4-D per gram of formulation per day. To provide a basis of comparison between laboratories, release rate data were determined in

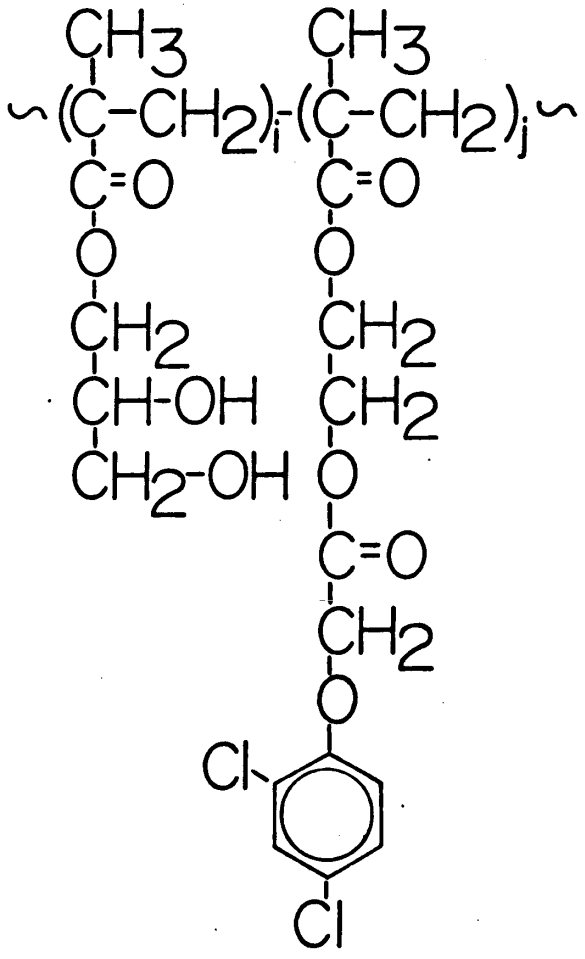

Figure 1. MOE 2,4-D/GMA Copolymer (2-methacryloyloxyethyl 2,4-dichlorophenoxyacetate copolymerized with glycerylmethacrylate)

pH 8 reconstituted water at 28°C ±2°.

10. Static reconstituted water. For these comparisons, treatments of copolymer calculated to release at the rate of 0.1 mg/ℓ per day were applied to 3.5 ℓ reconstituted distilled water containing 192 mg $NaHCO_3$, 120 mg $CaSO_4 \cdot 2H_2O$, 120 mg $MgSO_4$, and 8 mg KCl per litre. Treatments were compared with untreated controls of reconstituted water and were replicated three times.

11. Water samples were taken from each container at 1, 2, 3, 7, 28, 42, 90, 112, and 132 days after treatment. Samples taken through 42 days were concentrated on SEP-PAC C_{18} cartridges, eluted with acetonitrile, and esterified with methanol + boron trifluoride. Quantification of 2,4-D was by gas chromatography, and recovery efficiency was determined from fortified control samples. Samples taken at 90, 112, and 132 days were analyzed directly by high-pressure liquid chromatography (HPLC). Analytical standards of 2,4-D in deionized water were used as references.

12. Static natural water. Copolymer treatments, at rates identical to those in reconstituted water, were applied to 18.9 ℓ natural (pond) water filtered through a commercial sediment filter.* Treatments were replicated four times.

13. Water samples were taken at 1, 2, 3, 4, 7, 14, 28, 67, 78, 112, and 133 days after treatment. Samples taken through 28 days were filtered through 0.45-μ membrane filters, concentrated on SEP-PAK C_{18} cartridges, eluted with acetonitrile, washed with hexane, extracted with ether, and esterified with methanol + boron trifluoride. Analysis was by gas chromatography. Analytical standards of 2,4-D methyl ester in hexane were used as reference. Samples taken after 28 days were analyzed directly by HPLC and were compared with reference standards in deionized water.

Degradation of 2,4-D in
natural water in the laboratory

14. A rapid degradation of 2,4-D as it is released from CR

---

* Plymouth Supreme Mod S, Amtek Ply. Prod. Div., Sheboygan, Wis. 53081.

formulations would complicate the determination of consistency of release of the herbicide from experimental formulations. It consequently was deemed necessary to determine the effect of the culture system on the stability of the herbicide.

15. Commercial grade 2,4-D dimethylamine was applied to filtered natural water in 19-ℓ glass containers in the laboratory. The herbicide was applied with two replications, at rates of 0, 10, 25, 50, 100, and 250 µg/ℓ. Experiments were conducted under the same temperature and lighting conditions as experiments with plants.

16. Immediately after and at 1, 2, 7, 14, 28, 42, and 70 days posttreatment, three 50-ml water samples were removed from each container. Samples were prepared by the procedure described in Appendix A (paragraph A8).

17. Quantification of herbicide concentrations in 50-µl samples was determined by HPLC with chromatographic conditions as described in paragraph A9 using acetonitrile:1 percent acetic acid (25:75) for the mobile phase at a flow rate of 2.0 ml/min. The sensitivity of this method was 50 ng. Recovery of 2,4-D in spiked samples was greater than 90 percent.

Release of 2,4-D from
MOE/GMA CRHF into flowing water

18. MOE 2,4-D/GMA Copolymer, estimated to release 1.2 mg 2,4-D per gram of copolymer per day, was applied to water flowing through 19-ℓ glass culture vessels. The copolymer was applied at a rate calculated to maintain a 0.1-mg/ℓ concentration of 2,4-D in the flowing water. Treatments, replicated four times, were applied to vessels containing watermilfoil and to vessels without plants to determine the effect of plants and soil on herbicide concentrations. Culture vessels with and without plants to which treatments were not applied served as plant and water controls.

19. Regulated flowing water was delivered to the bottom of individual culture vessels by a multichannel tubing pump at a rate to provide one volume change in 24 hours. Wastewater flowed out through side arms near the top of the vessels and was carried outside (Figure 2). The

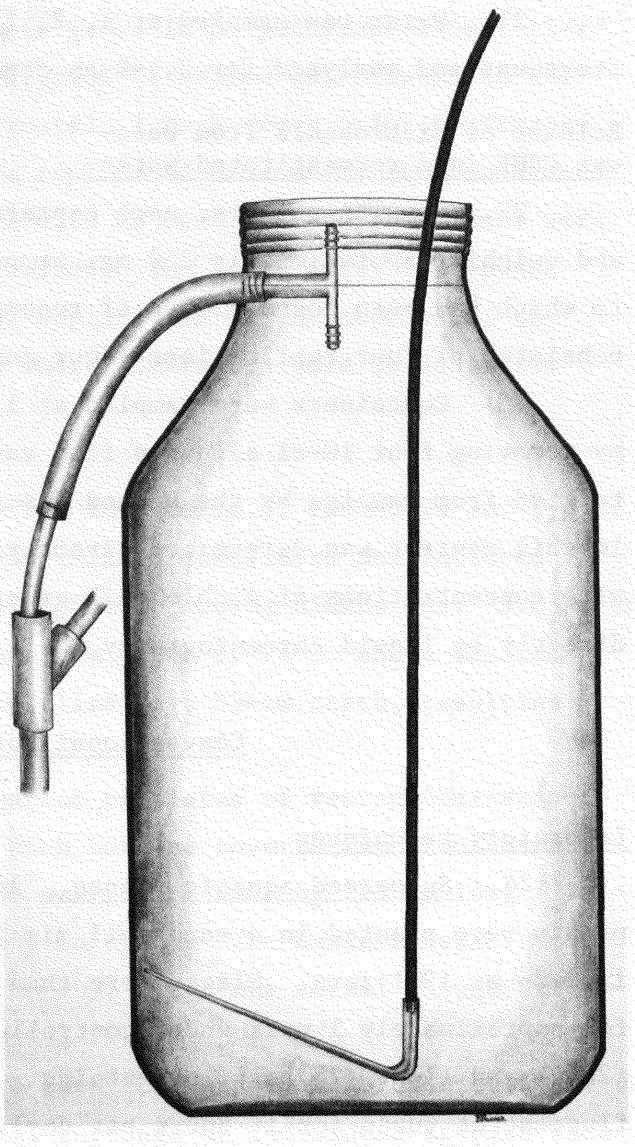

Figure 2. Illustration of 19-ℓ culture vessel used to provide a regulated flow of water for evaluation of CRHF's

wastewater was run through a series of three interconnected 19-ℓ containers filled with activated charcoal to remove residual 2,4-D. Water flow to vessels was stopped for the first 24 hours after treatment to allow concentrations to become established.

20. Watermilfoil apical stem sections 15 cm long were established in standard soil mix in 250-ml glass beakers. Five beakers, each containing three plant sections, were placed in the culture vessels and allowed to establish for 4 weeks before being treated. Culture vessels were subjected to 14 hours of 150 μeinsteins $\cdot$ m^{-2} $\cdot$ sec^{-1} light from

fluorescent tubes. Temperature was maintained at 28°C.

21. Water was sampled at 1, 2, 3, 4, 7, 28, 42, and 56 days after treatment and analyzed for 2,4-D as described for release rate studies.

Release of dichlobenil from beeswax CRHF into reconstituted water

22. Beeswax pellets, each containing 20 percent (a.i.) dichlobenil and weighing approximately 250 mg, were placed one per 3.8-ℓ container to which had been added 3500 g of reconstituted water. The experiment consisted of four replications (four containers with pellets).

23. Containers were sampled at 1, 3, 7, 14, 21, 28, and 56 days by removing four 10-ml aliquots from each jar. Dichlobenil was extracted from samples by the method described in paragraph A15. Dichlobenil content was determined directly by gas chromatography. Samples with concentrations of dichlobenil greater than 1.0 µg/ℓ were analyzed directly by liquid chromatography.

Conventional Formulations

Laboratory techniques

24. Submersed aquatic plants. Apical sections of submersed plants were planted in a sand-soil mix in small plastic pots and placed in 3.8- or 19-ℓ jars. Plants were than allowed to become established for approximately 1 week under controlled conditions of temperature (25°C) and light (25 to 40 µeinsteins $\cdot m^{-2} \cdot sec^{-1}$, from Grow-Lux fluorescent tubes for 14 hours per day). The plants were treated by injecting treatment solutions into the water with a hypodermic syringe. The treatments were then evaluated biweekly for phytotoxicity.

25. Growth inhibition of hydrilla propagules. Vegetative propagules (tubers) of hydrilla were planted in 5-cm pots (five tubers per pot), and three pots placed in a 3.8-ℓ jar filled with water. Chemical treatments were applied at the time of planting. Effects on germination were recorded along with phytotoxic response of sprouted plants. These tests were conducted in a growth laboratory under conditions of controlled light and temperature as described in the previous paragraph.

Greenhouse techniques

26. Plants to be treated were grown in polyethylene-lined, 12-ℓ-capacity plastic containers, and allowed to become established in the greenhouse for a period of approximately 1 to 4 weeks prior to treatment. For the application of each replicated treatment, the containers were placed in a 929-cm^2 enclosure with an open top and the plants were sprayed uniformly with a small atomizer. The total spray volume was equivalent to 935 ℓ/ha. Following application of the chemicals, the plants were moved to a greenhouse where treatments were periodically evaluated for phytotoxicity.

Outside aquaria techniques

27. Evaluations were conducted in aquaria of two sizes and types. One type consisted or circular, vinyl-lined containers manufactured for use as swimming or wading pools with the following dimensions: 3.05 m in diameter (7.3×10^{-4} ha) with a maximum depth of 74 cm and a maximum volume of 5400 ℓ. The pools were filled to a 53-cm depth, resulting in a volume of 3870 ℓ.

28. The second type of aquarium consisted of rectangular-shaped concrete boxes. The interior of each box was covered with two coats of white epoxy paint. The containers were 77 cm wide by 219 cm long (1.7×10^{-4} ha) with depth varying from 48 to 65 cm. The maximum capacity of these containers ranged from 815 to 1095 ℓ and the normal volume after adding soil was 500 to 825 ℓ.

29. When these aquaria were used to evaluate herbicide efficacy on submersed plants, 15-cm-long apical cuttings of individual species were planted in holes on 5.1-cm centers (428 stems/m^2) punched into a 15-cm layer of sand-organic soil mix on the bottom of each aquarium. Water levels were then slowly raised in the aquaria and the plants were subjected to a continuous water flow until treatments were applied. For evaluation of herbicide efficacy on floating plant species, field-collected plants were established for a minimum of 2 weeks in the aquaria and allowed to cover the water surface completely before treating.

30. All chemical treatment rates were replicated a minimum of three times and were applied on an area (kilograms per hectare) or volume

(milligrams per litre) basis. Phytotoxicity ratings, determined at various times after treatment, were made on a scale of 0 to 100 percent injury: 0 percent represented no injury, and 100 percent was complete elimination of live plant tissue.

31. Torpedograss was cultured in a simulated flooded (emersed) habitat by maintaining water depths at 40 cm with standpipes. Torpedograss was propagated by burying sections of rhizome containing 4 to 5 internodes in potting soil. Rhizome sections that produced shoots and roots were transplanted one per 30-cm-diam plastic pot with soil and allowed to become established for several weeks. Then 20 pots were emersed in each aquarium, or placed in dry aquaria.

32. Glyphosate at a 3.4-kg/ha rate was applied to the foliage of emersed and nonemersed plants, and observations were recorded over a 20-week period. Herbicide treatments were replicated three times with aquaria.

PART III: RESULTS AND DISCUSSION

Evaluation of Controlled-Release Formulations

Release of 2,4-D from MOE/GMA Copolymer

33. Reconstituted water. The concentration of 2,4-D in water increased with time indicating that release from the formulation had occurred (Table 2). Regression analysis of the release rate data revealed a significant relationship between release and time. The rate of release of the herbicide was estimated to be 3.2 mg 2,4-D per gram of copolymer per day (Figure 3).

34. Natural water. The release rate data from this investigation are given in Table 2. The release rate of 2,4-D from the copolymer into natural water was 2.07 mg/g/day, or approximately one third less than into reconstituted water (Figure 4). The factor or factors responsible for the decreasing rate of herbicide release in natural water should be identified so that their effect on field performance of the formulation can be evaluated.

Degradation of 2,4-D in natural water in the laboratory

35. Slow degradation of 2,4-D was observed over the 70-day sampling period (Table 3). Complete disappearance of the conventional herbicide occurred only in one replicate of each of two treatments: the 50- and 250-µg/ℓ (ppb) treatments.

36. The disappearance of 2,4-D in the two replicates did not appear to be related to concentration but appeared to be random. Disappearance was very abrupt and occurred after 14 days. Scatter diagrams and regression lines of the data from Table 4 show the gradual decline in herbicide concentration with time (Figures 5-9).

37. The linear regression equations from these analyses were used to estimate the degradation rate of 2,4-D in the various treatments. These rates were expressed in three different ways in Table 4: (a) as ppb 2,4-D per day; (b) as percent of amount initially applied per day;

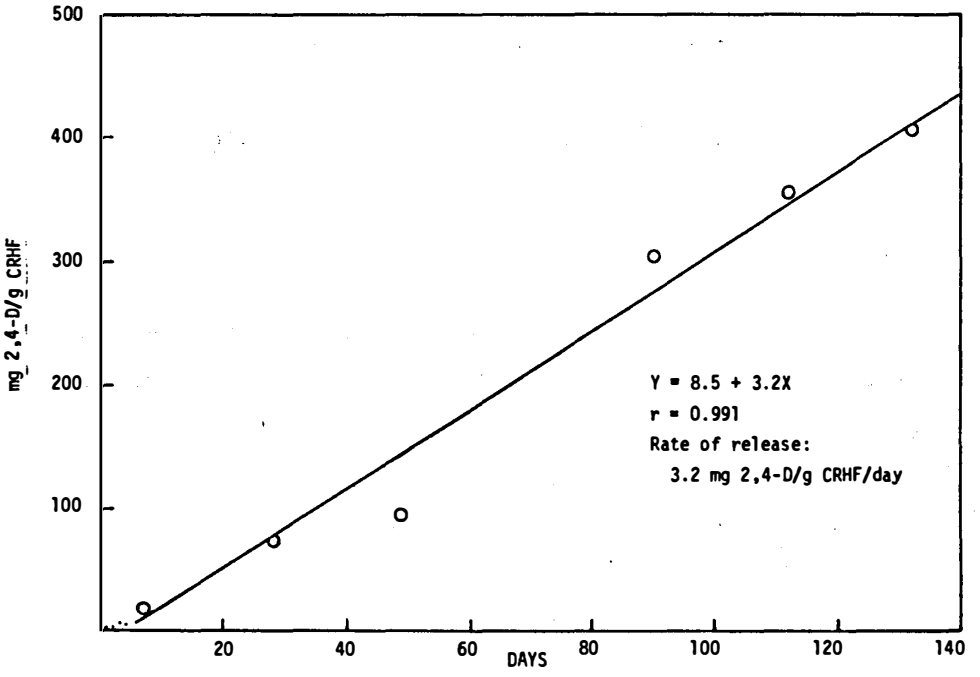

Figure 3. Release of 2,4-D from MOE/GMA CRHF into static reconstituted water

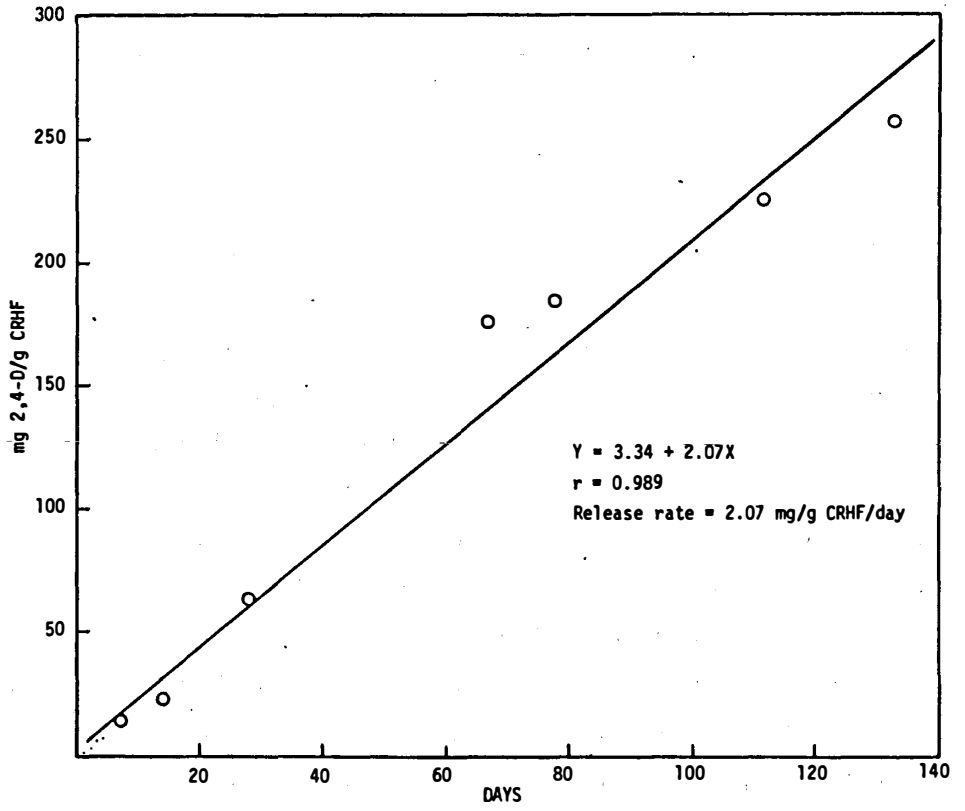

Figure 4. Release of 2,4-D from MOE/GMA CRHF into static natural water

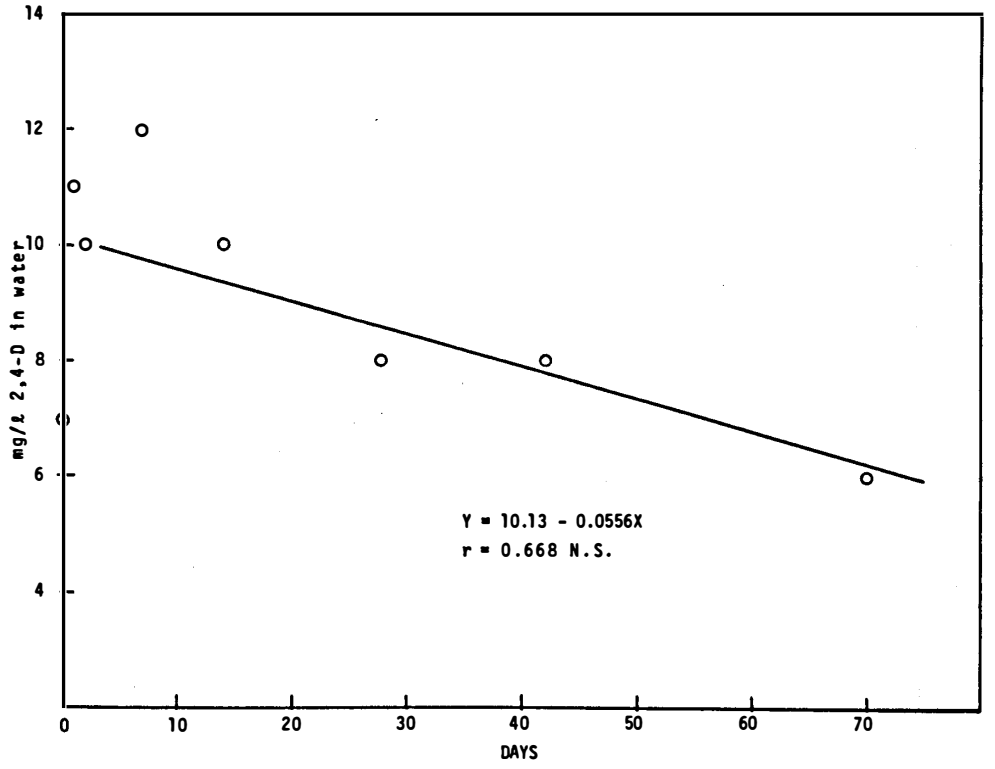

Figure 5. Degradation of 2,4-D applied at a 0.01-mg/ℓ rate in natural water in the laboratory

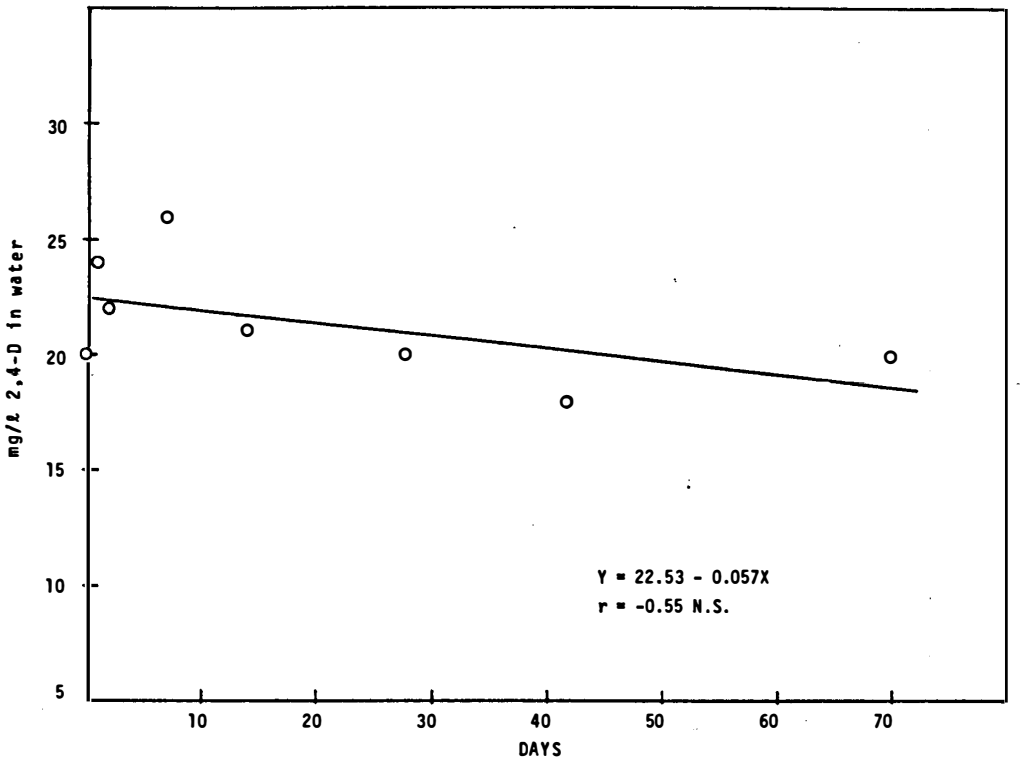

Figure 6. Degradation of 2,4-D applied at a 0.025-mg/ℓ rate in natural water in the laboratory

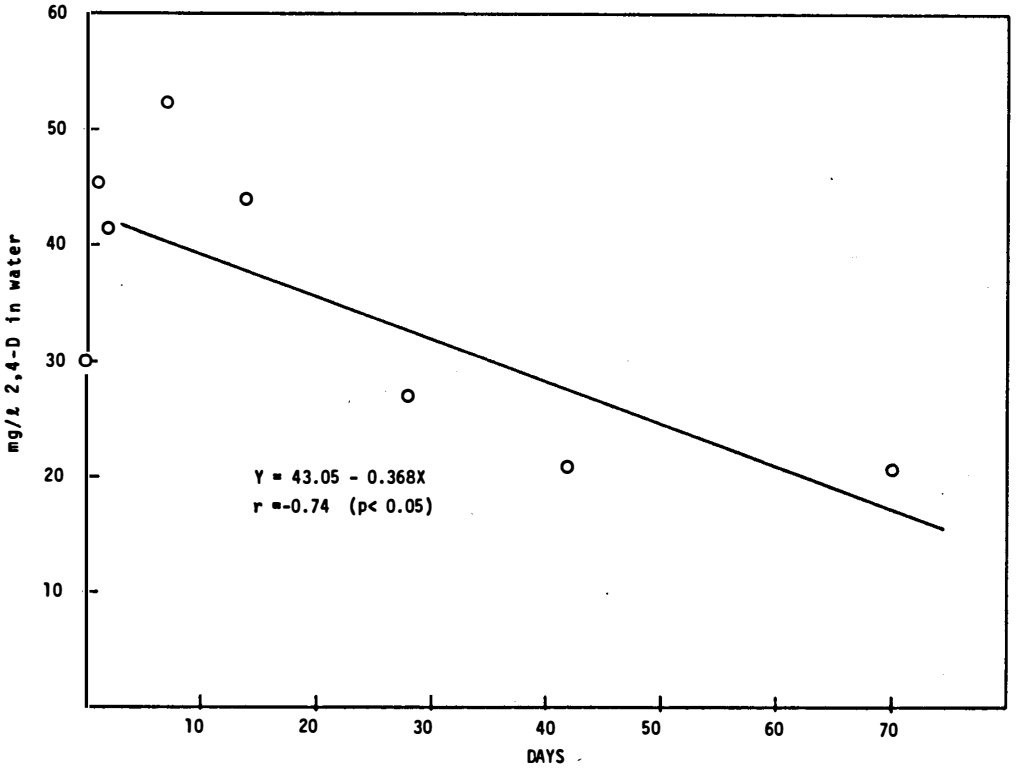

Figure 7. Degradation of 2,4-D applied at a 0.05-mg/ℓ rate in natural water in the laboratory

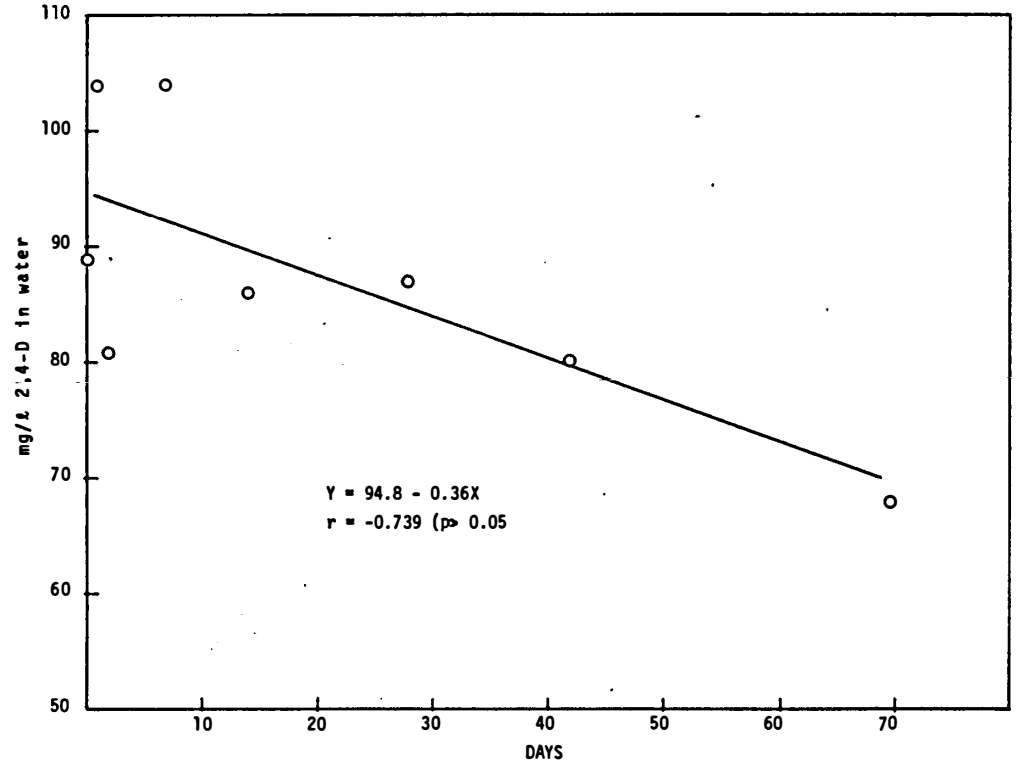

Figure 8. Degradation of 2,4-D applied at a 0.10-mg/ℓ rate in natural water in the laboratory

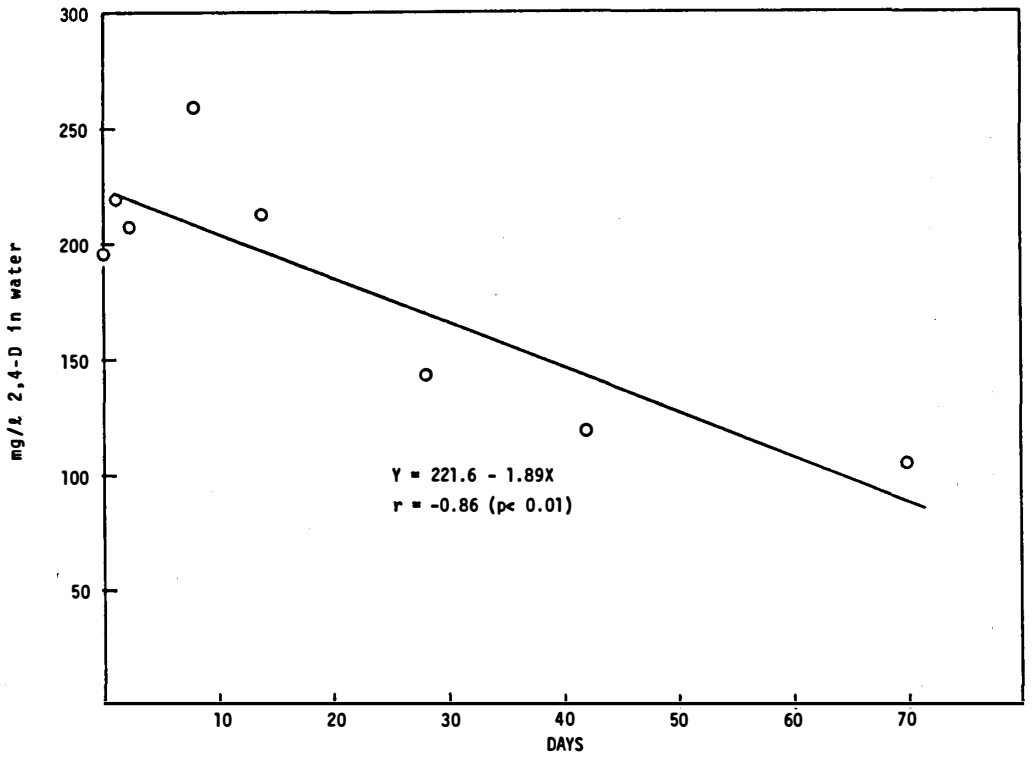

Figure 9. Degradation of 2,4-D in natural water in the laboratory at a 0.25-mg/ℓ rate

and (c) as time required for disappearance of one half of the amount originally applied.

38. Estimates for the 50- and 250-ppb treatments reflect the influence of the abrupt disappearance of 2,4-D in one of the replicate treatments. The half-lives for the replications in which abrupt degradation did not occur were 743 days for the 50-ppb and 414 days for the 250-ppb treatments.

39. The results of these investigations indicate that degradation was slow; and considering the short residence time of the chemical in the aquaria under flowing water conditions, the measurements of 2,4-D levels in this system can be considered accurate estimates of release.

Release of 2,4-D from MOE/GMA CRHF

40. Release into flowing water. Estimates of 2,4-D concentration in static water indicate that about two to three times the expected amount of 2,4-D was released from the CRHF after 24 hours (Figure 10).

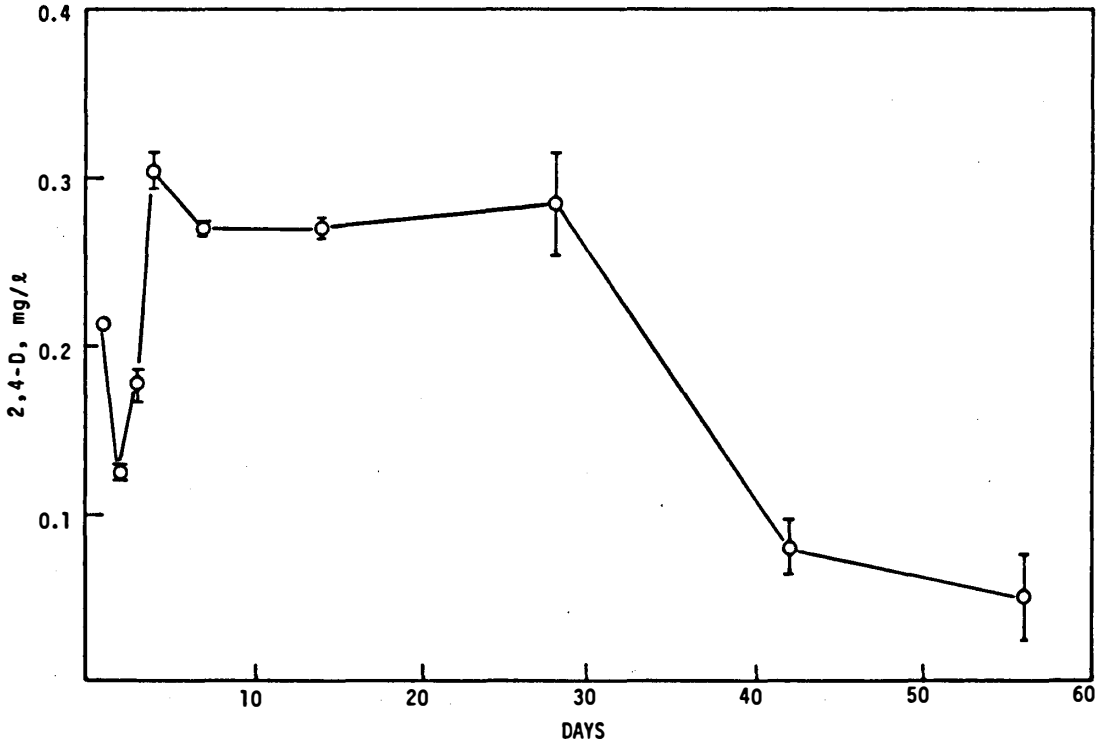

Figure 10. Release of 2,4-D from MOE/GMA copolymer into flowing natural water

The formulation was expected to release at the rate of 1.2 mg 2,4-D per gram of polymer per day. The release in containers without plants was estimated to be 2.6, 1.5, 2.1, 3.7, 3.2, 3.2, 3.4, 0.9, and 0.6 mg 2,4-D per gram of polymer per day at 1, 2, 3, 4, 7, 14, 28, 42, and 56 days, respectively.

41. After 24 hours in flowing water, 2,4-D concentrations decreased in treatments with and without plants (Figures 10 and 11). Concentrations of 2,4-D in treatments with plants continued to decrease for 72 hours, possibly due to adsorption of herbicide by plants and soil. After 72 hours in treatments with plants, 2,4-D levels increased, and after the first week the herbicide levels gradually decreased.

42. Concentration of 2,4-D increased in treatments without plants after 48 hours and peaked after 4 days. In these treatments, herbicide concentrations remained steady between 4 days and 4 weeks, after which a decline was observed.

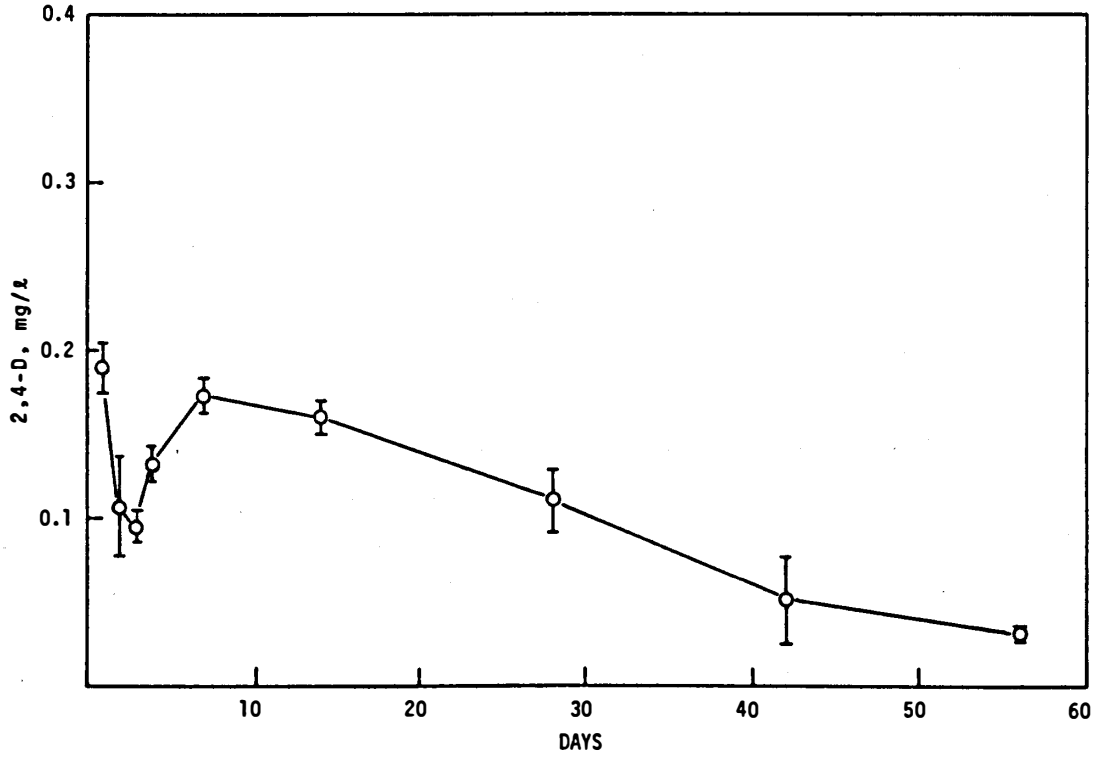

Figure 11. Influence of soil and watermilfoil on 2,4-D concentration in flowing water as released from MOE 2,4-D/GMA CRHF

43. Efficacy toward control of watermilfoil. In the early stages of the study, the response of watermilfoil plants to treatments was followed closely. A slight epinastic response of leaves was observed after 3 days and was very pronounced after 1 week.

44. At the end of 2 weeks, severe epinasty of leaves was observed near stem apices of treated plants. The internodes of the upper portions of stems appeared darkened and necrotic. The lower nodes near the base of stems appeared to be darkening and discoloring. Leaflets on treated plants were drooping when not undergoing epinasty. The average injury rating to treated plants was 45 percent (Table 5). Control plants appeared healthy and erect with good color. Leaflets were observed to be upright along all stems. Several new branches were observed to be developing along some stems. There were several necrotic leaflets on some stems apparently due to transplanting injury or shock. However, new branches were developing on all of these stems.

45. The average injury to treated plants after 4 weeks was 76 percent. Damage to stems had progressed to internodal areas and defoliation was severe. Injury to control plants had also increased slightly.

46. After 8 weeks, two of the four replicate treatments had produced 100 and 94 percent injury. A slight regrowth had occurred in the form of a single branch or one stem in the 94 percent replicate. Regrowths had occurred in the other two replicate treatments in the form of several new branches arising from the nodes of damaged stems. The reduced efficacy in those two replicates was responsible for the variability within the treatment that had occurred after 4 weeks.

47. In spite of the variable effect of the treatment on plant control, plant growth was inhibited. The fresh weight and growth in stem length was significantly lower in treated plants than in control plants (Table 6).

48. The 2,4-D MOE/GMA CRHF was shown in this investigation to be efficacious in consistency of release and in producing phytotoxicity to watermilfoil plants. This efficacy was confirmed through instrument analysis and bioassay.

49. Some difficulty was experienced with the polymer floating to the surface of culture vessels when treatments were applied. This may account for some of the variability in herbicide concentration that was encountered at various sampling periods, since part of the treatment could have floated out of the container. As a result of this experience, it is recommended that the polymer be formulated within an inert carrier, such as a clay granule, to provide more reliable delivery and distribution of the herbicide.

50. In the early stages of this study, some difficulty was experienced with erratic flow of wastewater from the culture containers. On two occasions, several jars overflowed due to blockages of the discharge lines; and on one occasion, approximately half the contents of several jars were siphoned when blockages were cleared. Loss of floating polymer when the jars overflowed could account for a portion of the variability in herbicide levels. Design modification of the discharge lines has eliminated the overflow and siphoning problems that were experienced

in the first trial of this prototype system.

Release of dichlobenil from beeswax CRHF into reconstituted water

51. Dichlobenil appeared to be slowly released from the beeswax pellet as indicated by the gradual increase in concentration with time (Table 7). A plot of the release rate data revealed a decreasing rate of release with time (Figure 12). The theoretical (designed) release

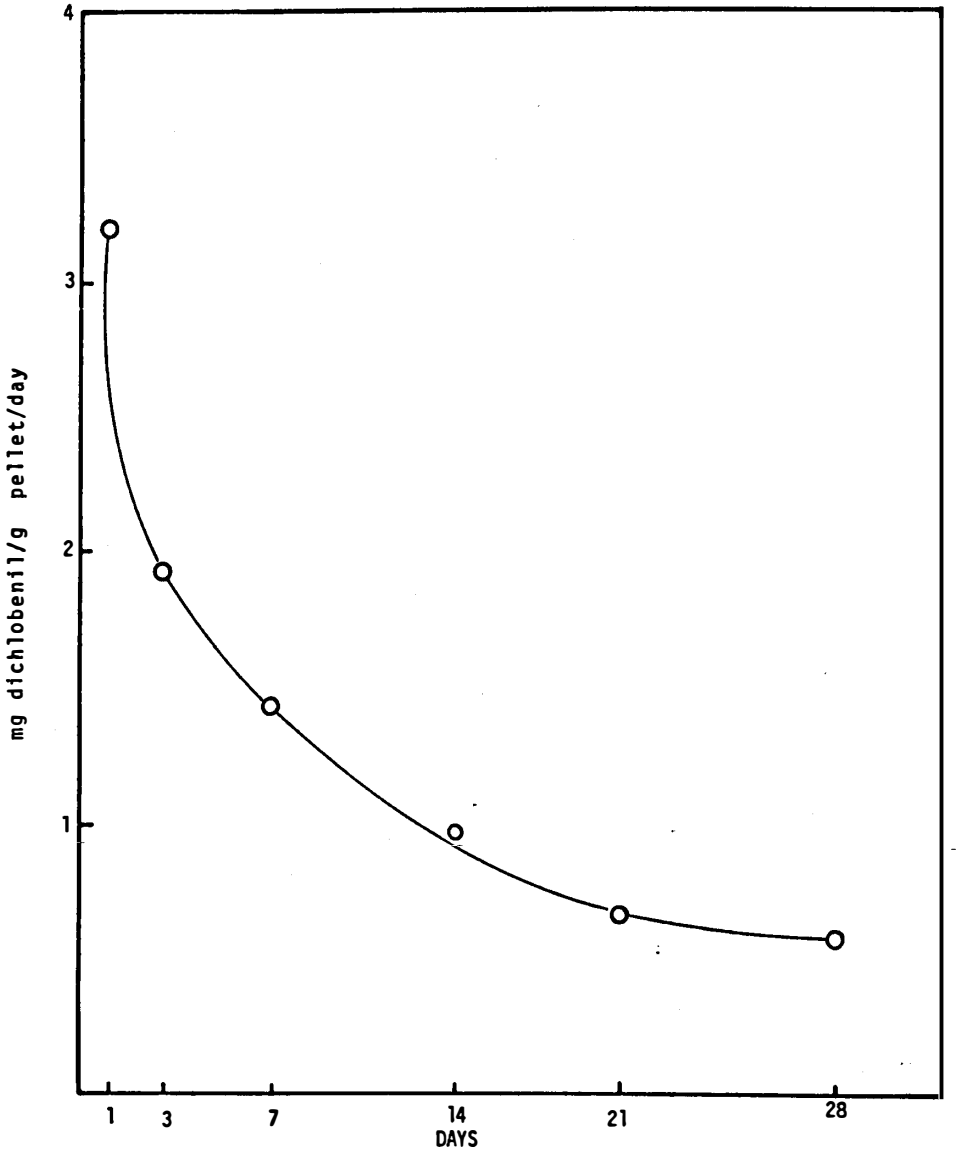

Figure 12. Release of dichlobenil from beeswax pellets into static reconstituted water

rate was expected to be 0.8 mg/g CRHF/day. The measured rate was four times greater than expected after the first day and decreased to approximately 74 percent of the expected release rate after 28 days. The data were corrected to account for possible rapid release of the chemical at or near the pellet surface by subtracting the amount released the first day that exceeded the expected amount. Inspection of these data indicated that the release rate was greater than expected the first 7 days, approximately as expected during the second week, and less than expected beyond 2 weeks.

52. These preliminary data indicate that release of dichlobenil from the beeswax CRHF was not constant but tended to decrease with time. Microscopic examination of the pellets revealed an accumulation of crystalline substance at the surface that may account for the initially high concentration of the herbicide in the water.

Laboratory Evaluations

Inhibition of hydrilla tuber germination

53. The results of the evaluation of RO 3-7042, a coded conventional herbicide from MAAG Agrochemicals, indicate that this compound had no effect on tuber germination (Table 8). Concentrations of 5.0 mg/ℓ and higher produced 87 percent or greater injury ratings to germinated plants. These levels, however, are generally considered to be economically infeasible for field applications.

Submersed plants

54. Several CR formulations of fenac and 2,4-D and the conventional RO 3-7042 formulation were evaluated for efficacy in controlling hydrilla, southern naiad, cabomba, watermilfoil, and chara (Table 9).

55. Three fenac CR formulations 7310-172-1, -2, and -3; four sinking CR formulations of 2,4-D, 7389-8-A to -D; and four floating CR formulations of 2,4-D, 7389-14-A to -D, were applied at treatment rates of 0.5, 1.0 and 2.0 mg/ℓ and were compared with conventional formulations of the same rates.

56. RO 3-7042 was evaluated at treatment rates of 0.5, 1.0, 5.0, 10.0, and 20.0 mg/ℓ.

57. Combinations of chelated iron complexes Aqua-vator (AV) -1, -2, and -3 were evaluated for enhancement of diquat (Table 10) or potassium endothall (Table 11) activity against hydrilla.

58. Hydrilla. Fenac CR formulations 7310-172-1 and -3 were comparable in efficacy to the conventional formulation after 10 weeks; 7310-172-2 was less effective than the reference formulation. None of the 2,4-D formulations were effective against hydrilla. RO 3-7042 was effective at a treatment rate of 1.0 mg/ℓ but was determined to be slow acting since a period of over 16 weeks was required to produce a 97 percent control rating.

59. The combination of all three of the AV compounds with the 0.025-mg/ℓ diquat rate appeared to enhance the efficacy of the diquat treatment since that rate of diquat alone produced a lower average injury rating. These tests did not demonstrate an enhancement of potassium endothall efficacy through the addition of the various AV compounds.

60. Southern naiad. Fenac CR formulations 7310-172-1 and -3 were more effective than the reference formulation at the 1.0-mg/ℓ treatment rate. None of the 2,4-D formulations were effective. RO 3-7042 produced 98 percent injury after 8 weeks at the 5.0-mg/ℓ treatment rate.

61. Cabomba. Evaluations of the fenac formulations were complicated by high injury ratings of controls. These evaluations were not repeated due to shortage of materials. None of the 2,4-D CR formulations were noticeably more effective than the reference formulation. RO 3-7042 had no significant effect on cabomba growth.

62. Watermilfoil. The fenac formulation appeared to produce greater injury to plants than was observed in the control plants. Comparison of the results of the 2,4-D 7389-8 evaluations was made impossible by loss of the control plants.

63. The 0.5-mg/ℓ treatment rate of the 2,4-D 7389-14 formulations and the reference formulations produced complete control after 2 weeks.

64. RO 3-7042 produced 100 percent control after 16 weeks at the 0.5-mg/ℓ treatment rate, confirming the observation

made on hydrilla of a slow response rate.

65. Chara. RO 3-7042 was effective against chara but only at an impractical 5.0-mg/ℓ or greater treatment rate.

Floating plants

66. RO 3-7042 was not effective against duckweed, was marginally effective against waterhyacinth (99 percent control at 4 kg/ha after 16 weeks), and very effective against waterlettuce (98 percent control at 1 kg/ha after 4 weeks) (Table 12).

67. Increasing treatment rates of EL-507 growth retardant progressively reduced the estimated growth of waterhyacinth (Table 13). The specific effects of the chemical on various growth parameters are indicated in Table 14. The 1.0-kg/ha application rate had a retarding influence on most of the measured paramters. All of the measured parameters that were influenced by the growth retardant were affected at the 2.0-kg/ha rate. The most sensitive indicator of retardant efficacy was root length:leaf length ratio. This was the only ratio that was decreased at the 0.25-kg/ha rate. Growth enhancement was observed in several of the parameters at the 0.25- and 0.50-kg/ha treatment rates. A relatively low treatment rate of 0.5 kg/ha produced a 36 percent average reduction in dry matter production. Additional study of the effects of this chemical on growth of other aquatic plants appears warranted.

Ditchbank plants

68. RO 3-7042 was not effective against torpedograss and only marginally effective against paragrass (Table 15).

69. The efficacy of glyphosate was reduced when applied to torpedograss cultured in outside aquaria under simulated flooded growth conditions (Figure 13). These results confirm earlier observations indicating that control of emersed torpedograss with glyphosate was generally of shorter duration than for dry habitats. Investigation of glyphosate translocation in plants growing in these diverse habitats in in progress.

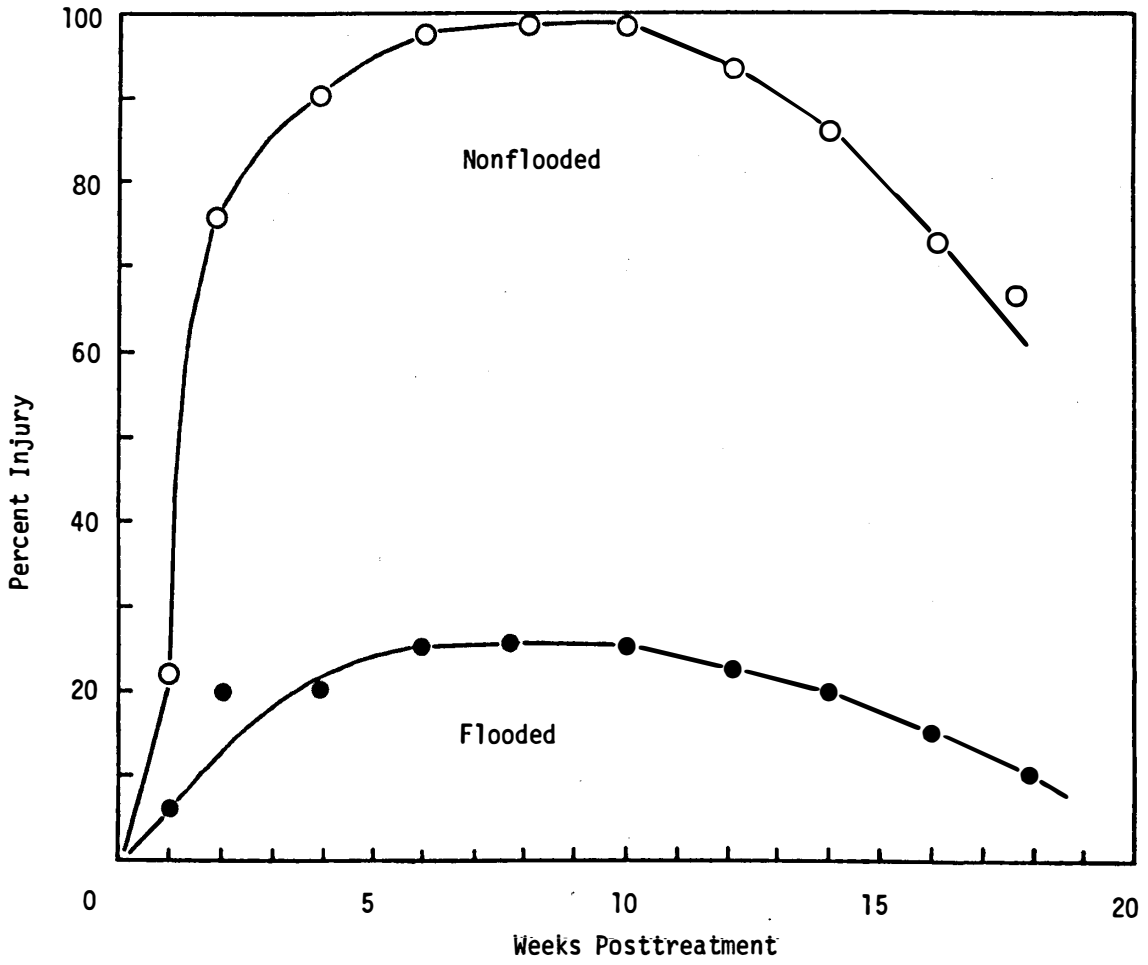

Figure 13. Response of flooded and nonflooded torpedograss in outdoor aquaria to 3.4-kg/ha treatments with glyphosate

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Table 1

Names and Sources of Chemicals Evaluated in Fiscal Year 1980

Common Name	Chemical Name	Source
AV-1, -2, -3 (Aqua-vator)	Undetermined iron complexes of natural organic chelating agents	Dixie Agricultural Chemical Co. P. O. Box 1227 Eustis, FL 32726
Diquat	6,7-dihydrodipyrido(1,2-a:2',1'c) pyrazinediium dibromide	Chevron Chemical Company Ortho Division 940 Hensley Street Richmond, CA 94804
Endothall	Dipotassium salt of 7-oxabicyclo (2.2.1)heptane-2,3-dicarboxylic acid	Pennwalt Corporation Agricultural Chemical Division 1630 East Shaw Avenue Fresno, CA 93710
EL-507	a-tert-butyl-a(p-fluorophenyl)-3-pyridine methanol	Elanco Division of Eli Lilly and Co. P. O. Box 708 Greenfield, IN 46140
Fenac	Salts of 2,3,6-trichlorophenyl-acetic acid	Union Carbide Agricultural Products Co., Inc. 300 Brookside Avenue Ambler, PA 19002
Fenac 7310-172-1 to -3		USDA, SEA, SRRC* 1100 Robert E. Lee Boulevard P. O. Box 19687 New Orleans, LA 70279

(Continued)

* Southern Regional Research Center, Dr. W. J. Connick.

Table 1 (Concluded)

Common Name	Chemical Name	Source
RO 3-7042	a-amino-6-methyl benzoic acid	MAAG Agrochemicals Research Development HLR Sciences, Inc. Kings Highway, P. O. Box X Vero Beach, FL 32960
2,4-D	Dimethylamine salt of 2,4-dichlorophenoxy acetic acid	Thompson Hayward Chemical Co. P. O. Box 2383 Kansas City, KS 66110
MOE 2,4-D/GMA	2-methacryloyloxyethyl 2,4-dichlorophenoxyacetate/glyceryl-methacrylate	Dr. Frank Harris Wright State University Dayton, OH
2,4-D 7389-8-A to -D 7389-14-A to -D		USDA, SEA, SRRC*

* Southern Regional Research Center, Dr. W. J. Connick.

Table 2

Release of 2,4-D from CRHF (MOE/GMA) into Static Water in the Laboratory

Days after treatment - Reconstituted water								
1	2	3	7	28	42	90	112	132
mg/ℓ 2,4-D								
0.14	0.36	0.58	1.32	6.12	7.81	25.4	29.6	34.0
mg 2,4-D/g CRHF								
1.7	4.3	7.0	15.8	73.4	93.7	304.8	355.2	408.0

Days after treatment - Natural water										
1	2	3	4	7	14	28	67	78	112	133
mg/ℓ 2,4-D										
0.15	0.27	0.51	0.59	1.23	1.99	5.24	14.7	15.4	18.8	21.4
mg 2,4-D/g CRHF										
1.77	3.26	6.10	7.06	14.7	23.8	62.7	175.8	184.2	224.9	256.0

Table 3

Degradation of 2,4-D in Static Natural Water in the Laboratory

Applied 2,4-D µg/ℓ		Days Posttreatment							
		0	1	2	7	14	28	42	70
		Measured 2,4-D, µg/ℓ*							
10	(A)	7	10	11	11	11	9	8	8
	(B)	6	11	10	13	8	7	8	5
	Avg.	7	11	11	12	10	8	8	6
25	(A)	23	26	22	26	21	22	19	19
	(B)	19	22	23	27	21	18	18	20
	Avg.	21	24	22	26	19	20	18	19
50	(A)	33	44	40	51	45	11	ND**	ND
	(B)	26	47	43	54	43	43	42	43
	Avg.	30	46	42	53	44	27	21	21
100	(A)	89	107	84	102	83	85	86	76
	(B)	89	100	78	105	88	89	73	61
	Avg.	89	104	81	104	86	87	80	63
250	(A)	235	234	208	261	232	228	228	212
	(B)	158	205	205	257	195	74	8	ND
	Avg.	196	220	206	259	214	142	118	106
Control	(A)	ND	ND	ND	ND	ND	ND	ND	ND
	(B)	ND	ND	ND	ND	ND	ND	ND	ND
	Avg.	ND	ND	ND	ND	ND	ND	ND	ND

* Average of three determinations per replicate.
** Not detected.

Table 4

Rate of 2,4-D Disappearance in Static Natural Water

Applied 2,4-D ppb	ppb/day	% of Applied per Day	Half-Life Days
10	0.056	0.56	92
25	0.057	0.23	177
50	0.368	0.70	25
100	0.350	0.36	123
250	1.89	0.76	24

Table 5

Phytotoxic Response of *M. spicatum* to Controlled Release of 2,4-D into Flowing Ambient Water

	Average* % Injury, Weeks Posttreatment		
	2 Wk	4 Wk	8 Wk
Treated	45	76	64
CV**	16%	14%	61%
Control	18	23	19
CV**	86%	53%	70%

* Mean of three replicates.
** Coefficient of variation.

Table 6

Effect of 2,4-D Released from MOE/GMA CRHF on *M. spicatum* Growth After 8 Weeks in Flowing Water

Group	Stems		Roots
	Fresh Wt. g	Length cm	Fresh Wt. g
Treated	0.36	9.5	0.56
Control	3.40	36.8	1.32
t-test	3.73**	4.18**	4.8***

** t-test @ 0.01 = 3.7.
*** t-test @ 0.005 = 4.3.

Table 7

Release of Dichlobenil from Beeswax Pellets into Static Reconstituted Water

Days after treatment						
1	3	7	14	21	28	56
mg/ℓ concentration						
0.22	0.40	0.71	0.91	0.97	1.14	--
Total mg released						
0.8	1.4	2.5	3.2	3.4	4.0	--
Expected mg released						
0.2	0.6	1.4	2.7	4.1	5.4	10.9
Corrected mg released						
0.2	0.8	1.9	2.6	2.8	3.4	--

Table 8

Laboratory Evaluation of RO 3-7042 for Phytotoxicity Toward Hydrilla Tubers

Date of Evaluation	Chemical Designation	Company or Source	Rate mg/ℓ	Posttreatment Response* 2 Days G	2 Days E	5 Days G	5 Days E	1 Wk G	1 Wk E	2 Wks G	2 Wks E	4 Wks G	4 Wks E	8 Wks G	8 Wks E	12 Wks G	12 Wks E
3/12/80	RO 3-7042	MAAG	0.5	0	0	0	0	6	0	7	0	9	0	9	0	9	6
			1.0	0	0	0	0	5	0	6	0	8	3	8	13	4	27
			5.0	0	0	0	0	7	5	8	22	10	65	10	87	10	72
			10.0	0	0	0	0	7	5	9	22	10	65	10	90	10	90
			20.0	0	0	0	0	7	10	8	32	9	77	9	96	9	95
			0.0	0	0	0	0	8	0	8	0	8	0	8	0	8	15

* G = Number germinated of 15 total; E = evaluation (% injury).

Table 9

Laboratory Evaluations of Various Herbicides for Phytotoxicity Toward Submerged Plants

Date of Evaluation	Chemical Designation	Company or Source	Rate mg/ℓ	Percent Control for Weeks Posttreatment														
				Hydrilla					Naiad					Cabomba				
				2	4	8	16	22	2	4	8	16	22	2	4	8	16	22
9/13/79	RO 3-7042	MAAG	0.5	0	0	1	20	37	0	0	1	1	1	0	0	0	0	33
			1.0	0	2	14	88	97	0	0	0	0	0	0	0	0	0	30
			5.0	0	9	85	100	100	1	57	98	100	100	0	0	0	0	19
			10.0	0	14	81	97	100	1	53	94	100	100	0	0	0	13	37
			20.0	0	15	76	100	100	5	60	93	100	100	0	0	0	0	38
			0.0	0	0	0	0	3	0	0	0	0	0	0	0	0	44	79
10/30/79	7310-172-1	SRRC	0.5	0	0	0	0	0	0	0	6	19	22	0	10	62	68	70
			1.0	0	8	10	23	12	3	77	89	90	90	0	15	68	100	100
			2.0	0	5	11	40	53	3	8	23	43	48	0	63	83	100	100
	7310-172-2	SRRC	0.5	0	2	2	3	5	0	13	37	37	39	0	1	5	10	98
			1.0	0	2	3	3	4	0	2	2	8	8	0	3	20	70	80
			2.0	7	9	15	17	18	0	18	36	37	41	0	10	67	97	100
	7310-172-3	SRRC	0.5	2	2	2	2	10	0	1	2	7	11	0	4	42	68	95
			1.0	4	10	43	53	73	0	0	3	47	86	1	7	7	40	63
			2.0	35	40	42	42	58	38	41	73	98	100	32	38	45	70	70
	Fenac (liquid)	UC	0.5	1	2	3	5	4	0	7	17	30	30	5	15	52	83	100
			1.0	4	5	9	9	10	0	2	4	5	11	0	5	10	42	76
			2.0	3	7	42	48	53	28	53	97	97	0	0	45	58	97	100
	Control		0.0	0	0	0	0	0	0	0	2	2	1	0	0	8	37	70
				Watermilfoil					Chara									
				2	4	8	16	22	2	4	8	16	22					
9/13/79	RO 3-7042	MAAG	0.5	0	1	78	99	100	0	0	0	0	0					
			1.0	0	3	92	100	100	3	17	0	0	12					
			5.0	0	20	93	100	100	7	46	83	93	99					
			10.0	0	12	92	100	100	30	55	95	97	100					
			20.0	0	29	96	100	100	25	34	60	98	100					
			0.0	0	37	25	40	40	0	0	0	0	0					

(Continued)

Table 9 (Continued)

Date of Evaluation	Chemical Designation	Company or Source	Rate mg/ℓ	Percent Control for Weeks Posttreatment: Watermilfoil 2	4	8	16	22	Chara 2	4	8	16	22
10/30/79	7310-172-1	SRRC	0.5	8	97	100	100	100	0	0	0	0	0
			1.0	32	100	100	100	100	0	0	0	0	0
			2.0	35	100	100	100	100	0	0	0	0	0
	7310-172-2	SRRC	0.5	77	100	100	100	100	0	0	0	0	0
			1.0	47	100	100	100	100	0	0	0	0	0
			2.0	33	100	100	100	100	0	0	0	0	0
	7310-172-3	SRRC	0.5	65	100	100	100	100	0	0	0	0	0
			1.0	60	100	100	100	100	0	0	0	0	0
			2.0	68	100	100	100	100	0	0	0	0	0
	Fenac (liquid)	UC	0.5	52	100	100	100	100	0	0	0	0	0
			1.0	73	100	100	100	100	0	0	0	0	0
				67	99	100	100	100	0	0	0	0	0
	Control		0.0	20	32	50	53	67	0	0	0	0	0

Date of Evaluation	Chemical Designation	Company or Source	Rate mg/ℓ	Hydrilla 2	4	8	16	22	Naiad 2	4	8	16	22	Cabomba 2	4	8	16	22
10/31/79	7389-8-A	SRRC	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			1.0	0	0	17*	17*	20*	0	0	13	17	20	0	0	32	32	35
			2.0	0	0	2	2	2	0	0	0	1	2	0	0	5	5	33
	7389-8-B	SRRC	0.5	0	0	0	0	0	0	0	0	0	0	0	0	12	12	33
			1.0	0	0	11	11	11	0	0	1	1	1	0	0	23	23	55
			2.0	0	0	3	3	2	0	0	3	3	2	0	0	3	3	5
	7389-8-C	SRRC	0.5	0	0	0	0	0	0	0	0	0	0	0	0	2	2	1
			1.0	0	0	3	3	2	0	0	0	0	0	0	0	4	4	3
			2.0	0	0	2	2	1	0	0	0	0	0	0	0	3	7	62
	7389-8-D	SRRC	0.5	0	0	0	0	0	0	0	0	3	2	0	0	10	0	8
			1.0	0	0	3	3	2	0	0	0	0	0	0	0	10	10	10
			2.0	0	0	2	2	1	0	0	0	2	0	0	0	3	4	17

(Continued)

* Insect damage.

Table 9 (Continued)

Date of Evaluation	Chemical Designation	Company or Source	Rate mg/ℓ	Percent Control for Weeks Posttreatment														
				Hydrilla					Naiad					Cabomba				
				2	4	8	16	22	2	4	8	16	22	2	4	8	16	22
10/31/79	2,4-D		0.5	0	0	0	0	0	0	0	0	0	0	0	0	5	5	13
			1.0	0	0	1	1	0	0	0	0	0	0	0	0	3	3	5
			2.0	0	0	6	6	3	0	13	12	17	18	0	0	4	7	7
	Control		0.0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	4
				Watermilfoil					Chara									
				2	4	8	16	22	2	4	8	16	22					
	7389-8-A	SRRC	0.5	67	100	100	100	100	0	0	0	0	0					
			1.0	87	100	100	100	100	0	0	0	0	0					
			2.0	98	100	100	100	100	0	0	0	0	0					
	7389-8-B	SRRC	0.5	80	100	100	100	100	0	0	0	0	0					
			1.0	77	100	100	100	100	0	0	0	0	0					
			2.0	78	100	100	100	100	0	0	0	0	0					
	7389-8-C	SRRC	0.5	78	100	100	100	100	0	0	0	0	0					
			1.0	97	100	100	100	100	0	0	0	0	0					
			2.0	98	100	100	100	100	33	33	33	33	35					
	7389-8-D	SRRC	0.5	78	100	100	100	100	0	0	0	0	0					
			1.0	96	100	100	100	100	0	0	0	0	0					
			2.0	99	100	100	100	100	0	0	0	0	0					
	2,4-D		0.5	77	100	100	100	100	0	0	0	0	0					
			1.0	92	100	100	100	100	0	0	0	0	0					
			2.0	96	100	100	100	100	0	0	0	0	0					
	Control		0.0	0	100	100	100	100	0	0	0	0	0					
				Hydrilla					Naiad					Cabomba				
				2	4	8	16	22	2	4	8	16	22	2	4	8	16	22
11/1/79	7389-14-A	SRRC	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			1.0	0	0	0	0	3	0	0	0	5	5	0	0	0	0	2
			2.0	0	0	0	1	1	0	0	0	0	0	0	0	2	7	38

(Continued)

Table 9 (Continued)

Date of Evaluation	Chemical Designation	Company or Source	Rate mg/ℓ	Percent Control for Weeks Posttreatment															
				Hydrilla					Naiad					Cabomba					
				2	4	8	16	22	2	4	8	16	22	2	4	8	16	22
11/1/79	7389-14-B	SRRC	0.5	0	0	0	0	0	0	0	0	0	0	0	0	6	8	28
				0	0	0	0	0	0	0	0	0	0	0	0	0	2	47
				0	0	2	0	2	17	20	3	11	25	0	0	0	21	62
	7389-14-C	SRRC	0.5	0	0	0	0	0	0	0	2	3	3	0	0	0	2	7
			1.0	0	0	0	0	0	7	10	10	11	14	0	0	0	2	8
			2.0	0	0	0	10	11	0	0	0	13	33	17	20	20	30	66
	8390-14-D	SRRC	0.5	0	0	0	0	0	0	17	33	33	33	0	0	0	0	2
			1.0	0	0	1	1	5	0	0	0	1	2	0	0	0	1	17
			2.0	0	0	2	4	11	0	0	0	8	8	0	0	0	3	8
	2,4-D (liquid)		0.5	0	0	0	0	1	0	0	0	3	3	0	0	0	23	60
			1.0	0	0	1	1	2	0	0	0	34	35	0	0	0	1	35
			2.0	0	0	1	0	2	0	10	40	40	63	0	0	0	0	22
	Control		0.0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	2
				Watermilfoil					Chara										
				2	4	8	16	22	2	4	8	16	22						
	7389-14-A	SRRC	0.5	100	100	100	100	100	0	0	0	0	0						
			1.0	100	100	100	100	100	0	0	0	0	0						
			2.0	100	100	100	100	100	0	0	0	0	0						
	7389-14-B	SRRC	0.5	96	100	100	100	100	0	0	0	0	0						
			1.0	100	100	100	100	100	0	0	0	0	0						
			2.0	99	100	100	100	100	0	0	0	0	0						
	7389-14-C	SRRC	0.5	100	100	100	100	100	0	0	0	0	0						
			1.0	99	100	100	100	100	0	0	0	0	0						
			2.0	100	100	100	100	100	0	0	0	0	0						

(Continued)

Table 9 (Concluded)

Date of Evaluation	Chemical Designation	Company or Source	Rate mg/ℓ	Percent Control for Weeks Posttreatment: Watermilfoil 2	4	8	16	22	Chara 2	4	8	16	22
11/1/79	7389-14-D	SRCC	0.5	100	100	100	100	100	0	0	0	0	0
			1.0	100	100	100	100	100	0	0	0	0	0
			2.0	100	100	100	100	100	0	0	0	0	0
	2,4-D (liquid)		0.5	100	100	100	100	100	0	0	0	0	0
			1.0	100	100	100	100	100	0	0	0	0	0
			2.0	99	100	100	100	100	0	0	0	0	0
	Control		0.0	0	0	0	2	7	0	0	0	0	0

Table 10

Evaluation of Experimental Iron Chelates for Enhancing Efficacy of Diquat Against Hydrilla

Date of Evaluation	Chemical Designation	Company or Source	Rate mg/ℓ	Percent Injury Weeks Posttreatment 2	4	6	8
4/16/80	Diquat		0.025	0	0	0	3
			0.050	6	73	92	93
			0.100	50	98	100	100
	Diquat + AV-1	Dixie	0.025 + 0.5	0	0	0	18
			0.025 + 1.0	1	2	2	22
			0.050 + 0.5	15	43	51	58
			0.050 + 1.0	27	65	86	87
			0.100 + 0.5	58	73	75	82
			0.100 + 1.0	86	98	100	100
	Diquat + AV-2	Dixie	0.025 + 0.5	0	7	8	12
			0.025 + 1.0	27	50	50	53
			0.050 + 0.5	23	48	73	79
			0.050 + 1.0	10	18	52	65
			0.100 + 0.5	80	96	99	99
			0.100 + 1.0	88	98	98	98
	Diquat + AV-3		0.025 + 0.5	0	10	13	27
			0.025 + 1.0	2	3	5	6
			0.050 + 0.5	20	62	63	73
			0.050 + 1.0	48	80	87	92
			0.100 + 0.5	67	98	100	99
			0.100 + 1.0	62	95	97	98
	Control			0	0	0	0

Table 11

Evaluation of Experimental Iron Chelates for Enhancing Efficacy of Potassium Endothall Against Hydrilla

Date of Evaluation	Chemical Designation	Company or Source	Rate mg/ℓ	Percent Injury Weeks Posttreatment 2	4	6	8
4/17/80	Potassium-Endothall	Dixie	0.25	2	17	19	45
			0.50	37	53	62	72
			1.00	67	79	99	99
	Potassium-Endothall + AV-1	Dixie	0.25 + 0.5	17	25	25	30
			0.25 + 1.0	13	17	17	35
			0.50 + 0.5	13	32	38	57
			0.50 + 1.0	38	47	53	70
			1.00 + 0.5	65	80	93	98
			1.00 + 1.0	75	90	97	99
	Potassium-Endothall + AV-2	Dixie	0.25 + 0.5	2	2	3	5
			0.25 + 1.0	6	17	17	34
			0.50 + 0.5	27	37	50	58
			0.50 + 1.0	33	38	50	58
			1.00 + 0.5	37	55	77	83
			1.00 + 1.0	75	90	97	99
	Potassium-Endothall + AV-3	Dixie	0.25 + 0.5	1	1	5	16
			0.25 + 1.0	3	4	13	38
			0.50 + 0.5	13	27	57	68
			0.50 + 1.0	38	57	60	68
			1.00 + 0.5	75	90	97	100
			1.00 + 1.0	65	78	82	84
	Control			0	0	0	4

Table 12

Greenhouse Evaluation of RO 3-7042 for Phytotoxicity Toward Floating Plants

Date of Evaluation	Chemical Designation	Company or Source	Rate kg/ha	Percent Control Weeks Posttreatment 2	4	8	16	20
			Waterhyacinth					
10/4/79	RO 3-7042	MAAG	1.0	0	0	0	0	7
			2.0	0	0	0	0	4
			4.0	0	25	77	99	100
			6.0	0	25	65	96	100
			10.0	0	25	68	89	99
			0.0	0	0	0	0	0
			Waterlettuce					
9/17/79	RO 3-7042	MAAG	1.0	83	98	96	--	--
			2.0	95	100	100	--	--
			4.0	85	98	100	--	--
			6.0	97	100	100	--	--
			10.0	95	98	100	--	--
			0.0	0	0	34	--	--
			Duckweed					
				1	2	4	6	
9/17/79	RO 3-7042	MAAG	1.0	3	3	33	33	--
			2.0	0	0	0	0	--
			4.0	0	0	13	0	--
			6.0	0	0	65	66	--
			10.0	0	0	7	35	--
			0.0	0	0	35	90	--

Table 13

Growth Response of Waterhyacinth to Growth Retardant EL-507

Date of Evaluation	Chemical Designation	Company or Source	Rate kg/ha	Percent Control Weeks Posttreatment 1	2	4	6	8
4/10/80	EL-507	Elanco	0.25	0	2	2	2	0
			0.50	0	3	4	5	2
			1.00	0	5	9	23	17
			2.00	0	20	25	30	40
			4.00	0	20	35	77	72
			0.0	0	0	0	0	0

Table 14

Preliminary Study of Waterhyacinth Growth Response to Various Rates of EL 507

10 Weeks Posttreatment

Growth Parameter	Application Rate of EL 507, kg/ha*						
	0	0.25	0.50	1.00	2.00	4.00	F(DF)
Root length, cm	22.6^{bc}	25.2^{c}	18.3^{b}	6.3^{a}	3.9^{a}	4.4^{a}	24.0(5,21)
Leaf length, cm**	18.7^{c}	22.4^{d}	22.2^{d}	12.2^{b}	9.1^{a}	7.0^{a}	36.4(5,20)
Petiole length, cm**	13.8^{c}	16.4^{d}	16.8^{d}	9.1^{b}	6.4^{a}	4.8^{a}	36.8(5,20)
Lamina length, cm**	5.4^{b}	5.9^{b}	5.4^{b}	2.9^{a}	2.7^{a}	2.3^{a}	29.9(5,20)
Petiole width, cm**	1.3^{bc}	1.0^{ab}	1.2^{ab}	0.8^{a}	1.2^{ab}	1.7^{c}	4.0(5,20)
Lamina width, cm**	6.6^{c}	6.8^{c}	5.7^{c}	4.5^{b}	4.5^{b}	3.3^{a}	13.0(5,20)
Petiole length:width**	11.8^{b}	17.9^{c}	21.1^{c}	13.0^{b}	7.6^{a}	4.4^{a}	25.4(5,20)
Lamina length:width**	0.9^{b}	0.9^{b}	1.1^{c}	0.9^{b}	0.7^{a}	0.7^{a}	7.7(5,201)
Root:leaf length**	1.4^{d}	1.0^{c}	0.8^{bc}	0.6^{ab}	0.5^{a}	0.6^{ab}	11.6(5,201)
No. of leaves/stem	6.5^{b}	6.7^{b}	7.4^{b}	6.7^{b}	5.3^{a}	5.0^{a}	5.1(5,212)
No. of inflorescences/stem	1.05^{c}	1.22^{c}	0.40^{b}	0.4^{b}	0.0^{a}	0.0^{a}	27.6(5,212)
No. of stems/container	12.7^{a}	11.7^{ab}	10.0^{a}	15.7^{b}	11.0^{ab}	11.0^{ab}	1.2(5,12)
Dry weight, g/container	67.8^{a}	62.2^{a}	43.1^{b}	29.0^{c}	14.5^{d}	21.0^{cd}	19.9(5,12)

* Means followed by the same letter not significantly different, p = 0.05, as determined by Least Significant Difference (LSD) test.

** Data from the third leaf on each plant.

Table 15

Greenhouse Evaluation of RO 3-7042 for Phytotoxicity Toward Ditchbank Plants

Date of Evaluation	Chemical Designation	Company or Source	Rate kg/ha	Percent Control Weeks Posttreatment									
				Paragrass					Torpedograss				
				2	4	8	16	20	4	8	16	20	34
3/10/79	RO 3-7042	MAAG	1.0	5	10	3	0	5	5	0	0	5	5
			2.0	10	15	13	12	20	10	5	0	3	5
			4.0	10	35	67	87	88	25	32	30	20	7
			6.0	10	75	82	98	98	25	35	40	40	10
			10.0	10	75	80	97	98	50	55	55	50	22
			0.0	0	0	0	0	7	0	0	0	7	5

APPENDIX A: HERBICIDE ANALYSES METHODS DEVELOPMENT

1. Method development for evaluating controlled release formulations of 2,4-D and dichlobenil included sample preparation and quantification of herbicide content by laboratory techniques. For reliable measurement of trace amounts of herbicide residues in natural waters, it was necessary to develop methods for extraction, concentration, and/or esterification of samples as well as optimum chromatographic conditions for analyses with liquid and gas chromatographs.

2. Method development of sample collection and preparation for the quantification of 2,4-D and dichlobenil was carried out as follows:

a. Determination of chromatographic conditions for analytical standard analysis.

b. Development of efficient techniques to be used for sample preparation.

c. Determination of chromatographic conditions for the quantification of herbicides released from controlled release formulations.

The following apparatus were used:

a. Liquid chromatograph (HPLC):

(1) Perkin Elmer Model Series 3 B pump system with Rheodyne 7125 injector and HCODS SIL X (reverse phase) column.

(2) Perkin Model LC 75 variable wavelength detector.

(3) Perkin Elmer Model LC 75 auto control.

b. Gas chromatograph (GC): Perkin Elmer Model 3920 gas chromatograph equipped with ^{63}Ni electron capture detector. Column:

(1) 1.5 percent OV 17/1.95 percent QF-1 on 80/100 mesh Chrom Q.

(2) 1.5 percent SP 2250/1.95 percent SP 2401 on 100/120 mesh Suppelcoport.

Carrier Gas: N_2 at 60 ml/min.

c. Recorder-integrators:

(1) Varian Model 9176 recorder.

(2) Perkin Elmer Sigma 10 Data station.

Determination of Chromatographic Conditions for 2,4-D Standard Analyses by HPLC

3. Available published methods for the analyses of 2,4-D by HPLC were evaluated initially. Chromatographic conditions described by Skelly, Stevens, and Mapes (1977) using a mobile phase of acetonitrile and a phosphate buffer solution provided both poor resolution and significant variability in retention times--important factors in accurate quantification. The conditions recommended by Waters Associates (1978), substituting deionized water for the buffer solution, stabilized retention times without significantly improving resolution. When 2 percent acetic acid was substituted for deionized water, improved separation and quantification of 2,4-D in water samples were observed (Gard, undated). To increase sensitivity and lower detection limits, quantification was made at various wavelengths and with different ratios of acetonitrile:acetic acid mobile phase. Optimum chromatographic conditions for the analysis of 2,4-D in deionized water samples were determined to be as follows:

a. Mobile phase - Acetonitrile: 1 percent acetic acid in ultrapure water (20:80).

b. Flow rate: 2.5 ml/min.

c. Detection: UV 285 nm 0.1 AUFS.

4. A series of 2,4-D standards in deionized water, natural water, and acetonitrile were quantitated to determine detection limits and linearity at concentrations of 0.5, 1.0, 2.0, 4.0, 6.0, and 10 mg/ℓ. It was determined that the minimum amount of 2,4-D reliably measured was 50 ng (50-µl injection of 1.0 mg/ℓ). Linearity was observed throughout the whole range.

5. When samples of 2,4-D in natural wate were analyzed at a later time, it was necessary to modify the chromatographic conditions to improve resolution and to decrease analysis time. The conditions used for samples from the evaluations of 2,4-D release rates from 2,4-D MOE/GMA Copolymer in reconstituted or natural water taken after 60 days were as follows:

a. Mobile phase - Acetonitrile: 1 percent acetic acid in ultrapure water (35:65).

b. Flow rate: 1.5 ml/min.

c. Detection: UV 285 nm 0.1 AUFS.

Preparation of Water Samples for 2,4-D Analysis

6. A method was developed using SEP-PAK C_{18} cartridges for concentrating 2,4-D in water samples. Optimum conditions were determined for both kind and amount of eluant to provide a 50-times increase in concentration.

7. Fifty-millilitre samples of natural water spiked with 2,4-D analytical standard at the rates of 0.01, 0.03, 0.05, 0.10, and 0.30 mg/ℓ were concentrated on SEP-PAK C_{18} cartridges, eluted with 1 ml acetonitrile, and analyzed by the method developed for liquid chromatography. The lower detection limit was determined to be 0.02 mg/ℓ 2,4-D for a 50-ml sample of water.

8. This method was used for the preparation of samples from the first 2,4-D degradation study. Results (not reported) from this study led to the conclusion that reliable measurement of the lower concentrations could be improved by increasing sample size and/or increasing the concentration factor. During the early part of this investigation it was not possible to take larger samples; consequently, methods to increase the concentration factor were developed. It was determined that the 2,4-D concentration could be increased 100 times with good recovery by evaporating the eluted sample to dryness under vacuum at 80°C and bringing to volume with 0.5 ml deionized water. Accurate quantification of 0.01 mg/ℓ 2,4-D was made for 50-ml samples prepared by the following method:

a. Filtration through 0.45-μ Metricel membrane filters.

b. Acidification with H_2SO_4 to pH 3.

c. Concentration of 2,4-D on SEP-PAK C_{18} cartridges.

d. Elution with 1.5 ml acetonitrile.

e. Evaporation to dryness under vacuum at 80°C.

f. Reconstitution with 0.5 ml deionized water.

g. Quantification of 50-µl samples by liquid chromatography.

It is possible to prepare at least 50 samples/day for analyses with this procedure.

9. Samples from the second 2,4-D degradation study were prepared by this method. During this study, a method for quantification using an internal standard, 2,4,5-T, was developed to increase efficiency and accuracy. The dried, concentrated samples were brought to volume with 0.5 ml of 10 mg/ℓ 2,4,5-T aqueous solution. Chromatographic conditions were modified to improve resolution of the two compounds. These modified conditions were as follows:

a. Mobile phase - Acetonitrile: 1 percent acetic acid (25:75).

b. Flow rate: 2.0 ml/min.

c. Detection: 0.1 AUFS.

Determination of Chromatographic Conditions for 2,4-D Methyl Ester Analyses by GC

10. The U. S. Environmental Protection Agency (EPA) (1971) approved GC method for the quantification of 2,4-D residues in water samples was evaluated with 2,4-D methyl ester standards in toluene and hexane. The following chromatographic conditions were optimized for available equipment with column 1: injector temperature, 225°C; column temperature, 195°C; interface temperature, 250°C; detector temperature, 275°C. Standards of 2,4-D methyl ester, 0.1-10 ppm in hexane, were quantitated to determine detection limits. It was determined that the lower detection limit was 0.002 mg/ℓ for a 50-ml sample concentrated 50 times (5-µl injections = 0.5 ng). Saturation was observed when injections were greater than 10 ng.

11. The initial quantification by peak height measurement compared favorably with areas integrated with the Sigma 10 data station. Other chromatographic conditions evaluated with different available column packings were observed to be less sensitive with poorer resolution.

Preparation of Water Samples for the Analysis of 2,4-D by GC

12. The preparation of water samples for the analysis and quantification of 2,4-D by GC included concentration, extraction, and esterification. The method approved by EPA for the preparation and esterification of 2,4-D in water samples was evaluated and found to be complicated with relatively low recovery from 1-ℓ samples when the required cleanup steps for natural water were used (EPA 1971). For accurate measurement of the trace amounts of 2,4-D (less than 0.01 mg/ℓ) in the 50-ml size samples feasible in initial laboratory tests, it was necessary to develop a method for their preparation and esterification.

13. Fifty-millilitre samples of deionized water spiked with 2,4-D standard at various rates were concentrated on SEP-PAK C_{18} cartridges and eluted with acetonitrile. The eluant was used to evaluate various published procedures for the esterification of 2,4-D with boron trifluoride methanol and with boron trichloride 2-chloroethanol (Woolson and Harris 1967, Agemian and Chau 1977, Woodham et al. 1972). A methylating procedure for 2,4-D acid with boron trifluoride methanol was developed that proved to be efficient and relatively rapid with greater than 90 percent recovery. When natural water samples spiked with 2,4-D were prepared by this method, organic impurities in the water interfered with quantification. It was necessary to use several cleanup steps to remove the impurities from the concentrated samples prior to esterification. (Impurities were removed after concentration more efficiently with less equipment than by conventional methods.)

14. The method developed for the preparation and esterification of 2,4-D in water samples for the analysis is as follows:

a. 50-ml samples filtered through 0.45-μ Metricel membrane filters.

b. Acidification with H_2SO_4 to pH 3.

c. Concentration of 2,4-D on SEP-PAK C_{18} cartridges.

d. Elution with 1.5 ml acetonitrile.

e. Wash eluant with 7 percent sodium bicarbonate.

f. Extraction with 1 ml hexane 2X.

g. Acidification to pH 0 with 50 percent hydrochloric acid.

h. Extraction with 1 ml diethylether 3X.

i. Evaporation of ether under airflow.

j. Esterification with 0.5 ml methanol + 1 ml boron trifluoride methanol at 70°C for 1 hour.

k. Extraction of 2,4-D methyl ester in 1 ml hexane.

l. Wash hexane fraction with 7 percent sodium sulfate.

m. Quantification of 5-µl samples by gas chromatography (Column 2).

Samples from tests evaluating 2,4-D release rates from 2,4-D MOE/GMA Copolymer in static reconstituted water, in static natural water, and flowing natural water were prepared by this method.

Determination of Chromatographic Conditions for the Analysis of Dichlobenil by GC

15. Chromatographic conditions were developed for the analysis of dichlobenil with standards of 0.005, 0.01, 0.05, and 0.10 mg/ℓ in hexane. Chromatographic conditions used for the analysis of 2,4-D methyl ester were modified to quantitate dichlobenil using 2,4-D ME as internal standard (Bowmer et al. 1976). Detection limit was <0.05 ng, 5 µl of 0.01 mg/ℓ dichlobenil. The following chromatographic conditions (Column 2) were used: injector temperature, 230°C; interface temperature, 250°C; detector temperature, 275°C; column temperature, 140-195°C at 32°/min.

Preparation of Water Samples Spiked With Dichlobenil for Analysis by GC

16. Deionized water samples were spiked with dichlobenil standards at the rates of 0.005, 0.01, 0.05, and 0.1 mg/ℓ. It was determined that more than 98 percent dichlobenil was extracted by shaking 30 min with hexane on a wrist-action shaker. The hexane was spiked with 2,4-D ME for use as an internal standard. Analysis with a gas chromatograph

was made by injecting 2 to 5 µl from the hexane layer. The lower detection limit was <0.01 mg/ℓ.

17. Samples from evaluation of dichlobenil release from beeswax and from the degradation study were prepared by this procedure.

Determination of Chromatographic Conditions for the Analysis of Dichlobenil by HPLC

18. Chromatographic conditions are being developed and optimized for the HPLC analysis of dichlobenil residues in water. Samples of the higher concentrations of dichlobenil from the degradation and release rate studies were analyzed by the following chromatographic conditions:

a. Mobile phase Acetonitrile: 2.5 percent acetic acid in ultrapure water (40:60).

b. Flow rate: 1.0 ml/min.

c. 50-µl injections.

d. Detection: UV 293 nm

Although no detection limit has been determined, it is less than 50 ng (50 µl of 1 mg/ℓ dichlobenil).